Carcinogenesis vol.29 no.5 pp.964–970, 2008
doi:10.1093/carcin/bgn056
Advance Access publication February 22, 2008

Iron intake, oxidative stress-related genes (*MnSOD* and *MPO*) and prostate cancer risk in CARET cohort

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Iron overload may increase prostate cancer risk through stimulation of oxidative stress, and endogenous pro- and antioxidant capabilities, i.e. manganese superoxide dismutase (MnSOD) and myeloperoxidase (MPO), may modify these associations. We investigated this hypothesis in the Carotene and Retinol Efficacy Trial cohort in a nested case–control study. Although there was no association between iron intake and risk overall, there was a suggestion of increased risk of clinically aggressive prostate cancer with higher iron intake [odds ratio (OR) = 1.4, 95% confidence interval (CI) = 0.9–2.0]. Associations were most notable for men with aggressive prostate cancer who were below the median consumption of total fruits and vegetables (OR = 1.8, 95% CI = 1.1–3.2). Associations between *MPO* −463 *G* to *A* genotype (rs2333227) and prostate cancer risk were only noted among men with aggressive cancer, with more than a 2-fold risk reduction among men with *AA* genotypes (OR = 0.4, 95% CI = 0.2–1.0); *MnSOD* was not associated with risk overall, but the *MnSOD T* to *C* (Val-9Ala, rs4880) polymorphism modified associations between risk of clinically aggressive prostate cancer and dietary iron intake (*P* for interaction = 0.02). Among aggressive cancer cases with the *TT* genotype, higher iron intake level was associated with >2-fold increase in risk (OR = 2.3, 95% CI = 1.0–4.9), whereas there was no association among men with *CC* genotypes (OR = 0.9, 95% CI = 0.4–2.3). Although interactions were not significant, there were similar patterns for *MPO* genotype, iron intake and risk. These findings suggest that higher iron intake may be associated with risk of clinically aggressive prostate cancer, and that endogenous antioxidant capabilities may modify these associations.

Introduction

The persistent generation of reactive oxygen species (ROS) in cells is stimulated by carcinogens, infection, inflammation, environmental toxicants, nutrients and mitochondrial respiration and is an inevitable consequence of aging in aerobic organisms (1–3). Age-related elevation of free radicals has been associated with increased risk for cancer (4). Prostate cancer is a progressive disease in which tumor cells under oxidative stress may manifest continuous genetic alterations that may lead to carcinogenesis (5,6). Recent experimental studies support a role for ROS in prostate cancer with human variation in response to ROS damage and repair exacerbating ROS-related DNA damage in the prostate (7,8).

Excessive iron intake from either foods or dietary supplements can be a source of ROS, although results from epidemiologic studies have been inconsistent (9–15). Iron, the most prevalent metal in the body, reacts with hydrogen peroxide and catalyzes the generation of highly reactive hydroxyl radicals, thereby increasing oxidative stress, which in turn increases free iron concentrations by the Fenton and Haber–Weiss reaction (16). The oxidative stress produced by dietary intake of iron might be modified by endogenous oxidant and antioxidant capabilities that may act in concert to provide a coordinated network of protection against ROS accumulation and oxidative damage (17).

Manganese superoxide dismutase (MnSOD) may play an important role in mediating oxidative stress resulting from high iron intake. Superoxide radicals can attack the iron–sulfur cluster of various enzymes releasing free iron (ferric iron), which can subsequently react with hydrogen peroxide to generate increased levels of ROS (18). MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, and an *MnSOD* valine to alanine substitution at amino acid −9 (*T* to *C*, rs4880) allows more efficient MnSOD uptake into the mitochondria and thus may result in higher activity compared with the *Ala* allele (19). Given its role in producing hydrogen peroxide, the *MnSOD Ala* variant has been associated with risk of prostate (20,21), breast (22,23) and bladder (24) cancers, but not all studies have noted associations (25–29).

Myeloperoxidase (MPO) is a lysosomal enzyme located in neutrophils and monocytes and facilitates conversion of hydrogen peroxide to hypochlorous acid, a cytotoxic antimicrobial agent. An *MPO* −463 *G* to *A* substitution (rs2333227) located in the consensus binding site of a SP1 transcription factor in the 5′ upstream region (30), confers lower transcriptional activation than the −463 *G* common allele *in vitro*, due to disruption of the binding site (31,32). Hypochlorous acid reacts with other biological molecules to generate secondary oxidation products, which in synergy with iron increase oxidative damage (33).

Given the potential role of iron as a pro-oxidant to provoke DNA damage and lead to subsequent carcinogenesis, dietary iron intake may play a role in prostate cancer etiology. In this study, we evaluated associations between dietary iron intake and risk of overall prostate cancer as well as clinically aggressive prostate cancer. Although we reported previously no main effects with regards to *MnSOD* polymorphism and prostate cancer risk in the Carotene and Retinol Efficacy Trial (CARET) cohort (34), we assessed whether genetic polymorphisms known to affect the activity of *MnSOD* and *MPO* modified potential relationships between iron intake as a source of ROS and prostate cancer risk.

Abbreviations: BMI, body mass index; CARET, Carotene and Retinol Efficacy Trial; CI, confidence interval; FDR, false discovery rate; FFQ, food frequency questionnaire; MnSOD, manganese superoxide dismutase; MPO, myeloperoxidase; OR, odds ratio; ROS, reactive oxygen species.

Materials and methods

Study participants

CARET was a multicenter randomized, double-blind placebo-controlled chemoprevention trial to test β-carotene plus vitamin A (retinol) for the prevention of lung cancer among 18 314 heavy smokers, former heavy smokers and asbestos-exposed workers (35–37). Briefly, CARET began in 1985 and ended in 1996 when interim analysis found evidence that the supplements increased the risk of lung cancer and total mortality (35). Active follow-up of all participants continued until 2005 and included the collection of disease end point data. Age, sex, race/ethnicity, education, smoking history, alcohol use, diet, general health history and body mass index (BMI) were collected at each participant's first CARET clinic visit, with updates provided at all CARET contacts. Fasting blood was collected at annual study center visits and was separated into aliquots and frozen for later analysis; whole blood suitable for DNA extraction was available for 68% of CARET participants. All participants provided written informed consent at recruitment and throughout the study. The Institutional Review Board of the Fred Hutchinson Cancer Research Center and each of the five other participating institutions approved all procedures for the study, and for this study, additional Institutional Review Board approval was obtained from Roswell Park Cancer Institute.

A total of 778 prostate cancer cases were confirmed by 2005 through the medical records and pathology reports reviewed by one of the coauthors (G.G.) and considered for a series of nested case–control studies. After excluding men with prior cancer history at the baseline visit, 724 cases were eligible for this study. Staging information and Gleason scores were available for 627 (87%) and 674 (93%) of the study cases, respectively, with no information for 23 cases. Aggressive prostate cancer was defined as that which was diagnosed with extraprostatic extension or metastasis (stage III or IV) or with Gleason sum of ≥7. Eligible controls were men who were free of both prostate cancer and lung cancer (the primary end point in CARET), and had available whole blood or extracted DNA. Cases and controls were frequency matched on age (5 year increments), race/ethnicity and follow-up time in CARET, resulting in a total of 724 cases and 1474 controls. Among them, there was lack of dietary data on 117 participants [63 cases (8.7%) and 114 controls (7.7%), $P = 0.433$ by chi-square test], and there was insufficient DNA or genotyping failure for 190 men [159 cases (24.1%) and 6 controls (0.4%), $P < 0.001$ by chi-square test]. There were no significant differences between individuals with food frequency questionnaire (FFQ) data or with genotyping data and those included in initial nested case–control study in any of the variables assessed as described previously (34). Thus, the current study was restricted to 661 cases and 1360 controls with dietary information for overall analysis and slightly smaller numbers with genotyping data (469 cases and 1279 controls with *MnSOD* and 493 cases and 1332 controls with *MPO*).

Dietary assessment

Dietary intake over the previous year was assessed at baseline and every 2 years thereafter with a self-administered FFQ, which was reviewed for completion by CARET study staff. As published previously (38), the CARET FFQ was designed to be especially sensitive to the measurement of fruits and vegetables and their nutrients. The three sections of the FFQ included seven adjustment questions on types of food and preparation techniques, 110 line items on frequency and portion size and two summary questions, used to reduce measurement error that can often occur when participants are asked to respond to long lists of foods. The nutrient database was derived from the University of Minnesota Nutrition Coordinating Center database (39) and included the United States Department of Agriculture-Nutrition Coordinating Center carotenoid database for USA foods (40). Iron (milligram) is one of >130 standard nutrient variables available in the database and the iron values are originally derived from analyzes at the United States Department of Agriculture Nutrient Composition Laboratory (39). For these analyses, we used the dietary intakes averaged across all FFQs completed prior to the prostate cancer diagnosis date for the cases. For the matched controls, we used all FFQs completed up until the reference diagnosis date of the matched case. Participants were excluded from any dietary analyses if their reported energy intake was either <800 or >5000 kcal/day because these energy estimates were considered unreliable.

DNA extraction and genotyping

Genomic DNA was extracted from whole blood samples with the use of QIAamp DNA blood Midi kits (Qiagen, Valencia, CA) and genotyping was performed by BioServe Biotechnologies (Laurel, MD) with Sequenom's (San Diego, CA) high-throughput matrix-assisted laser desorption/ionizing time-of-flight mass spectrometry, as described previously (41,42). There was excellent interassay agreement among the 8% of randomly selected duplicates of genotyping results that were included for quality control purposes (κ statistic: 0.95) with <1% assay failure rate.

Statistical analysis

Distributions of putative risk factors among cases and controls were assessed by the chi-square test for categorical variables or Student's *t*-test for continuous variables, after log transformation to approximate a normal distribution for nutrients, which typically have a skewed distribution.

Iron intake was modeled as a continuous variable and then categorized into tertiles based on the distributions in the controls. Unconditional logistic regression was performed to test the association of dietary iron intake with prostate cancer risk while adjusting for potential confounding variables. Associations between iron intake and risk by disease severity (aggressive versus non-aggressive) were tested using polychotomous logistic regression. Tests for linear trend across the tertiles were based on a two-sided likelihood ratio test.

Multivariate models were simultaneously adjusted for covariates that were significant in the initial logistic regression model ($P < 0.2$), those used in previous CARET reports (34,43) or known confounders of iron-related ROS production (44). These included age at enrollment (continuous), race/ethnicity (Caucasian, African-American, others), intervention randomization assignment (β-carotene + retinol, placebo), history of prostate cancer in first-degree relatives (yes, no), smoking status (current, former or never), smoking pack-years (<40, 40–60, ≥60), alcohol consumption (non-drinker, <median, ≥median), BMI (<25.0, 25.0–29.9, ≥30.0), total energy intake (continuous, log transformed) and dietary antioxidants (total carotenoids and vitamin C, continuous variables, log transformed). Among dietary antioxidants, total carotenoids were obtained by summing α-carotene, β-carotene, β-cryptoxanthin, lutein + zeaxanthin and lycopene. Tocopherols and selenium were omitted in the model because they could not be reliably measured with a FFQ.

We stratified the data to investigate whether the main effects of iron intake were modified by oxidative stress-related exposures (CARET randomization assignment, dietary supplements, alcohol consumption, BMI, smoking status and pack-years) or dietary antioxidants (continuous variables of fruit, vegetables and specific dietary antioxidants were dichotomized at the median in controls).

Genotype distributions in the controls were tested for Hardy–Weinberg equilibrium to evaluate possible selection bias or genotyping errors. The risk of prostate cancer was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) by unconditional logistic regression models adjusting for the variables described above. We first examined associations for *MnSOD* genotypes *TC* and *CC*, using *TT* as the reference group, and *MPO* genotypes *GA* and *AA*, using *GG* as the reference group. Subsequently, because the results for *MPO GG* and *GA* genotypes were similar, the two were collapsed into a single reference group. There were no differences between *MnSOD TT* and *TC* genotypes compared with *CC* genotype, and the two were also collapsed into a single reference as a recessive model to have consistency to *MPO* in the analysis. To explore whether main effects of iron intake were modified by genotypes, data were stratified by each genotype, with associations between iron intake and prostate cancer risk examined using a single cell as a reference group (i.e. men with *MnSOD TT* genotype and the lowest tertile of iron intake).

To test statistical interactions on a multiplicative scale, a cross-product term of the ordinal score for tertiles or a linear variable (log transformed) of iron intake and various factors was included in multivariate models. The difference of two $-2\log L$ values of logistic models with and without cross-product terms was evaluated by the likelihood ratio test with one degree of freedom.

To correct for multiple comparison testing, adjusted *P* values were calculated using the false discovery rate (FDR) method of Benjamini *et al.* (45) at each genotype. FDR is defined as the expected proportion of true null hypotheses rejected out of the total number of null hypotheses rejected. In practice, with the true FDR unknown, an estimated FDR can serve as a criterion to evaluate the performance of various statistical methods under the condition that the estimated FDR approximates the true FDR well or at least it does not improperly favor or disfavor any particular method.

All statistical analyses were performed using STATA version 9.0 (Stata Corporation, College Station, TX) except for FDR, which was done by SAS (version 9.1, SAS Institute, Cary, NC).

Results

Table I shows demographic, lifestyle characteristics and diet among the participants with FFQ data in this nested case–control study. Cases and controls were similar in age, race, CARET randomization assignment, education, smoking and BMI. Prostate cancer cases were more likely to have history of prostate cancer in a first-degree relative and to drink more alcohol and were less likely to use dietary supplements at baseline than controls.

As shown in Table I, overall, mean iron intake was not significantly different between cases and controls (14.4 versus 14.3 mg/day, respectively), and we found no overall association between iron intake and prostate cancer risk (OR = 1.1, 95% CI = 0.8–1.4 for third versus first tertile) (Table II). However, among men with higher iron intake, the risk of clinically aggressive prostate cancer (stage ≥3 or Gleason score ≥7) was slightly increased (OR = 1.4, 95% CI = 0.9–2.0 for third versus first tertile of iron, $P_{\text{trend}} = 0.07$), although of borderline significance.

When consumption of fruits, vegetables and specific dietary antioxidants were dichotomized at the median among controls, associations between iron intake and aggressive prostate cancer risk were greatest among men who consumed low amounts of food-based antioxidants (data not shown) with a 1.6- to 1.9-fold increase in risk with higher iron intake among men who were below the median of fruit, vegetable or vitamin C consumption (total fruits: OR = 1.6, 95% CI = 1.0–2.8; total fruits and vegetables: OR = 1.7, 95% CI = 1.0–2.9 and vitamin C: OR = 1.9, 95% CI = 1.1–3.2 for third versus first tertile of iron). There was no increase in risk with iron

Table I. Selected characteristics of 661 prostate cancer cases and 1360 controls in the CARET cohort, 1985–1996

Variables	Cases, $N = 661$	Controls, $N = 1360$	P value[a]
Age at enrollment (years)			
<55	132 (20.0)	273 (20.1)	
55–59	174 (26.3)	360 (26.5)	
60–64	189 (28.6)	392 (28.8)	
≥65	166 (25.1)	335 (24.6)	1.000
Race (*n*, %)			
Caucasian	594 (89.9)	1204 (88.5)	
African-American	43 (6.5)	108 (7.9)	
Others	24 (3.6)	48 (3.5)	0.51
Random assignment			
Intervention	342 (51.7)	690 (50.7)	
Placebo	319 (48.3)	670 (49.3)	0.67
Family history of prostate cancer (*n*, %)			
No	617 (93.3)	1318 (96.9)	
Yes	44 (6.7)	42 (3.1)	<0.001
Education			
<12 years	98 (14.8)	212 (15.6)	
High school graduate	142 (21.5)	332 (24.4)	
Some college	168 (25.4)	406 (29.9)	
4 year college degree or higher	156 (23.6)	294 (21.6)	0.27
Missing	97 (14.7)	116 (8.5)	—
Any vitamin supplement at baseline			
No	435 (65.8)	831 (61.2)	
Yes	226 (34.2)	528 (39.8)	0.04
BMI			
<25.0	143 (21.8)	311 (23.0)	
25.0–29.9	317 (48.3)	658 (48.7))	
≥30.0	197 (30.0)	382 (28.3)	0.68
Smoking status at baseline[b]			
Never/former	327 (49.5)	635 (46.7)	
Current	334 (50.5)	725 (53.3)	0.24
Smoking pack-year			
<40	235 (35.6)	506 (37.2)	
40–60	246 (37.2)	494 (36.4)	
>60	180 (27.2)	359 (26.4)	0.76
Alcohol consumption			
Non-drinker	169 (25.6)	359 (26.4)	
Below median (10 g/day)	202 (30.6)	500 (36.8)	
At or above median (10 g/day)	290 (43.9)	501 (36.8)	0.005
Disease status			
Non-aggressive prostate cancer	339 (51.3)		
Aggressive prostate cancer[c]	299 (45.2)		
Unknown	23 (3.5)		
Total energy intake, mean (SD), kcal	1782 (594)	1800 (615)	0.73
Total fruit intake, mean (SD), median portion/day	1.1 (0.8)	1.0 (0.8)	0.47
Total vegetable intake, mean (SD), median portion/day	1.7 (0.8)	1.7 (0.8)	0.57
Iron intake, mean (SD), mg/day	14.3 (6.5)	14.4 (7.2)	0.74
Carotenoid intake, mean (SD), mcg/day	10 293 (5666)	10 243 (5057)	0.84
Vitamin C intake, mean (SD), mg/day	80.2 (45.6)	80.0 (44.8)	0.92

[a]*P* value tested by chi-square test (categorical variables) or Student's *t*-test (continuous variables after log transformation, nutrients and diet).
[b]Number of non-smokers was 37 (1.7%).
[c]Stage ≥3 or Gleason score ≥7.

intake among men who were at or above the median for antioxidant intake (total fruits: OR = 1.1, 95% CI = 0.6–2.0; total fruits and vegetables: OR = 1.1, 95% CI = 0.6–1.9 and vitamin C: OR = 1.0, 95% CI = 0.6–1.8 for third versus first tertile of iron). We also evaluated alcohol consumption as an oxidative stress-related exposure as well as a significant risk factor in this cohort. Drinking did not significantly change relationships between iron intake and prostate cancer, and similarly, no associations were noted for other oxidative stress-related exposures (BMI, smoking status and pack-years smoked) (data not shown). There were no significant interactions between iron intake and the above factors.

As shown in Table III and published previously for *MnSOD* (34), there were no significant associations between *MnSOD* and *MPO* genotypes on prostate cancer risk overall; however, among men with aggressive prostate cancer, *MPO AA* genotypes were associated with more than a 2-fold reduction in risk (OR = 0.4, 95% CI = 0.2–1.0) compared with *MPO GG* genotypes. There were no gene dosage effects for either genotype (non-significant *P* for trend) or combined genotypes (data not shown). Because distributions of *MPO* genotypes were significantly different between races/ethnicities, we conducted analysis among Caucasians only; however, risk relationships were essentially unchanged. Because the cell sizes were too small for other race/ethnicity, we included all cases and controls in the analysis and adjusted for race/ethnicity. *MnSOD* genotypes did not differ between race/ethnicities. There were no significant differences between strata of alcohol consumption and other oxidative stress-related factors.

Genetic variation modified the associations between iron intake and prostate cancer risk, especially for *MnSOD* (*P* for interaction = 0.01 for all and 0.02 for aggressive cases; Table IV). In stratified analysis by the *MnSOD* genotype, risk increased as iron intake increased among men homozygous for *TT* genotypes (*P* for trend = 0.02 overall; *P* for trend = 0.01 in aggressive cases), whereas there was decreased risk among men with CC genotypes as iron intake increased (*P* for trend = 0.04 overall; *P* for trend = 0.34 in aggressive cases). In the results presented in Table IV using a single reference group combining genotypes and dietary iron, we observed significantly increased risk of clinically aggressive prostate cancer among men with *TT* genotypes and the highest tertile of iron intake (OR = 2.3, 95% CI = 1.0–4.9); however, there were no associations (OR = 0.9, 95% CI = 0.4–2.3) for those with *CC* genotypes. The interaction between *MnSOD* genotypes and iron intake on risk of prostate cancer remained significant after adjusting for multiple comparisons by the FDR method (adjusted *P* for interaction = 0.05 overall). Similar associations were noted for *MPO* although they were weaker; risk of aggressive prostate cancer was increased among men with *MPO GG* genotypes who were in the highest tertile of iron intake (OR = 1.8, 95% CI = 1.1–3.1), but there were no associations among men with high iron intake and *MPO AA* genotypes (OR = 1.0, 95% CI = 0.3–3.6). Interactions between *MPO* polymorphism and iron intake were not statistically significant (Table IV).

Discussion

In this case–control study nested in the CARET cohort, we found that higher dietary iron was not associated with risk of overall prostate cancer, but significantly increased risk of clinically aggressive prostate cancer. There was some evidence that associations were stronger among men with low dietary intake of antioxidant-rich foods, such as fruits and vegetables. Although there were no main effects for *MnSOD* genotypes on prostate cancer risk, we found a significant interaction between genotypes and iron intake on risk. *MPO AA* genotypes reduced the risk of aggressive prostate cancer, but there were no significant interactions with iron intake.

Iron intake has been examined in relation to risk of cancers of the colon (11–13) and lung (14,15) with inconsistent results. There have been few studies examining associations between iron intake and prostate cancer risk, although levels of red meat consumption, a source of iron intake, has been associated with increased risk of prostate cancer in large cohort studies (46,47). We (A.R.K.) investigated previously the association between iron-containing dietary supplements and prostate cancer risk in a population-based case–control study with middle-aged men, finding a non-significant increase in risk with iron supplementation (10). However, the prevalence of supplemental iron intake was low in this population, with <4% of both cases and controls reporting use. In contrast, Vlajinac *et al.* (9) reported an OR of 0.34 (95% CI = 0.1–1.0) comparing the highest tertile of dietary iron

Table II. Associations between iron intake and prostate cancer risk in 661 nested cases and 1360 controls from CARET cohort, 1985–1996

Dietary iron (mg/day)	Dietary intake of iron				*P* for trend
	Continuous[a]	Tertile			
		First <10.7	Second 10.7–15.7	Third ≥15.8	
Controls, *N*		452	454	454	
All cases, *N*		222	205	234	
Age- and race-adjusted OR (95% CI)	1.0 (0.9–1.2)	1.0 (reference)	0.9 (0.7–1.2)	1.0 (0.8–1.3)	0.70
Fully adjusted OR (95% CI)[b]	1.0 (0.9–1.3)	1.0 (reference)	0.9 (0.7–1.2)	1.1 (0.8–1.4)	0.54
Non-aggressive cases, *N*		122	107	110	
Age- and race-adjusted OR (95% CI)	1.0 (0.8–1.2)	1.0 (reference)	0.9 (0.7–1.2)	0.9 (0.7–1.2)	0.43
Fully adjusted OR (95% CI)[b]	1.0 (0.8–1.3)	1.0 (reference)	0.9 (0.6–1.2)	0.9 (0.6–1.3)	0.67
Aggressive cases, *N*		90	90	119	
Age- and race-adjusted OR (95% CI)	1.1 (0.9–1.3)	1.0 (reference)	1.0 (0.7–1.4)	1.3 (1.0–1.8)	0.07
Fully adjusted OR (95% CI)[b]	1.1 (0.9–1.4)	1.0 (reference)	1.0 (0.7–1.5)	1.4 (0.9–2.0)	0.07

The ORs in overall were estimated by unconditional logistic regression model and the ORs among subgroups of cases according to disease status were tested by polychotomous logistic regression model adjusting for variables described below.
[a]OR per 10 mg/day.
[b]Adjusted for race, random assignment, family history of prostate cancer in first-degree relatives, alcohol consumption (non-drinker, below median, at or above median), smoking status (current versus former or never), smoking pack-year (<40, 40–60, ≥60), age at enrollment (continuous), BMI (<25.0, 25.0–29.9, ≥30.0), total energy intake (continuous, log transformed) and dietary antioxidants (carotenoids and vitamin C, continuous variables, log transformed).

Table III. Association between *MnSOD* rs4880 T/C and *MPO* rs2333227 G/A genotypes and prostate cancer risk in 502 nested cases and 1354 controls from CARET cohort, 1985–1996

				P for trend		
MnSOD rs4880	*TT*	*TC*	*CC*		*TT+TC*	*CC*
Control, N	327	635	317		962	317
All cases, N	119	245	105		364	105
OR (95% CI)[1]	1.0 (reference)	1.1 (0.8–1.4)	0.9 (0.7–1.3)	0.58	1.0 (reference)	0.9 (0.7–1.1)
Nonaggressive cases, N	63	128	53		191	53
OR (95% CI)[2]	1.0 (reference)	1.0 (0.7–1.5)	0.9 (0.6–1.3)	0.49	1.0 (reference)	0.8 (0.6–1.2)
Aggressive cases, N	53	108	50		161	50
OR (95% CI)[2]	1.0 (reference)	1.1 (0.7–1.5)	1.0 (0.7–1.5)	0.99	1.0 (reference)	1.0 (0.7–1.4)
MPO rs2333227	GG	GA	AA		GG+GA	AA
Control, N	807	447	78		1254	78
All cases, N	301	174	18		475	18
OR (95% CI)[1]	1.0 (reference)	1.1 (0.9–1.3)	0.6 (0.4–1.1)	0.51	1.0 (reference)	0.6 (0.4–1.1)
Nonaggressive cases, N	149	89	12		238	12
OR (95% CI)[2]	1.0 (reference)	1.1 (0.8–1.5)	0.9 (0.5–1.6)	0.88	1.0 (reference)	0.8 (0.4–1.6)
Aggressive cases, N	141	83	5		224	5
OR (95% CI)[2]	1.0 (reference)	1.1 (0.8–1.5)	0.4 (0.2–1.0)	0.36	1.0 (reference)	0.4 (0.2–0.9)

[a]The ORs in overall were estimated by unconditional logistic regression model adjusting for race, random assignment, family history of prostate cancer in first-degree relatives, alcohol consumption (non-drinker, below median, at or above median), smoking status (current versus former or never), smoking pack-year (<40, 40–60, ≥60), age at enrollment (continuous), BMI (<25.0, 25.0–29.9, ≥30.0), total energy intake (continuous, log transformed) and dietary antioxidants (carotenoids and vitamin C, continuous variables, log transformed).
[b]The ORs among subgroups of cases according to disease status were tested by polychotomous logistic regression model adjusting for the same covariates listed above.

intake with the lowest tertile, although hospital-based case–control studies may be prone to both recall and information bias.

Iron availability in the body is affected by many factors, such as iron form (heme iron versus non-heme iron) and dietary sources of iron. Heme iron is more easily absorbed than non-heme iron. However, it is difficult to distinguish heme from non-heme iron by FFQs because there is no available nutrient database that specifies heme and non-heme iron, with heme iron content varying between foods from 17 to 80% of total iron content (48). Available databases also do not consider loss of heme iron during cooking (49). In addition, absorption of non-heme iron is notably dependent on other dietary factors. For example, absorption is increased with certain vitamins (vitamin C and vitamin A) and meat protein factors and impaired with proteins from egg, milk and dietary products. Absorption is also decreased with fiber, polyphenol and phytic acid intake (49). FFQs do not have information on meal patterns or foods consumed together, and thus it is difficult to determine the non-heme iron availability.

As a significant oxidative stress-related exposure in this cohort, alcohol consumption was tested whether it could modify the association between iron intake, *MnSOD* genotypes and prostate cancer risk. Although considering the influence of ethanol consumption on iron metabolism (50) and the fact that MnSOD is inducible by ethanol (51), there were no differences as other factors related to oxidative stress. Hypothetically, antioxidants can scavenge or suppress free radicals or ROS damage (52), thereby reducing the risk of prostate cancer attributable to oxidative stress. We found that relationships between aggressive prostate cancer risk and iron intake were modified by intake of dietary antioxidants, although previous studies have

Table IV. Association between iron intake and *MnSOD* rs4880 T/C and *MPO* rs2333227 G/A genotypes on prostate cancer risk in 502 nested cases and 1354 controls from CARET cohort, 1985–1996

Genotypes	Dietary iron tertile								
	First			Second			Third		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
MnSOD rs4880									
All[a]									
TT	35	108	1.0 (reference)	33	114	0.8 (0.5–1.5)	51	105	1.4 (0.8–2.5)
TC	78	208	1.2 (0.7–1.9)	75	215	1.0 (0.6–1.6)	92	212	1.3 (0.8–2.1)
CC	43	105	1.3 (0.8–2.2)	35	105	1.0 (0.6–1.8)	27	107	0.7 (0.4–1.2)
TT + TC	113	316	1.0 (reference)	108	329	0.8 (0.6–1.2)	143	317	1.2 (0.8–1.7)
CC	43	105	1.2 (0.8–1.8)	35	105	0.9 (0.6–1.5)	27	107	0.6 (0.4–1.0)
P for interaction									0.01
Non-aggressive[b]									
TT	21	108	1.0 (reference)	18	114	0.7 (0.4–1.4)	24	105	1.0 (0.5–2.0)
TC	45	208	1.1 (0.6–1.9)	38	215	0.8 (0.4–1.4)	45	212	0.9 (0.5–1.8)
CC	22	105	1.1 (0.6–2.1)	16	105	0.7 (0.3–1.5)	15	107	0.6 (0.3–1.2)
TT + TC	66	316	1.0 (reference)	56	329	0.7 (0.5–1.1)	69	317	0.9 (0.6–1.4)
CC	22	105	1.0 (0.6-1.8)	16	105	0.7 (0.4–1.3)	15	107	0.5 (0.3–1.0)
P for interaction									0.14
Aggressive[b]									
TT	13	108	1.0 (reference)	14	114	1.0 (0.5–2.4)	26	105	2.3 (1.0–4.9)
TC	31	208	1.3 (0.6–2.6)	33	215	1.3 (0.6–2.6)	44	212	1.9 (0.9–3.9)
CC	19	105	1.6 (0.7–3.4)	19	105	1.7 (0.8–3.7)	12	107	0.9 (0.4–2.3)
TT + TC	44	316	1.0 (reference)	47	329	1.0 (0.6–1.6)	70	317	1.7 (1.0–2.8)
CC	19	105	1.3 (0.7–2.4)	19	105	1.4 (0.8–2.6)	12	107	0.8 (0.4–1.6)
P for interaction									0.02
MPO rs2333227									
All[a]									
GG	84	261	1.0 (reference)	102	281	1.1 (0.8–1.6)	115	265	1.3 (0.9–1.9)
GA	65	152	1.4 (0.9–2.0)	48	142	1.0 (0.7–1.6)	61	153	1.2 (0.8–1.9)
AA	6	27	0.7 (0.3–1.8)	4	23	0.5 (0.2–1.6)	8	28	0.9 (0.4–2.1)
GG + GA	149	413	1.0 (reference)	150	423	0.9 (0.7–1.3)	176	418	1.1 (0.8–1.5)
AA	6	27	0.6 (0.3–1.6)	4	23	0.5 (0.2–1.4)	8	28	0.8 (0.3–1.8)
P for interaction									0.85
Non-aggressive[b]									
GG	46	261	1.0 (reference)	50	281	0.9 (0.6–1.5)	53	265	1.0 (0.6–1.7)
GA	37	152	1.4 (0.9–2.4)	23	142	0.8 (0.5–1.5)	29	153	1.0 (0.6–1.7)
AA	5	27	1.1 (0.4–3.0)	2	23	0.5 (0.1–2.1)	5	28	0.9 (0.3–2.6)
GG + GA	83	413	1.0 (reference)	73	423	0.8 (0.5–1.1)	82	418	0.9 (0.6–1.3)
AA	5	27	0.9 (0.4–2.5)	2	23	0.4 (0.1–1.8)	5	28	0.8 (0.3–2.2)
P for interaction									0.99
Aggressive[b]									
GG	34	261	1.0 (reference)	49	281	1.4 (0.8–2.3)	58	265	1.8 (1.1–3.1)
GA	27	152	1.4 (0.8–2.5)	24	142	1.4 (0.8–2.5)	32	153	1.7 (1.0–3.1)
AA	1	27	0.3 (0.04–2.3)	1	23	0.3 (0.04–2.7)	3	28	1.0 (0.3–3.6)
GG + GA	61	413	1.0 (reference)	73	423	1.2 (0.8–1.8)	90	418	1.6 (1.0–2.4)
AA	1	27	0.3 (0.04–2.0)	1	23	0.3 (0.04–2.3)	3	28	0.8 (0.2–3.0)
P for interaction									0.50

[a]The ORs for all participants were estimated by unconditional logistic regression model adjusting race, random assignment, family history of prostate cancer in first-degree relatives, alcohol consumption (non-drinker, below median, at or above median), smoking status (current versus former or never), smoking pack-year (<40, 40–60, ≥60), age at enrollment (continuous), BMI (<25.0, 25.0–29.9, ≥30.0), total energy intake (continuous, log transformed) and dietary antioxidants (continuous, log transformed, carotenoids and vitamin C).

[b]The ORs among subgroups of cases according to disease status were tested by polychotomous logistic regression model adjusting for the same covariates as above.

shown little evidence for an association between dietary antioxidants and prostate cancer risk (reviewed in refs 53,54). While our subgroup findings may be due to chance, it is possible that aggressive and non-aggressive prostate cancers differ in their etiology and that combining these groups may obscure associations (55,56). Several studies have observed differential associations with antioxidants or oxidative stress on aggressive versus non-aggressive prostate cancer (27,57,58).

We reported previously no main effects of the *MnSOD* polymorphism on risk of prostate cancer (34), which is consistent with results from the Physicians Health Study cohort (27), but contrary to recent results from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (20), which showed *MnSOD Ala/Ala* genotype conferred slightly increased risk of prostate cancer in Caucasians but not in African-Americans. This difference in results between studies may be due to differing participant characteristics, as discussed in more detail below. Our findings of decreased risk of aggressive prostate cancer for men with *MPO AA* genotypes, associated with lower transcription and presumably lower levels of ROS, are consistent with findings for several cancers (59–61), but not all (62). The frequency of the *MPO* variant allele differed >10% between Caucasian (n = 1181) and African-American (n = 106) controls, which is consistent with a previous report in a community-based setting (63). To our knowledge, there have been no investigations of associations between prostate cancer risk and *MPO* polymorphisms, and this is

the first to examine the effects of *MnSOD* and *MPO* genotypes on relationships between dietary iron intake and prostate cancer risk.

There is mechanistic support for an interaction between *MnSOD* genotypes and iron on prostate cancer risk. Ferric acid promotes the formation of ROS with hydrogen peroxide via Haber-Weiss chemistry including the Fenton reaction. Thus, we hypothesized that the higher activity (Ala) variant of *MnSOD* would increase prostate carcinogenesis when iron intake is high. Our results, however, are in conflict with this hypothesis. A possible explanation may be that the *Val* variant, with reduced activity, may increase superoxide anion levels in mitochondria, leading to the release of ferric acid from iron-containing enzymes (i.e. mitochondria aconitase, complex I and succinate dehydrogenase) and increased Fenton reactions (18). Consistent with this study, Valenti *et al.* (64) reported that patients carrying low activity *MnSOD Val* allele had higher prevalence of cardiomyopathy associated with hereditary hemochromatosis, which is characterized by excessive iron deposition and is the consequence of increased ROS due to hepatic iron overload. Perez *et al.* (65) also showed that overexpression of MnSOD prevents iron-related oxidative damage in *in vitro*. In contrast, a study of 162 patients with alcoholic cirrhosis showed that patients with high-activity *MnSOD Ala* allele frequently develop hepatic iron accumulation and have a high risk of hepatocellular carcinoma (66). The inconsistent findings may be due to the different profile of factors in the hepatocellular carcinoma study that influence accumulation of iron: younger age (progressive accumulation) and female gender (loss of iron through menstruation).

MPO produces hypochlorous acid from hydrogen peroxide and chloride anion (Cl^-) during the neutrophil's respiratory burst, which is the rapid release of ROS from immune cells. It requires heme as a cofactor, which consists of an iron atom contained in the center of a large heterocyclic organic ring (67). Thus, the *MPO* G allele might produce more ROS when iron intake is high. Two studies investigated the combined role of *MPO* and iron levels on cancer development, with reports that were consistent with our results. Osterreicher *et al.* (68) assessed the impact of *MPO* genetic polymorphisms on the development of cirrhosis with hereditary hemochromatosis. They found that *MPO GG* genotype was more common in patients with cirrhosis than in those without, modifying the clinical penetrance of hepatic iron overload with respect to hepatic fibrosis in hereditary hemochromatosis. Ekmekci *et al.* (69) observed an association between plasma iron levels and MPO levels on bronchial asthma, an indicator of airway inflammation, finding higher plasma MPO and iron concentrations in asthmatic individuals.

There are limitations of this study that should be considered. First, these findings cannot be easily generalized to other populations because the study was specifically conducted among participants who were heavy smokers or who had occupational exposure to asbestos. Previous studies indicate that expressions of antioxidant enzymes are induced by oxidants, cytokines, asbestos fibers and cigarette smoking (70,71), and thus, results in this cohort exposed to high oxidative stress factors may be specific to those with chronic pro-oxidative exposures. Second, iron intake was assessed by FFQ, and we did not measure the biological markers of iron store (i.e. transferrin saturation, serum/plasma iron, serum/plasma ferritin and hemoglobin) or iron load in prostate tissue. Iron accumulation in liver tissue was an independent risk factor for hepatocellular carcinoma among alcohol-induced cirrhosis patients (66), but there have been no studies in prostate cancer. Previous studies have shown that dietary iron intake is a determinant of plasma ferritin levels (72). Thus, despite the limitations of FFQ, they remain an important tool for the dietary assessment of nutrients without biomarkers. Third, we did not measure supplementary iron intake in the FFQ, although the association between dietary iron intake and prostate cancer risk did not change depending on vitamin supplement status. Fourth, controlling for multiple comparisons, associations were null except for the interaction between *MnSOD* genotypes and iron intake. Although the genotypes in this study selected a priori because of their known biological functions, the results will need to be verified by replication. The strengths of our study include its prospective design, a nested age and race-matched case–control study set, multiple measures of dietary assessment, end point ascertainment that utilized medical records and cancer registry files and a relatively large number sample size. Diet and lifestyle data were collected before diagnosis, which avoid or minimize recall bias.

In conclusion, these findings suggest that higher iron intake is associated with risk of clinically aggressive prostate cancer. These associations are significantly modified by endogenous capacity to handle an oxidative load, as well as other nutrients such as antioxidants, that when consumed in lower quantities interact to further increase the iron-associated risk. Replication of these novel findings could further our understanding and prevention of prostate cancer.

Funding

National Cancer Institute (R01 CA96789).

Acknowledgements

Conflict of Interest Statement: None declared.

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Received October 22, 2007; revised January 22, 2008;
accepted February 15, 2008