Iron in Relation to Gastric Cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

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Abstract

Background: Iron is an essential micronutrient that can have carcinogenic effects when at high or low concentrations. Previous studies of iron in relation to gastric cancer have not assessed subtype-specific relationships. We used the prospective Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study to assess whether iron metrics were associated with gastric cardia cancer (GCC) and gastric noncardia cancer (GNCC).

Methods: We selected 341 incident gastric cancer cases (86 cardia, 172 noncardia, and 83 nonspecified), accrued during 22 years of follow-up, and 341 individually matched controls. We measured prediagnostic serum iron, ferritin, unsaturated iron binding capacity, and C-reactive protein. Total iron-binding capacity (TIBC) and transferrin saturation were estimated from these metrics. Dietary iron exposures were estimated from a food frequency questionnaire. Multivariable logistic regression was used for analysis.

Results: Serum iron metrics were not associated with GCC, except for a potential “u”-shaped relationship with TIBC (global $P = 0.038$). GNCC was inversely associated with serum ferritin (global $P = 0.024$), serum iron (global $P = 0.060$) and, possibly, transferrin saturation. TIBC appeared to share a “u”-shaped relationship with GNCC (global $P = 0.033$). Dietary iron exposures were not associated with either subsite. Adjustment for Helicobacter pylori and gastric atrophy had little effect on observed associations.

Conclusions: We found little evidence for the involvement of iron exposure in the pathogenesis of GCC. GNCC was associated with an iron profile similar to that of iron deficiency.

Impact: Our findings indicate that inverse associations between iron metrics and gastric cancer are driven by associations with GNCC. Further elucidation of potential mechanisms is warranted. Cancer Epidemiol Biomarkers Prev; 21(11); 2033–42. ©2012 AACR.

Introduction

Iron is an essential micronutrient involved in oxygen transport and cellular oxidative metabolism. The concentration of iron in humans accumulates with age in most populations, a result of dietary iron exceeding loss and the lack of a biologic mechanism to excrete excess levels (1). This is important because iron can induce oxidative DNA damage via free radical generation (2, 3) and high iron levels have been positively associated with cancer risk (4, 5). In addition, heme iron can catalyze endogenous formation of N-nitroso compounds, which are potent carcinogens. Increasing the complexity of the potential carcinogenic roles of iron is evidence that deficient levels may also increase risk of malignancy.

Gastric cancer is one such malignancy that has been associated with low levels of serum iron (6–8). Although all previous studies have assessed total gastric cancer, the pathology of this malignancy is now recognized to be subsite specific. Helicobacter pylori infection leading to gastric atrophy and then cancer is the de facto multistep pathway of gastric noncardia cancer (GNCC; ref. 9). Gastric cardia cancer (GCC), meanwhile, presents a distinct risk profile more aligned with that of esophageal adenocarcinoma—such as gastroesophageal reflux, increased body mass index, and tobacco smoking—which may result from heterogeneous etiologies within this single subsite (10). If iron is associated with gastric malignancies, it is possible that H. pylori infection could mediate this association, given that it is positively associated with...
biomarkers of iron and its bound state, which included

total number of eligible cases, based on these criteria, was
to have at least 500

defined according to the International Classification of

total iron binding capacity (TIBC) and transferrin saturation; and dietary

total iron, and a proxy of heme iron intake measured using a FFQ.

All serologic iron assays were conducted by Drs. Richard Wood and Gayle Petty at Tufts University. Serum ferritin was quantitated using an immunoradiometric assay (Count-A-Count Ferritin IRMA: Diagnostic Products Los Angeles). Serum iron and UIBC were measured using a standard ferrozine-based iron colorimetric assay (Olympus AU 400e auto analyzer). TIBC (µg/dL) was calculated as the sum of UIBC (µg/dL) and serum iron (µg/dL). Transferrin saturation (%) was calculated as [serum iron (µg/dL)/TIBC(µg/dL)] × 100. Serum ferritin is an indicator of iron stored in the body and is a correlate of heme iron intake (22). Serum iron represents the amount of circulating iron bound to transferrin. UIBC is the amount of transferrin not currently bound to iron; the residual capacity. TIBC represents the blood’s capacity to bind iron with transferrin. Transferrin saturation is the percentage of transferrin currently bound to iron. Ferritin is an acute phase reactant, thus we also measured high-sensitivity C-reactive protein (CRP), a marker of systemic inflammation, to enable adjustment (23). CRP was quantitated using a latex particle enhanced immunoturbidimetric assay kit (K-ASSAY CRP Ultra, Equal Diagnostics).

For quality control purposes we included 36 serum samples aliquoted from a single large pool of serum from ATBC Cancer Prevention Study participants. These 36 samples were distributed among the 9 assay plates (4 samples per plate). On the basis of these samples, the coefficients of variation (CV) were 3.8% for ferritin, 25.5% for serum iron, 7.7% for UIBC, and 5.7% for CRP. However, 3 of the serum iron measurements were clearly outliers at values of 109 (plate 2), 147 (plate 2), and 175 (plate 3) compared with the mean and standard deviation (Mean: 75.9, SD: 1.9) of the remaining 33 samples. When these 3 samples were excluded, the CV for serum iron was 2.5%. There were no differences in overall, control or case distributions of iron amongst the 9 analytic plates. Furthermore, internal low and high iron concentration standards were stable across all analytic plates and produced CVs of 1.0% and 2.0%, respectively.

The FFQ aimed to assess the usual frequency of consumption of foods over the past 12 months. Study subjects were also asked to approximate portion sizes of foods, using a provided guide with 3 to 5 different portion sizes for each of the different food types. The FFQ was linked to a food-composition database of the National Public Health Institute in Finland, to estimate intake of: dietary iron (dietary iron); total iron (dietary iron and supplemental iron); heme iron proxy (dietary pork, beef, liver, and other organ meats); as well as the intake of potential enhancers (e.g., meat and vitamin C) and inhibitors (e. g., alcohol, fiber, and calcium) of iron absorption. Dietary iron, total iron, and heme iron proxy exposure variables were adjusted for energy intake (kcal) using the nutrient

GNCC (11–14), potentially inversely associated with GCC (12–15), and has been associated with reduced iron levels in the human body (16, 17).

To investigate the relationships between iron and gastric cancer subsites, including the potential effects of H. pylori, we conducted a nested case-control study in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, a prospective cohort of more than 29,000 men in Finland.

Methodology

The rationale, design, and results of the ATBC Cancer Prevention Study have been described in detail (18). In brief, the ATBC Cancer Prevention Study was a randomized, double-blind, placebo-controlled, 2 × 2 factorial primary prevention trial that tested whether daily supplementation with alpha-tocopherol (50 mg) and/or beta-carotene (20 mg) could reduce the incidence of lung and other cancers (19). A total of 29,133 Caucasian, male smokers, aged 50 to 69 years and living in southwestern Finland, were recruited from 1985 to 1988. All subjects provided written informed consent. During the 2 clinical visits before randomization, study participants completed a life-style factor questionnaire and a 276-item food frequency questionnaire (FFQ). In addition, fasting blood samples were collected from participants, the sera of which were stored at −70°C. The intervention concluded on April 30, 1993, but the participants continue to be followed as a cohort using the Finnish Cancer Registry which provides almost 100% case coverage (20). The ATBC Cancer Prevention Study was approved by the institutional review boards of the National Cancer Institute, Bethesda, Maryland, and the National Public Health Institute, Helsinki, Finland.

Study subjects

Cases were subjects of the ATBC Study cohort who were diagnosed with gastric cancer through April 30, 2006, a follow-up of up to 21 years. Gastric cancers were defined according to the International Classification of Diseases, 9th Revision (21) code 151, and were subclassified as GCC if they involved the esophagogastric junction and as GNCC if they did not. Eligible cases were required to have at least 500 µL of serum available for analysis. The total number of eligible cases, based on these criteria, was 341. Eighty-six of these gastric cancer cases were coded as GCC and 172 as GNCC; the remaining 83 were coded as gastric cancer not otherwise specified. Controls were matched to cases in a 1:1 ratio using the variables age at diagnosis of their matched case.
density model (e.g., [dietary iron/kcal] × 1,000) so that the variable was expressed as units (e.g., grams) per 1,000 kcal. Dietary information was available only for 312 cases, 320 controls, and 292 matched sets, and for a few of these subjects some individual dietary responses were also missing.

For purposes of statistical adjustment and effect modification analyses, we also assessed serum for biomarkers of *H. pylori* infection and gastric atrophy. *H. pylori* seropositivity was assessed using immunoglobulin G antibodies against *H. pylori* whole cell by an enzyme linked immunosorbent assay (Biohit ELISA kit). Each plate included 2 quality control samples provided by the kit (a negative control and a positive control) and 3 blinded quality control samples from a single serum pool from the ATBC Cancer Prevention study. Cases, controls, and QC samples were all measured in duplicate. Seropositivity was defined as 30 enzyme-immunosorbent units or more. Concordance between QC samples was 100%. Serum pepsinogen I (PGI) is a serologic marker of gastric atrophy. PGI was measured using a radioimmunoassay, as previously described (24), and subjects with PGI < 25 μg/L were defined as having gastric atrophy (24, 25). PGI measurements were available for only 218 cases, 310 controls, and 206 matched sets.

**Statistical analysis**

Primary exposure variables were assessed for correlation. These primary exposures were analyzed as ordinal variables (quartiles) with categorical cut points based on control distributions. Conditional logistic regression models and unconditional logistic regression models, adjusted for matching factors, were conducted to estimate ORs and 95% confidence intervals (CI); results from both sets of models were similar, thus we present the unconditional models herein as they allowed inclusion of a greater number of subjects. Minimally adjusted models included the covariates age at randomization, date of blood draw, and trial intervention (alpha-tocopherol and beta-carotene; each dichotomous). Additional covariates for the fully adjusted models were chosen by whether they altered an exposure’s estimate by more than 10%. Because of the interrelatedness amongst serum iron metrics, and amongst dietary iron metrics, chosen additional covariates were repeated for other models within the same exposure category (serum/dietary). Unless otherwise specified, covariates were modeled as continuous metrics. Fully adjusted models for serum exposures included the covariates age at randomization, date of blood draw, CRP (mg/L), fiber intake (g/d), education (categorical), and total vitamin C intake (diet and supplements; mg/d). Fully adjusted models for dietary exposures included the covariates age at randomization, date of blood draw, trial intervention, fiber intake, education, total calcium intake (diet and supplements; mg/d), vegetable intake (g/d), energy intake (kcal/d), cigarettes per day smoked, and years of cigarette smoking. Further models, for both serum and dietary exposures, were adjusted for the additional variables of *H. pylori* (dichotomous) and gastric atrophy (dichotomous). Note that when adjustment was made for dietary exposures some individuals could not be included because of missing data. Because of the fact that many of the categorical results presented nonlinear exposure-disease relationships, we calculated global *P* values using the likelihood ratio test to compare nested models to main models with the addition of the quartiled categorical exposure of interest. All logistic regression models were conducted for the outcomes gastric cancer and subsite-specific groups (GCC and GNCC).

To test for differences in serum markers of iron by *H. pylori* seropositivity and presence of gastric atrophy, we used the *t* test for transformed normally distributed exposures. In addition, we estimated the global *P* value (as previously described) with adjustment for case-type and case–control status. We also conducted analyses to determine whether exposures and/or exposure–outcome relationships were different when stratified by *H. pylori* seropositivity, gastric atrophy, and CRP level. To investigate the possibility of reverse causation, we conducted sensitivity analyses whereby cancers diagnosed within 3 years postblood draw were excluded. Finally, to investigate potential short-term effects mediated by these exposures, we conducted sensitivity analyses whereby cancers diagnosed *after* 15 and 12.5 years postblood draw were excluded. Two-sided *P* < 0.05 were considered to be statistically significant. All analyses were conducted using STATA version 11.2 (Stata-Corp LP).

**Results**

Table 1 shows the descriptors of all participants for each variable pertinent to this analysis of iron and gastric cancer. As can be seen, GCC and GNCC cases smoked slightly more cigarettes per day than controls. In addition, GNCC cases had fewer years of schooling, were more likely to be *H. pylori* seropositive and positive for gastric atrophy, consumed slightly less fiber, and had lower levels of serum ferritin and serum iron, compared with control subjects.

Table 2 shows the correlations between age at blood draw, serum iron markers, and dietary iron exposures. Moderate to high correlations were observed for the following pairs of variables: serum iron and transferrin saturation (*r* = 0.87, *P* < 0.001); transferrin saturation and UIBC (*r* = −0.67, *P* < 0.001); and, TIBC and UIBC (*r* = 0.82, *P* < 0.001).

Table 3 shows the results of the fully adjusted logistic regression models; minimally adjusted models showed similar patterns of relationships between exposures and outcomes (data not shown). Serum ferritin shared a "u"-shaped relationship with gastric cancer, with the third quartile and the global *P* value being statistically significant (OR3rd quartile, 0.52; 95% CI, 0.33–0.82; *P* = 0.005; global *P* = 0.037). All other serum measures and dietary...
iron were not associated with all gastric cancer cases combined.

Site-specific analyses, meanwhile, provided fairly distinct results. There was limited evidence for associations of iron metrics with GCC—only TIBC was associated with this outcome with an apparent “n”-shaped relationship (global P = 0.038). For GNCC, there was stronger evidence for associations with iron metrics. Both serum ferritin and serum iron shared inverse, or possibly “u”-shaped, relationships with this malignancy (serum ferritin OR2nd quartile, 0.36; 95% CI, 0.18–0.71; P = 0.003; global P = 0.024; serum iron OR2nd quartile, 0.39; 95% CI, 0.19–0.78; P = 0.008; global P = 0.060). In addition, TIBC appeared to share a “u”-shaped relationship with GNCC (OR2nd quartile, 0.51; 95% CI, 0.26–1.00; P = 0.051; global P = 0.033). Finally, the point estimates for transferrin saturation were suggestive of an inverse, or decreased risk with a threshold-effect, relationship with GNCC, albeit none of these P values were less than 0.05.

Adjustment for H. pylori and then gastric atrophy did not materially affect a majority of the estimates (data not shown)—the only exception was that the relationship between ferritin and GNCC was attenuated (OR2nd quartile, 0.92; 95% CI; 0.39–2.16; P = 0.85; OR3rd quartile, 0.46; 95% CI, 0.18–1.17; P = 0.11; OR4th quartile, 1.02; 95% CI, 0.45–2.31; P = 0.96; global P = 0.31). There was little evidence for direct associations between iron metrics and H. pylori seropositivity or gastric atrophy (low pepsinogen I)—only serum ferritin appeared to share a relationship with these variables, and this relationship was stronger between ferritin and gastric atrophy (Table 4). Restricting analyses to individuals with low CRP levels (<10 mg/L; 308 [90.3% of cases, 314 [92.1% of] controls), individuals seropositive for H. pylori, or individuals without gastric atrophy did not materially alter the results (data not shown). There were too few individuals who were H. pylori negative or gastric atrophy positive to permit analysis of such groups. Sensitivity analyses with exclusion of cancers within 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 341)</th>
<th>Cases (n = 341)</th>
<th>P</th>
<th>Cases (n = 86)</th>
<th>P</th>
<th>Cases (n = 172)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58 (5)</td>
<td>58 (5)</td>
<td>0.935</td>
<td>59 (5)</td>
<td>0.502</td>
<td>59 (5)</td>
<td>0.533</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 (4)</td>
<td>26 (4)</td>
<td>0.834</td>
<td>27 (4)</td>
<td>0.167</td>
<td>26 (4)</td>
<td>0.473</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>19 (8)</td>
<td>21 (9)</td>
<td>0.003</td>
<td>22 (10)</td>
<td>0.007</td>
<td>20 (8)</td>
<td>0.022</td>
</tr>
<tr>
<td>Years smoked</td>
<td>36 (8)</td>
<td>37 (9)</td>
<td>0.138</td>
<td>38 (7)</td>
<td>0.344</td>
<td>37 (10)</td>
<td>0.089</td>
</tr>
<tr>
<td>Education (% junior high or above)</td>
<td>18 (14–22)</td>
<td>12 (9–15)</td>
<td>0.025</td>
<td>20 (11–28)</td>
<td>0.735</td>
<td>10 (5–14)</td>
<td>0.014</td>
</tr>
<tr>
<td>H. pylori, %</td>
<td>73 (68–78)</td>
<td>88 (84–91)</td>
<td>&lt;0.001</td>
<td>74 (65–84)</td>
<td>0.782</td>
<td>91 (87–96)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gastric atrophy, %</td>
<td>9 (6–13)</td>
<td>21 (15–26)</td>
<td>&lt;0.001</td>
<td>16 (6–26)</td>
<td>0.117</td>
<td>22 (14–31)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ever daily use of aspirin, %</td>
<td>16 (12–20)</td>
<td>14 (9–18)</td>
<td>0.514</td>
<td>18 (8–28)</td>
<td>0.684</td>
<td>13 (6–19)</td>
<td>0.466</td>
</tr>
<tr>
<td>Dietary intake (daily)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy, kcal</td>
<td>2,666 (695)</td>
<td>2,655 (735)</td>
<td>0.567</td>
<td>2,566 (665)</td>
<td>0.253</td>
<td>2,649 (725)</td>
<td>0.543</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>19 (10)</td>
<td>17 (9)</td>
<td>0.006</td>
<td>18 (9)</td>
<td>0.101</td>
<td>17 (8)</td>
<td>0.011</td>
</tr>
<tr>
<td>Alcohol, g</td>
<td>16 (18)</td>
<td>16 (19)</td>
<td>0.666</td>
<td>15 (16)</td>
<td>0.822</td>
<td>18 (21)</td>
<td>0.560</td>
</tr>
<tr>
<td>Total vitamin C, mg</td>
<td>144 (173)</td>
<td>125 (148)</td>
<td>0.360</td>
<td>118 (117)</td>
<td>0.670</td>
<td>136 (184)</td>
<td>0.340</td>
</tr>
<tr>
<td>Total calcium, mg</td>
<td>1,423 (575)</td>
<td>1,377 (521)</td>
<td>0.368</td>
<td>1,370 (489)</td>
<td>0.586</td>
<td>1,360 (530)</td>
<td>0.239</td>
</tr>
<tr>
<td>Supplemental iron use, %</td>
<td>9 (6–12)</td>
<td>9 (6–12)</td>
<td>0.990</td>
<td>11 (4–17)</td>
<td>0.690</td>
<td>9 (5–14)</td>
<td>0.930</td>
</tr>
<tr>
<td>Dietary iron, mg</td>
<td>18 (5)</td>
<td>18 (6)</td>
<td>0.128</td>
<td>17 (6)</td>
<td>0.208</td>
<td>18 (5)</td>
<td>0.101</td>
</tr>
<tr>
<td>Total iron, mg</td>
<td>22 (27)</td>
<td>20 (14)</td>
<td>0.148</td>
<td>20 (9)</td>
<td>0.470</td>
<td>20 (18)</td>
<td>0.088</td>
</tr>
<tr>
<td>Heme iron proxy, g</td>
<td>69 (31)</td>
<td>67 (31)</td>
<td>0.462</td>
<td>65 (32)</td>
<td>0.250</td>
<td>68 (28)</td>
<td>0.951</td>
</tr>
</tbody>
</table>

NOTE: The mean and standard deviation of each variable are provided, unless the variable is stated to be a percentage in which case the percentage and 95% CI are provided, or unless the variable is a serum marker in which case the median and interquartile range (IQR) are provided.
Discussion

In this analysis of serologic and dietary metrics of iron exposure in the prospective ATBC Cancer Prevention Study, we found limited evidence for association of iron metrics with GCC. For GNCC, we observed inverse relationships with the exposures serum ferritin, serum iron, and, possibly, transferrin saturation—an iron profile similar to that of iron deficiency.

There have been 7 previously published articles from 5 studies that have assessed associations between serum iron metrics and gastric cancer (Table 5). Four of the articles represent 3 cohort studies: a mobile health clinic study from Finland, 120 incident male gastric cancers and 76 incident female gastric cancers (28), and a cohort of the Kaiser Permanente Multiphasic Health Check-up Evaluation Study (28). The remaining 3 previously published articles represent 3 cohort studies: a mobile health clinic study from Finland, 120 incident male gastric cancers and 76 incident female gastric cancers (28), and a cohort of the Kaiser Permanente Multiphasic Health Check-up Evaluation Study (28). The remaining 3 previously published articles represent 3 cohort studies: a mobile health clinic study from Finland, 120 incident male gastric cancers and 76 incident female gastric cancers (28), and a cohort of the Kaiser Permanente Multiphasic Health Check-up Evaluation Study (28). 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The remaining 3 previously published articles represent 3 cohort studies: a mobile health clinic study from Finland, 120 incidental male gastric cancers and 76 incident female gastric cancers occurred during a mean follow-up of 14 years (4). The relationship in males was statistically significant (RR<sub>4th vs. 1st quartile</sub> 0.66; P for trend < 0.01; case mean, 107.0 μg/dL; control mean, 115.7 μg/dL; P < 0.05), whereas in females, the estimate was similar albeit not statistically significant (RR<sub>4th vs. 1st quartile</sub> 0.59; P for trend = 0.17; case mean, 95.7 μg/dL; control mean, 99.9 μg/dL; P ≥ 0.05), likely because of the smaller number of accrued cases. In an 18-year
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Cases</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>Cases</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>Cases</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme iron proxy, g/1,000 kcal/db</td>
<td>&lt;14.50</td>
<td>80 79 87</td>
<td>0.72</td>
<td>0.68</td>
<td>1.11</td>
<td>0.56</td>
<td>0.005</td>
<td>0.003</td>
<td>&lt;14.50</td>
<td>80 79 87</td>
<td>0.72</td>
<td>0.68</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>&lt;21.46</td>
<td>79 83 88</td>
<td>0.88</td>
<td>0.86</td>
<td>1.36</td>
<td>0.75</td>
<td>0.029</td>
<td>0.027</td>
<td>&lt;21.46</td>
<td>79 83 88</td>
<td>0.88</td>
<td>0.86</td>
<td>1.36</td>
</tr>
<tr>
<td>Total iron, mg/1,000 kcal/db</td>
<td>&lt;1.25</td>
<td>80 103 87</td>
<td>0.20</td>
<td>0.19</td>
<td>1.58</td>
<td>0.77</td>
<td>0.027</td>
<td>0.026</td>
<td>&lt;1.25</td>
<td>80 103 87</td>
<td>0.20</td>
<td>0.19</td>
<td>1.58</td>
</tr>
<tr>
<td>Dietiet iron, mg/1,000 kcal/db</td>
<td>&lt;1.22</td>
<td>80 95 95</td>
<td>0.00</td>
<td>0.00</td>
<td>1.22</td>
<td>0.17</td>
<td>0.035</td>
<td>0.033</td>
<td>&lt;1.22</td>
<td>80 95 95</td>
<td>0.00</td>
<td>0.00</td>
<td>1.22</td>
</tr>
<tr>
<td>Iron, μg/d(^a)</td>
<td>&lt;92</td>
<td>81 91 91</td>
<td>0.80</td>
<td>0.58</td>
<td>1.40</td>
<td>0.87</td>
<td>0.151</td>
<td>0.139</td>
<td>&lt;92</td>
<td>81 91 91</td>
<td>0.80</td>
<td>0.58</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>≥92</td>
<td>78 77 77</td>
<td>0.55</td>
<td>0.40</td>
<td>0.87</td>
<td>0.57</td>
<td>0.141</td>
<td>0.129</td>
<td>≥92</td>
<td>78 77 77</td>
<td>0.55</td>
<td>0.40</td>
<td>0.87</td>
</tr>
<tr>
<td>Transferrin saturation, %(^a)</td>
<td>&lt;28.65</td>
<td>78 89 89</td>
<td>0.80</td>
<td>0.58</td>
<td>1.40</td>
<td>0.87</td>
<td>0.151</td>
<td>0.139</td>
<td>&lt;28.65</td>
<td>78 89 89</td>
<td>0.80</td>
<td>0.58</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>≥28.65</td>
<td>78 77 77</td>
<td>0.55</td>
<td>0.40</td>
<td>0.87</td>
<td>0.57</td>
<td>0.141</td>
<td>0.129</td>
<td>≥28.65</td>
<td>78 77 77</td>
<td>0.55</td>
<td>0.40</td>
<td>0.87</td>
</tr>
<tr>
<td>Global P value</td>
<td>&lt;1.02</td>
<td>380 381 381</td>
<td>1.00</td>
<td>0.99</td>
<td>1.02</td>
<td>0.99</td>
<td>0.789</td>
<td>0.789</td>
<td>&lt;1.02</td>
<td>380 381 381</td>
<td>1.00</td>
<td>0.99</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>≥1.02</td>
<td>350 351 351</td>
<td>1.00</td>
<td>0.99</td>
<td>1.02</td>
<td>0.99</td>
<td>0.789</td>
<td>0.789</td>
<td>≥1.02</td>
<td>350 351 351</td>
<td>1.00</td>
<td>0.99</td>
<td>1.02</td>
</tr>
</tbody>
</table>

\(^a\)Logistic regression models adjusted for age at randomization, date of blood draw, randomization (alpha-tocopherol and beta-carotene), energy intake (kcal/d), serum C-reactive protein (mg/L), fiber intake (g/d), education (categorical), and total vitamin C intake (mg/d).

\(^b\)Logistic regression models adjusted for age at randomization, date of blood draw, randomization (alpha-tocopherol and beta-carotene), fiber intake (g/d), education (categorical), total calcium intake (mg/d), vegetable intake (g/d), energy intake (kcal/d), cigarettes per day smoked, and years of cigarette smoking.
The last serum iron metric for which we found an association was TIBC, and this appeared to be a "u"-shaped relationship. This is not supported by the result of the Finnish cohort, which included 230 cases and 547 controls, found no evidence for association (35); of the 2 smaller case–control studies, the study from France found evidence (36) and the US study found tentative evidence (37) for an inverse association between dietary iron and gastric cancer. In addition, there is scant evidence that red meat, a major food source of heme iron, is associated with gastric cancer (38) or subsites thereof (33). Of the 3 case–control studies to assess dietary iron in relation to gastric cancer, the largest study, which included 230 cases and 547 controls, found no evidence for association (35); of the 2 smaller case–control studies, the study from France found evidence (36) and the US study found tentative evidence (37) for an inverse association between dietary iron and gastric cancer. In addition, there is scant evidence that red meat, a major food source of heme iron, is associated with gastric cancer (38) or subsites thereof (33).

Taken together, the results from previous studies and our own indicate an association between a serum iron profile consistent with iron deficiency and risk of GNCC. Whether this relationship is because of a causal mechanism or confounding remains unknown. Residual confounding via H. pylori bacterium to: induce hemorrhagic gastritis resulting in iron loss; induce gastric atrophy which reduces gastric cancer (RR4th vs. 1st quartile: 1.29; P for trend < 0.05; case mean, 350.5 μg/dL; control mean, 340.5 μg/dL; P < 0.05) and NHANES I found a similar difference in means (natural log for ferritin and CRP; square-root for iron, transferrin saturation and UIBC; and inverse of the square-root for TIBC).
Table 5. Studies which have assessed serum iron metrics in relation to gastric cancer

<table>
<thead>
<tr>
<th>First author</th>
<th>Year of publication</th>
<th>Study</th>
<th>Country</th>
<th>Period of blood draw</th>
<th>Last year of follow-up</th>
<th>Number of cases</th>
<th>Number of controls</th>
<th>Gastric cancer subsite</th>
<th>Serum ferritin</th>
<th>Serum iron</th>
<th>Transferrin saturation</th>
<th>TIBC</th>
<th>UIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knekt, P.</td>
<td>1994</td>
<td>Mobile Health Clinic Study</td>
<td>Finland</td>
<td>1966–72</td>
<td>1984</td>
<td>120 men, 21,085 men,</td>
<td>GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Arrows represent the general direction of associations between serum markers and gastric cancer (\(\wedge\)\) indicates an "n"-shaped relationship and \(\sqrt{\wedge}\) indicates a "u"-shaped relationship). An asterix indicates an association which was statistically significant at \(\alpha = 0.05\). Blue represents associations in men, red in women, and a black asterix indicates that the result also pertains to men and women combined. A dash indicates a null finding. A blank cell indicates that a study did not assess that particular serum marker.

*aThis manuscript.
acidity and ascorbic acid levels leading to poor absorption of iron; and sequester iron from the host for growth. Although we adjusted for *H. pylori* seropositivity, this biomarker is suboptimal in that it represents current or past exposure, time to seroreversion is variable (39), and it does not provide information as to the severity of infection. Arguing against confounding is the fact that associations changed very little: when adjusted for *H. pylori* and gastric atrophy; when restricted to *H. pylori* seropositive or gastric atrophy negative individuals; or when cancers diagnosed in the first 3 years were excluded from analysis. It is equally plausible that the mechanism of association is causal. Iron deficiency may lead to increased levels of oxidative stress, decreased antioxidant defenses, reduced enzymatic activity leading to increased DNA damage, and increased genomic instability (6, 40). However, these mechanisms are still poorly understood and further research is required to further elucidate potential causal pathways of the observed associations presented herein.

Strengths of this study include that this is the largest study of the topic to date, it is the only study to evaluate both overall gastric cancer and the anatomic subsites thereof, and it used the most comprehensive set of iron assessment metrics. In addition, it was nested in a prospective study with long-term follow-up which enabled use of prediagnostic serum and a detailed and validated FFQ (41). Finally, we included sex-specific analyses only (male cohort) which avoided combination of the sexes which could result in type I or type II errors given the (male cohort) which avoided combination of the sexes which could result in type I or type II errors given the

Having few individuals negative for *H. pylori* or positive for gastric atrophy: lack of a female cohort as a comparison for the male results; and a population which includes only smokers, although we did adjust for duration and rate of exposure, where applicable.

In conclusion, this analysis of serologic and dietary metrics of iron exposure in the prospective ATBC Cancer Prevention Study finds little evidence for the involvement of iron exposure or homeostasis with GCC. GNCC was associated with an iron profile similar to that of iron deficiency, but reasons for this association remain unclear.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: M.B. Cook, F. Kamangar, P.R. Taylor, C.C. Abnet, S.M. Dawsy

Development of methodology: M.B. Cook, F. Kamangar

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.B. Cook, D. Albanes, J. Virtamo, P.R. Taylor, R. J. Wood, G. Petty, S.M. Dawsey

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.B. Cook, F. Kamangar, C.C. Abnet, A.J. Cross, S.M. Dawsey

Writing, review, and/or revision of the manuscript: M.B. Cook, F. Kamangar, S.J. Weinstein, D. Albanes, J. Virtamo, P.R. Taylor, C.C. Abnet, R.J. Wood, G. Petty, A.J. Cross, S.M. Dawsey

Administrative, technical, or material support (i.e., reporting or organizing data, constructing database): M.B. Cook, S.J. Weinstein, D. Albanes, J. Virtamo

Study supervision: C.C. Abnet

Analyzed specimens for biochemical markers: G. Petty

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**References**


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