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Body iron status and gastric cancer risk in the EURGAST study

Ana Fonseca-Nunes[1], Antonio Agudo[1], Núria Aranda[2], Victoria Arija[2], Amanda J. Cross[3], Esther Molina[4,5], Maria Jose Sanchez[4,5], H. B(as) Bueno-de-Mesquita[6,7,8,9], Peter Siersema[6,7,8,9], Elisabete Weiderpass[10,11,12,13], Vittorio Krogh[14], Amalia Mattiello[15], Rosario Tumino[16], Calogero Saieva[17], Alessio Naccarati[18], Bodil Ohlsson[19], Klas Sjöberg[19], Marie-Christine Boutron-Ruault[20,21,22], Claire Cadeau[20,21,22], Guy Fagherazzi[20,21,22], Heiner Boeing[23], Annika Steffen[23], Tilman Kühn[24], Verena Katzke[24], Anne Tjønneland[25], Anja Olsen[25], Kay-Tee Khaw[26], Nick Wareham[26], Tim Key[27], Yunxia Lu[3], Elio Riboli[3], Petra H. Peeters[28], Diana Gavrila[5,29,30], Miren Dorronsoro[31], José Ramón Quirós[32], Aurelio Barricarte[33,34,35], Mazda Jenab[34], Raúl Zamora-Ros[34], Heinz Freisling[34], Antonia Trichopoulou[35,36], Pagona Lagiou[35,36], Christina Bamia[35,36] and Paula Jakszyn[1]

[1] Unit of Nutrition and Cancer, Catalan Institute of Oncology-ICO, IDIBELL, L'Hospitalet De Llobregat, Barcelona, Spain
[2] IISPV, Departament De Ciències Mèdiques Bàsiques, Universitat Rovira I Virgili (URV), Tarragona, Spain
[3] Department of Epidemiology and Biostatistics, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom
[4] Escuela Andaluza De Salud Pública, Instituto De Investigación Biosanitaria Ibs.GRANADA, Hospitales Universitarios De Granada/Universidad De Granada, Granada, Spain
[5] CIBER De Epidemiología Y Salud Pública (CIBERESP), Madrid, Spain
[6] Department of Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[7] Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands
[8] Department of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, London, United Kingdom
[9] Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[10] Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, the Arctic University of Norway, Tromsø, Norway
[11] Department of Research, Cancer Registry of Norway, Oslo, Norway
[12] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[13] Samfundet Folkhälsan, Helsinki, Finland
[14] Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milano, Italy
[15] Dipartamento Di Medicina Clinica E Chirugia, Federico II University, Naples, Italy
[16] Cancer Registry and Histopathology Unit, Civic - M.P.Arezzo Hospital, ASP Ragusa, Italy
[17] Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy
[18] Molecular and Genetic Epidemiology Unit, HuGeF - Human Genetics Foundation – Torino, Torino, Italy
[19] Department of Clinical Sciences, Division of Internal Medicine, Skåne University Hospital Malmo, Lund University, Malmo, Sweden
[20] Inserm, Centre for Research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health Team, Villejuif, France
[21] Université Paris Sud, UMRS 1018, Villejuif, France
[22] Institut Gustave Roussy, Villejuif, France
[23] Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, Germany
[24] Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[25] Danish Cancer Society Research Center, Copenhagen, Denmark
[26] MRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom

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Correspondence to: Paula Jakszyn, MPH, PhD, Nutritionist, Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Av Gran via 199-203 (08907), L'Hospitalet de Llobregat, Barcelona, Spain, Tel.:+34-93-260-74-01, Fax: +34-93-260-77-87, E-mail: paujak@iconcologia.net

[27] Cancer Epidemiology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
[28] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[29] Department of Epidemiology, Regional Health Council, Murcia, Spain
[30] IMIB-Arrixaca, Murcia, Spain
[31] Basque Regional Health Department, Public Health Direction and Biodonostia-Ciberesp, San Sebastian, Spain
[32] Public Health Directorate, Asturias, Spain
[33] Navarre Public Health Institute, Pamplona, Spain
[34] International Agency for Research on Cancer (IARC-WHO), Lyon, France
[35] WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece
[36] Hellenic Health Foundation, Athens, Greece

Although it appears biologically plausible for iron to be associated with gastric carcinogenesis, the evidence is insufficient to lead to any conclusions. To further investigate the relationship between body iron status and gastric cancer risk, we conducted a nested case–control study in the multicentric European Prospective Investigation into Cancer and Nutrition (EPIC) study. The study included 456 primary incident gastric adenocarcinoma cases and 900 matched controls that occurred during an average of 11 years of follow-up. We measured prediagnostic serum iron, ferritin, transferrin and C-reactive protein, and further estimated total iron-binding capacity (TIBC) and transferrin saturation (TS). Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of gastric cancer by iron metrics were estimated from multivariable conditional logistic regression models. After adjusting for relevant confounders, we observed a statistically significant inverse association between gastric cancer and ferritin and TS indices ($OR_{log2} = 0.80$, 95% CI = 0.72–0.88; $OR_{10\%increment} = 0.87$, 95% CI = 0.78–0.97, respectively). These associations appear to be restricted to noncardia gastric cancer (ferritin showed a *p* for heterogeneity = 0.04 and TS had a *p* for heterogeneity = 0.02), and no differences were found by histological type. TIBC increased risk of overall gastric cancer ($OR_{50\ \mu g/dl} = 1.13$, 95% CI = 1.02–1.2) and also with noncardia gastric cancer (*p* for heterogeneity = 0.04). Additional analysis suggests that time between blood draw and gastric cancer diagnosis could modify these findings. In conclusion, our results showed a decreased risk of gastric cancer related to higher body iron stores as measured by serum iron and ferritin. Further investigation is needed to clarify the role of iron in gastric carcinogenesis.

What's new?

Iron is highly reactive, iron levels in the body rise with inflammation, and the iron-overload disorder hemochromatosis is associated with an increased risk of gastric cancer. Thus, one might predict that high levels of iron will increase the risk of cancer. However, in this study from a large European population, the authors found that increased body iron stores actually decreased the risk of gastric cancer. These results suggest that further investigation is needed to clarify the role of iron in gastric carcinogenesis.

Almost 1 million new cases of gastric cancer were estimated to occur in 2012, making it the fifth most common malignancy in the world and the third leading cause of cancer death worldwide.[1] Infection with *Helicobacter pylori* (*Hp*) is the strongest established risk factor for gastric noncardia adenocarcinoma, but only a minority of infected individuals develop cancer of the stomach.[1] Tobacco smoking[2] is another recognized risk factor for gastric cancer. Although the evidence relating the risk of gastric cancer to consumption of red meat is considered to be limited by the World Cancer Research Fund & the American Institute for Cancer Research,[3] newer research suggests that red meat intake could also be a risk factor for gastric cancer.[4] One of the hypotheses behind meat intake and increased risk of gastric cancer relates to dietary iron, especially the heme type.[5] Heme iron could increase endogenous formation of *N*-nitroso compounds, which are well-known carcinogens.[6] Furthermore, redox cycling of iron is closely related to the production of reactive oxygen species (ROS) such as hydroxyl radicals, a highly reactive molecule able to induce lipid peroxidation and oxidative damage to DNA.[7] If iron plays a role in carcinogenesis, subjects who have elevated total body iron stores or iron overload could be at a greater risk of developing cancer. Hereditary hemochromatosis is the most severe clinical expression of iron overload and hemochromatosis gene mutations has been associated with gastric cancer in the EPIC study.[8]

Iron, an essential element for human life but also toxic when in excess,[7] has a very well-regulated metabolism.[9] Only a small percent of dietary iron is absorbed, and dietary iron is not necessarily related to body iron status.[10] The latter is frequently assessed by measuring unbound (free) iron, ferritin, total iron-binding capacity (TIBC) and transferrin saturation (TS). Iron is mainly bound to hemoglobin and about 30% of iron is stored in the form of ferritin. Most circulating iron is bound to transferrin and TIBC indirectly measures the extent to which transferrin is saturated. TS is a direct measure of the transferrin-binding sites that are occupied.[11] Ferritin, representing the levels of iron in liver, spleen and bone marrow, is decreased in iron deficiency and increased in iron overload, liver disease, infection, inflammation and other disorders.[12]

Although it appears biologically plausible for iron to be associated with gastric carcinogenesis, the evidence is insufficient to lead to any conclusions and might differ according to tumor location.[13] A recent study measured iron biomarkers in 341 male Finnish cases and 341 matched controls, and found an inverse association between ferritin and gastric noncardia cancer risk.[14] Two cohorts studying Japanese subjects, one in Japan[15] and the other in Hawaii,[16] had previously reported a significant decrease in risk of cancer of the stomach associated with lower levels of serum ferritin. Regarding transferrin, a significant decreased risk was observed in men (but not in women) in a Finnish cohort[17] and a decreased but no significant risk was found in the two Japanese cohorts.[15,16] While a significant increase in the risk was found in an American cohort only among women.[12]

To further investigate the relationship between body iron status and gastric carcinogenesis, we conducted a nested case–control study in the multicentric European Prospective Investigation into Cancer and Nutrition (EPIC) study as measured by markers of body iron status.

Material and Methods

Study subjects

The subjects in this study were participants from the EPIC study, and were selected according to a nested case–control design. The methodology and rationale behind the EPIC study has been described elsewhere.[18] In summary, EPIC is a multicenter prospective cohort of over 500,000 participants, recruited between 1992 and 2000 in 23 centers from ten European countries (Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom). Participants provided information on diet and lifestyle factors, and anthropometric data and blood samples were collected at recruitment. This study was approved by the Ethical Committees at the International Agency for Research on Cancer (IARC) and in each of the EPIC centers.

Case identification and control selection

Case identification was based on record linkage with population cancer registries except in France, Germany, Greece and Naples, where the centers have used a combination of methods such as health insurance records, cancer and pathology hospital registries and active follow-up with study subjects. Cancer of the stomach included cancers coded as C16 according to the tenth Revision of the International Classification of Diseases (ICD-10). Cases for our study were subjects with no previous cancer, newly diagnosed with primary gastric adenocarcinoma during the follow-up, from recruitment through 2010 depending on the study center. A total of 665 gastric adenocarcinoma cases were diagnosed during this follow-up period. We excluded 113 cases from Denmark as the biological samples were not available at the time of the study. Furthermore, we excluded six cases from Norway because of the small number of cases provided and 75 cases from Malmö (Sweden) as the center decided not to participate in this study. In total, 471 incident primary gastric adenocarcinoma cases were included. They were also classified according to both anatomic location (cardia and noncardia) and Lauren histological type (intestinal and diffuse).

For each case, two controls were randomly selected among cohort subjects alive and cancer free at the time of diagnosis of the case, matched by center, sex, age at baseline ($\pm$2.5 years) and date of blood collection ($\pm$45 days). According to these criteria, 942 controls were selected.

From the 471 cases and 942 controls there were available biological samples for 460 cases and 905 controls. We then proceeded to exclude nine subjects, including four cases and five controls, because of biomarkers' incoherent values, lipemic samples and incomplete risk sets. We finally had 456 cases and 900 controls for the analyses.

Laboratory procedures and measurements of biomarkers of iron status

All biomarkers in this study were quantified using blood drawn at baseline. Serum ferritin levels were measured by electrochemiluminescence immunoassay (ECLIA) by an Elecsys analyzer (Roche Diagnostics, Mannheim, Germany). Serum iron was measured by immune (chemiluminescence) assay and serum transferrin by immunoturbidimetric assay using a Modular Analytics P800 chemistry analyzer (Roche Diagnostics). All these analyses were carried out at the "Laboratory de Referència Sud de Catalunya" (Tarragona, Spain). The TIBC (μg/dl) was calculated using the measured transferrin value [Tf(g/l) $\times$ 143].[19] TIBC was then used to calculate the TS as iron/TIBC, expressed as percent.

Complementary biomarkers

Serum vitamin C was measured owing to its role as an enhancer of iron absorption (especially the inorganic iron fraction). Serum vitamin C was measured using liquid chromatography coupled to mass spectrometry. Vitamin C was extracted from the serum by liquid–liquid extraction using an acidified organic solvent mixture, and quantified in a 1290 UHPLC Series Liquid Chromatograph coupled to a 6490 QqQ-MS/MS (Agilent Technologies). The technique uses 50

μl of serum and was performed in the Center for Omic Sciences (COS), Universitat Rovira i Virgili (URV), Reus (Tarragona, Spain).

As ferritin can be increased in inflammatory conditions or infections, high-sensitive C-reactive protein (hsCRP) was measured to rule out inflammation as a cause of increased levels of ferritin by immunoturbidimetric assay on a Modular Analytics P800 chemistry analyzer (Roche Diagnostics). hsCRP was measured simultaneously with the biomarkers of iron status, using the same aliquot.

Other factors

Anthropometric measurements, smoking status, physical activity and educational level were collected in the cohort baseline questionnaire; measured height and weight were then used to compute the body mass index (BMI, kg/m^2). Data on habitual dietary intake such as meat and vitamin C were collected from validated country-specific dietary questionnaires in the cohort. Heme iron intake was estimated multiplying the estimated heme iron content by the mean daily intake of related food sources (meat and fish) for each subject.[5] Pepsinogen 1 level was determined by ELISA using the kits from Biohit (Helsinki, Finland). We considered pepsinogen 1 levels <22 μg/l as an indicator of severe chronic atrophic gastritis (SCAG). This information was available for 123 cases and 240 controls. Moreover, we assessed *Hp* serology by Immunoblot using the HELICOBLOT 2.1 kit (Genelab Diagnostics, Singapore). Detailed information can be found elsewhere.[20]

Statistical analysis

Indicators of body iron status tested as potentially related to gastric cancer risk were ferritin, serum iron, TIBC and TS. As most of these variables were not normally distributed, we used the median and interquartile range to describe them. Dietary variables (daily intake of meat, total iron and heme iron) were expressed as nutrient density per 2,000 kcal (*e.g.*, [g/kcal] $\times$ 2,000). The distributions of baseline characteristics in cases and controls were compared through χ^2 test for categorical variables, Student's *t*-test (normally distributed) and Wilcoxon rank-sum tests (non-normally distributed) for continuous variables. Correlations between variables were calculated using Pearson's test for normally distributed variables and Spearman test if not normally distributed.

Associations between each iron biomarker and gastric cancer risk were assessed by the odds ratio (OR) and 95% confidence intervals (CIs) estimated by conditional logistic regression. Main exposure variables were used as both continuous and categorical in the models. As ferritin showed a non-normal distribution, we proceeded to a $\log_2$ transformation; the OR is interpreted as the increase in risk of gastric cancer related to a doubling of the ferritin concentration. Quartile cutoff points for iron biomarker variables were calculated using the distribution among controls. The *p* for trend was calculated by treating categorical variables as continuous in regression analyses. Parsimonious models were built by testing each covariate using the likelihood ratio (LR) test after elimination of each variable. The final model included educational level (no formal education, primary school, technical or professional training, university and not specified), cigarette smoking (never smoker, former smoker, current smoker and not specified) and heme iron intake. We tested for other potential confounders including hsCRP because of the role of inflammation on iron biomarkers levels, but as they did not change the estimated effect $>10\%$, we did not include them in the final model. In the subset of subjects with measured values of plasma pepsinogen 1, we further assessed the relationship between gastric cancer risk and body iron status biomarkers including pepsinogen 1 in the multivariable model.

We conducted subgroup analysis according to tumor localization: gastric cardia cancer (GCC) *vs*. gastric noncardia cancer (GNCC) and histology (intestinal *vs*. diffuse) using conditional logistic regression, and applying the Wald test to assess heterogeneity of the OR. Furthermore, we assessed the effect of biomarkers of iron status on gastric cancer risk according to the time elapsed from the date of blood drawing to date of incidence. We also explored the interaction or modifying effect of *Hp* infection, plasma levels of vitamin C, alcohol, tobacco smoking, sex and BMI by means of stratified analysis, using unconditional regression models including the matching variables, and testing these interactions with the LR test. And lastly, we defined groups of participants according to the World Health Organization (WHO) cutoff values.[21] Participants with ferritin levels below 15 ng/ml were considered to have depleted iron stores. Subjects were classified with iron deficiency if their TS was lower than 16% and ferritin lower than 15 ng/ml. Individuals with TS above 45%, and/or women with ferritin higher than 150 ng/ml and men than 200 ng/ml, had iron overload. We proceeded to analyze the associations between gastric cancer risk within these two groups of participants.

Results

Of the 456 gastric cancer cases, 116 were classified as GCC, 236 as GNCC and for 104 cases the site was unknown. Histological subtype was similar between the proportion of intestinal and diffuse tumors, with 154 classified as diffuse, 149 intestinal and 153 cases were unclassified. Table 1 describes the baseline characteristics of our study subjects. Gastric cancer cases overall had a significantly higher waist-to-hip ratio, proportion of current smokers, percentage of *Hp* positivity, but had a significantly lower proportion of more educated subjects. Cases also had a greater intake of heme iron and processed meat. Considering markers of iron body status, cases had significantly lower levels of ferritin and TS. When looking into descriptive analysis by subgroups, all of these differences concerning iron indices among cases and controls were only found in the GNCC type, and additionally showed significantly higher levels of TIBC among cases.

Table 1. Baseline characteristics of cases and matched controls of EURGAST study

Characteristics		All gastric cancer		Gastric cardia		Gastric noncardia		Diffuse		Intestinal	
	Controls ($n = 900$)	Cases ($n = 456$)	p^1	Cases ($n = 116$)	p^1	Cases ($n = 236$)	p^1	Cases ($n = 154$)	p^1	Cases ($n = 149$)	p^1
BMI (kg/m^2), mean (SD)	26.76 (3.89)	27.04 (4.01)	0.212	26.69 (3.71)	0.65	27.01 (3.90)	0.5351	27.04 (4.35)	0.3611	27.63 (3.59)	0.6462
Waist-to-hip ratio, mean (SD)	0.88 (0.09)	0.90 (0.09)	<0.05	0.92 (0.09)	0.11	0.89 (0.08)	0.1237	0.89 (0.09)	0.0437	0.91 (0.09)	0.4789
Smoking status, *n* (%)											
Never	418 (46.44)	177 (38.82)		31 (26.72)		104 (44.07)		68 (44.16)		53 (35.57)	
Former	296 (32.89)	134 (29.39)		41 (35.34)		57 (24.15)		33 (21.43)		49 (32.89)	
Current	177 (19.67)	136 (29.82)		40 (34.48)		73 (30.93)		50 (32.47)		45 (30.20)	
Unknown	9 (1.00)	9 (1.97)	<0.001	4 (3.45)	<0.001	2 (0.85)	0.004	3 (1.95)	0.006	2 (1.34)	0.08
Physical activity, *n* (%)											
Inactive	273 (30.33)	160 (35.09)		37 (31.90)		88 (37.29)		51 (33.12)		56 (37.58)	
Moderately inactive	282 (31.33)	139 (30.48)		36 (31.03)		72 (30.51)		51 (33.12)		42 (28.19)	
Moderately active	187 (20.78)	80 (17.54)		21 (18.10)		45 (19.07)		29 (18.83)		32 (21.48)	
Active	149 (16.56)	72 (15.79)		21 (18.10)		30 (12.71)		21 (13.64)		19 (12.75)	0.5
Missing	9 (1.00)	5 (1.10)	0.27	1 (0.86)	0.7	1 (0.42)	0.422	2 (1.30)	0.353		
Education level, *n* (%)											
None	74 (8.22)	38 (8.33)		4 (3.45)		25 (10.59)		11 (7.14)		19 (12.75)	
Primary school completed	344 (38.22)	198 (43.42)		42 (36.21)		117 (49.58)		70 (45.45)		70 (46.98)	
Technical/professional school	189 (21.00)	104 (22.81)		34 (29.31)		44 (18.64)		36 (23.38)		28 (18.79)	
Secondary school	103 (11.44)	43 (9.43)		14 (12.07)		15 (6.36)		13 (8.44)		14 (9.40)	
Longer education	164 (18.22)	54 (11.84)		14 (12.07)		31 (13.14)		20 (12.99)		14 (9.40)	
Not specified	26 (2.89)	19 (4.17)	<0.05	8 (6.90)	0.65	4 (1.69)	0.07	4 (2.60)	0.102	4 (2.68)	0.1
Helicobacter pylori, *n* (%)											
Positive	366 (65.59)	254 (86.10)		50 (66.67)		153 (95.03)		100 (90.91)		96 (85.71)	
Negative	192 (34.41)	41 (13.90)	<0.001	25 (33.33)	0.15	8 (4.97)	<0.001	10 (9.09)	<0.001	16 (14.29)	0.0
Gastric atrophy, *n* (%)											
Positive	9 (3.75)	10 (8.13)		1 (4.00)		8 (11.59)		4 (9.09)		4 (8.51)	
Negative	231 (96.25)	113 (91.87)	0.08	24 (96.00)	0.15	61 (88.41)	0.056	40 (90.91)	0.186	43 (91.49)	0.2
Dietary intake (median, IQR)											
Total energy (kcal/day)	2052.16 (1625.68–2504.70)	2022.27 (1666.15–2563.47)	0.8	2006.07 (1619.08–2508.66)	0.92	2034.56 (1679.79–2586.93)	0.71	2109.52 (1677.13–2619.21)	0.516	1999.46 (1587.33–2552.21)	0.63
Total iron (mg/2,000 kcal)	12.67 (11.01–14.42)	12.62 (10.93–14.42)	0.68	12.11 (10.53–14.02)	0.7	12.80 (10.94–14.68)	0.61	12.84 (10.95–14.52)	0.72	13.43 (11.52–15.04)	0.42
Heme iron (mg/2,000 kcal)	1.11 (0.75–1.58)	1.20 (0.79–1.71)	<0.05	1.09 (0.70–1.54)	0.18	1.28 (0.84–1.80)	0.0068	1.23 (0.82–1.71)	0.06	1.47 (0.92–180)	0.01
Red meat (g/2,000 kcal)	33.94 (17.58–54.17)	36.54 (18.80–56.83)	0.13	38.36 (18.09–63.52)	0.11	34.96 (20.50–54.38)	0.304	35.76 (20.79–53.73)	0.21	38.70 (23.39–60.34)	0.17

Table 1. Baseline characteristics of cases and matched controls of EURGAST study (Continued)

Characteristics		All gastric cancer		Gastric cardia		Gastric noncardia		Diffuse		Intestinal	
	Controls ($n = 900$)	Cases ($n = 456$)	p^1	Cases ($n = 116$)	p^1	Cases ($n = 236$)	p^1	Cases ($n = 154$)	p^1	Cases ($n = 149$)	p^1
Processed meat (g/2,000 kcal)	23.70 (10.16–42.31)	26.79 (12.37–47.79)	<0.05	25.66 (12.73–46.64)	0.28	27.76 (12.41–47.94)	0.04	31.21 (13.79–49.31)	0.009	25.75 (13.64–49.86)	0.03
Vitamin C (mg/2,000 kcal)	113.09 (80.45–158.77)	106.86 (75.05–157.82)	0.4	100.13 (74.32–143.15)	0.32	106.86 (67.99–162.07)	0.63	102.97 (74.45–157.81)	0.99	106.57 (72.44–163.51)	0.83
Serum markers (median, IQR)											
Ferritin (ng/ml) ♂♀	104.45 (57.32–193.8)	86.2 (44.82–161.5)	<0.001	98.92 (57.66–189.8)	0.31	82.93 (35.01–140.7)	<0.001	83.06 (41.28–143.5)	0.13	85.78 (37.69–179.1)	0.46
Ferritin (ng/ml) ♂	138.15 (76.71–251.5)	110.5 (64.52)−219.2)	<0.05	132.8 (69.8–217.9)	0.37	98.67 (48.42–192.4)	0.003	109.4 (67.69–251.5)	0.68	129.4 (57.66–269.5)	0.24
Ferritin (ng/ml) ♀	74.88 (40.75–131)	60.69 (29.46–103.9)	<0.05	62.82 (33.29–122.6)	0.44	61.85 (29.46–109.8)	0.3	62.16 (30.34–101.7)	0.08	53.72 (26.24–97.75)	0.12
Iron (µg/dl)	111 (89–140)	107 (84–133)	0.13	114 (90–139)	0.9	104 (83.5–130)	0.01	105 (85–132)	0.33	105.5 (81.5–136.5)	0.70
Transferrin saturation (%)	29.68 (23.34–37.77)	28.34 (22.20–35.73)	<0.05	30.92 (24.87)	0.8	27.94 (21.80–33.70)	0.02	28.31 (22.45–33.84)	0.29	27.88 (20.90–36.6)	0.32
TIBC (µg/dl)	371.8 (341.77–410.41)	383.24 (343.2–423.28)	0.09	376.09 (343.2–411.12)	0.44	384.67 (344.63–427.57)	0.04	379.66 (346.06–418.99)	0.77	387.53 (338.91–430.43)	0.06
CRP (mg/dl)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.16	0.2 (0.2–0.4)	0.03	0.2 (0.2–0.3)	0.97	0.2 (0.2–0.3)	0.75	0.2 (0.2–0.4)	0.97
Serum vitamin C (µg/dl)	7.25 (4.80–9.57)	6.80 (4.21–9.10)	0.08	6.39 (3.57–8.63)	0.07	7.02 (4.61–9.40)	0.26	7.24 (4.73–9.41)	0.35	6.59 (4.56–8.88)	0.97

[1]*p* values were calculated excluding subjects with missing values and by comparing cases with their own controls; data not shown for variables on which cases and controls were matched.

Table 2. Correlation matrix for biomarkers of iron status and dietary variables among control subjects

	Ferritin (ng/ml)	Iron (μg/dl)	Transferrin saturation (%)	TIBC (μg/dl)	CRP (mg/dl)	Serum vitamin C (μg/dl)	Total iron (mg)	Heme iron (mg)
Ferritin (ng/ml)	1							
Iron (μg/dl)	0.22 (<0.001)	1						
Transferrin saturation (%)	0.36 (<0.001)	−0.05 (0.16)	1					
TIBC (μg/dl)	−0.42 (<0.001)	−0.05 (0.16)	−0.43 (<0.001)	1				
CRP (mg/dl)	0.08 (0.01)	−0.11 (0.001)	−0.13 (<0.001)	0.02 (0.48)	1			
Serum vitamin C (μg/dl)	0.0059 (0.86)	0.05 (0.10)	0.03 (0.38)	0.07 (0.04)	−0.10 (0.003)	1		
Total iron (mg/day)	0.04 (0.16)	0.05 (0.11)	0.04 (0.20)	0.04 (0.26)	−0.03 (0.34)	0.003 (0.92)	1	
Heme iron (mg/day)	0.1151 (<0.001)	0.02 (0.56)	0.04 (0.19)	−0.03 (0.38)	−0.002 (0.94)	−0.11 (0.001)	0.62 (<0.001)	1
Red meat (mg/day)	0.1097 (0.001)	0.06 (0.05)	0.05 (0.11)	0.005 (0.87)	0.06 (0.07)	−0.0002 (0.99)	0.42 (<0.001)	0.63 (<0.001)
Processed meat (mg/day)	0.22 (<0.001)	−0.02 (0.60)	0.02 (0.59)	−0.09 (0.005)	0.02 (0.44)	−0.05 (0.16)	0.26 (<0.001)	0.44 (<0.001)
Vitamin C (mg/day)	−0.11 (0.001)	0.004 (0.90)	−0.02 (0.63)	0.10 (0.004)	0.02 (0.50)	0.21 (<0.001)	0.44 (<0.001)	0.19 (<0.001)

Non-normally distributed data—Spearman correlation; normally distributed data—Pearson correlation.

Table 2 shows the correlations between iron biomarkers, dietary iron and dietary sources of iron. Ferritin was correlated with serum iron ($r = 0.22$, $p < 0.001$) and TS ($r = 0.36$, $p < 0.001$), and inversely correlated with TIBC ($r = -0.42$, $p < 0.001$). As expected, TS was inversely correlated with TIBC ($r = -0.43$, $p < 0.001$). Dietary variables, such as red meat, total iron, heme iron and vitamin C, did not show correlations with iron indices except for processed meat which showed a positive correlation with ferritin ($r = 0.22$, $p < 0.001$).

Table 3 presents the results of the conditional logistic regression models for iron indices and all gastric cancer as well as gastric cancer subgroups for site and histology. Comparing the highest to the lowest quartiles of iron biomarkers we found statistically inverse associations between overall risk of gastric cancer and levels of ferritin, serum iron and TS indices (OR = 0.38, 95% CI = 0.25–0.57; OR = 0.62, 95% CI = 0.44–0.88; and OR = 0.60, 95% CI = 0.43–0.86, respectively). Using ferritin as a continuous variable (in the log2 scale), the observed OR was 0.80 (95% CI = 0.72–0.88), indicating a 20% decrease in risk of gastric cancer for each doubling of the concentration of ferritin (ng/ml). Moreover, there was evidence that the association between ferritin and gastric cancer differed by site and was stronger in GNCC (p for heterogeneity = 0.04). An increment of 10% of TS led to a reduction of 13% in all gastric cancer risk and 22% in GNCC (no association for GCC; p for heterogeneity = 0.02). Comparing those in the highest to those in the lowest quartile, TIBC was positively associated with all gastric cancer (OR = 1.65, 95% CI = 1.16–2.36) and with GNCC (OR = 2.01, 95% CI = 1.21–3.32). Furthermore, a 50 μg/dl increment of TIBC yielded an OR of 1.13 (95% CI = 1.02–1.25) in all gastric cancer and 1.22 (95% CI = 1.06–1.41) in GNCC (no association in GCC; p for heterogeneity = 0.04). There was no evidence that the association differed by histology of the tumor. We further defined categories for iron status according to OMS cutoff points. In our study, comparing participants with iron deficiency to those within the normal range, iron-deficient subjects appear to be in a greater risk of suffering from gastric cancer, with a borderline significant OR = 1.87 (95% CI = 1.01–3.47). Subjects within the iron overload category showed a statistically significant inverse association with gastric cancer risk when compared to participants with normal values (OR = 0.67, 95% CI = 0.51–0.89) (data not shown).

For almost two-thirds of cases (289 of 456) the diagnosis of cancer took place more than 5 years after blood collection; for about one-quarter (111 cases) this period of time was 2–5 years, and 56 cases were diagnosed within 2 years after blood drawing. The analysis of the effect of biomarkers of iron status according to time since blood collection is shown in Table 4. Concerning ferritin the effect was stronger for cases diagnosed after a short period of blood collection; however, the inverse association remained significant even for cases diagnosed after 5 years since blood draw (p for

Table 3. ORs and 95% CI for the association between gastric cancer and iron metrics in the EURGAST study

		All gastric cancer				Gastric cardia[1]				Gastric noncardia[1]				Diffuse[2]				Intestinal[2]			
	Controls	Cases	OR[3]	95% CI	*p* trend	Cases	OR	95% CI	*p* trend	Cases	OR	95% CI	*p* trend	Cases	OR	95% CI	*p* trend	Cases	OR	95% CI	*p* trend
Ferritin (ng/ml)																					
≤57.09	225	147	Referent			32	Referent			84	Referent			45	Referent			48	Referent		
57.09–104.3	225	120	0.70	0.50–0.97		26	0.66	0.32–1.36		59	0.57	0.36–0.91		45	0.93	0.50–1.72		35	0.70	0.39–1.28	
104.4–193.7	225	104	0.54	0.38–0.77		36	0.91	0.45–1.86		58	0.51	0.31–0.85		34	0.57	0.30–1.08		35	0.62	0.32–1.20	
≥193.8	225	84	0.38	0.25–0.57	<0.001	21	0.38	0.15–0.95	0.366	35	0.26	0.14–0.47	<0.001	30	0.49	0.25–0.99	0.062	30	0.45	0.20–1.00	0.039
Log_2 ferritin	900	455	0.80	0.72–0.88		115	0.88	0.73–1.07		236	0.73	0.64–0.84		154	0.87	0.74–1.03		148	0.80	0.67–0.96	
Iron (μg/dl)																					
≤89	233	145	Referent			33	Referent			76	Referent			45	Referent			47	Referent		
89–111	219	99	0.69	0.50–0.97		21	0.74	0.35–1.56		61	0.74	0.47–1.16		41	0.77	0.43–1.41		32	0.77	0.43–1.37	
112–140	227	119	0.80	0.58–1.09		33	1.09	0.57–2.11		54	0.61	0.38–0.97		41	0.81	0.46–1.42		34	0.76	0.43–1.36	
≥141	220	91	0.62	0.44–0.88	0.034	28	1.00	0.49–2.02	0.973	45	0.54	0.33–0.89	0.029	27	0.52	0.26–1.02	0.153	35	0.88	0.49–1.57	0.435
Per 10 μg/dl	899	454	0.97	0.94–1.00		115	1.01	0.95–1.07		236	0.95	0.91–1.00		154	0.96	0.91–1.02		148	1.00	0.95–1.06	
TS (%)																					
≤23.35	225	136	Referent			28	Referent			73	Referent			42	Referent			49	Referent		
23.35–29.69	225	117	0.78	0.56–1.08		25	1.08	0.51–2.29		70	0.82	0.53–1.27		49	0.84	0.45–1.54		34	0.75	0.43–1.31	
29.69–37.78	225	105	0.75	0.53–1.05		31	1.44	0.67–3.08		48	0.56	0.35–0.92		37	0.80	0.42–1.50		30	0.72	0.39–1.33	
≥37.78	224	95	0.60	0.43–0.86	0.021	30	1.26	0.59–2.67	0.576	45	0.47	0.29–0.78	0.01	26	0.48	0.25–0.94	0.073	35	0.67	0.36–1.24	0.179
Per 10%	899	453	0.87	0.78–0.97		114	1.05	0.85–1.31		236	0.78	0.67–0.92		154	0.88	0.73–1.07		148	0.91	0.76–1.10	
TIBC (μg/dl)																					
≤341.77	226	111	Referent			32	Referent			54	Referent			35	Referent			40	Referent		
341.78–371.8	230	79	0.72	0.50–1.04		25	0.71	0.35–1.42		44	0.87	0.52–1.45		33	0.97	0.53–1.78		27	0.73	0.38–1.41	
371.9–410.41	222	116	1.16	0.82–1.65		31	1.28	0.62–2.67		56	1.09	0.66–1.82		51	1.38	0.77–2.46		31	0.89	0.45–1.78	
≥410.42	221	149	1.65	1.16–2.36	0.002	28	0.96	0.48–1.96	0.957	82	2.01	1.21–3.32	0.007	35	0.96	0.50–1.85	0.474	51	1.45	0.77–2.75	0.156
Per 50 μg/dl	899	455	1.13	1.02–1.25		116	0.93	0.75–1.16		236	1.22	1.06–1.41		154	1.01	0.84–1.21		149	1.29	1.06–1.56	

[1]Site unknown, $n = 104$.
[2]Histology unclassified, $n = 153$.
[3]Matched for age at recruitment, sex, center, date of blood extraction and further adjusted for education (categorical), tobacco smoking (categorical) and heme iron intake (mg/2,000 kcal).

Table 4. OR and 95% confidence interval for gastric cancer and iron metrics by time between blood draw and diagnosis for all gastric cancer cases

	Time between blood draw and gastric cancer diagnosis (years)					
	<2 years ($n = 56$)		2–5 years ($n = 111$)		>5 years ($n = 289$)	
	OR	95% CI	OR	95% CI	OR	95% CI
Ferritin (ng/ml)						
Log_2 ferritin	0.62	0.51–0.76	0.76	0.65–0.89	0.88	0.79–0.98
Iron (μg/dl)						
Per 10 μg/dl	0.86	0.80–0.93	1.03	0.98–1.08	0.98	0.94–1.01
TS (%)						
Per 10%	0.75	0.65–0.87	1.02	0.93–1.11	0.94	0.88–1.00
TIBC (μg/dl)						
Per 50 μg/dl	1.24	0.99–1.55	1.10	0.92–1.30	1.10	0.98–1.22

heterogeneity < 0.05). On the other hand, the inverse association with gastric cancer risk for iron and TS remained statistically significant only for cases diagnosed within 2 years of blood collection (p for heterogeneity < 0.001). Associations were not statistically significant for TIBC in any of the follow-up categories.

Measurement of plasma pepsinogen 1 was available for a subset of 363 subjects (123 cases and 240 controls), among which only 19 had levels below 22 μg/l the cutoff used for diagnosis of chronic atrophic gastritis (CAG).[20] Although concentrations of ferritin were consistently lower in subjects with CAG, there were too few individuals to assess the effect of interaction between CAG and iron biomarkers on gastric cancer risk. However, we explored the potential effect of physiological features associated with CAG by assessing the effect of body iron status indicators taking into account the pepsinogen 1 levels within a subset of 363 individuals with measured plasmatic pepsinogen 1. Adjusting for pepsinogen 1 levels did not affect risk estimates for gastric cancer risk for any of the indicators of body iron status (data not shown).

Finally, we further analyzed possible interaction of iron biomarkers with relevant variables, such as *Hp* infection, plasmatic levels of vitamin C and self-reported alcohol intake, tobacco smoking, sex, BMI and waist-to-hip ratio, but interactions were not statistically significant (data not shown).

Discussion

The results of this study represent the largest prospective analysis of the association of serum iron biomarkers with risk of gastric adenocarcinoma in European Populations. In this analysis, higher levels of ferritin, iron and TS, and lower levels of TIBC were inversely associated with risk of gastric adenocarcinoma. Moreover, these associations appear to be restricted to GNCC. It is important to mention that the differences between gastric cancer subgroups could be due to the number of cases we had available for each group. In fact, GNCC was the group with the biggest number of cases and this could have influenced the precision of associations found for this site. Moreover, GNCC and GCC have different etiological factors so it is expected to see some differential effects when analyzing both subsites. Also, the same pattern between gastric cancer sites has been shown in other studies researching iron intake.[5]

Overall, our findings are consistent with those from previous studies, and appear to suggest an inverse association between body iron status and gastric cancer risk, with ferritin—considered as the most specific biomarker for body iron stores—showing the strongest association amongst all iron indices, reaching a 27% reduction in risk for each doubling of the concentration of ferritin for GNCC. In a Finnish cohort study of 341 male participants, serum ferritin was inversely associated with gastric cancer and GNCC, albeit not in a strict dose-dependent manner.[14] Four other prospective studies have examined the association between iron biomarkers and all gastric cancers combined, although the predominant subsite was GNCC. Two nested case–control studies in Japanese populations[16,17] assessed ferritin levels and found a statistically significant inverse association with gastric cancer. In both studies the risk of gastric cancers was lower for higher levels of transferrin but the association was not significant. Another Finnish cohort, with 196 cases,[17] reported significant association of gastric cancer risk with lower levels of transferrin and ferritin and higher levels of TIBC in men but not in women. On the contrary, one American cohort, with 67 cases,[11] reported a borderline statistically significant positive association between gastric cancer and TS for women, but no association in men.

There is definitive evidence that iron overload induces stress and DNA damage, which can enhance carcinogenic risk.[7,22,23] Nevertheless, when we looked into the OMS categories for iron status, we found that a profile compatible with iron overload showed a statistically significant inverse association with gastric cancer risk. Other evidence suggests

that iron deficiency and anemia could also lead to increased levels of oxidative stress and DNA damage, increasing the carcinogenic risk, especially in the gastrointestinal tract. Iron deficiency may negatively influence several iron-dependent metabolic functions that are associated with genome protection and maintenance (*e.g.*, immune responses against cancer-initiated cells, metabolism of toxic compounds and redox regulation of DNA biosynthesis and repair).[24] Our results show a borderline statistically significant positive association between participants with iron deficiency and gastric cancer risk. However, the number of cases within this category is low which could alter the precision of associations.

It has been suggested that the inverse association between ferritin and gastric cancer risk could be at least in part explained by achlorhydria or hypochlorhydria, which decreases the absorption of nonheme iron in the gastric mucosa.[15] This condition often accompanies CAG, which is strongly associated to gastric cancer. In our study very few subjects had been diagnosed with atrophic gastritis, but ferritin levels were lower in those subjects, both among cases and controls. However, our sample size was too small to assess a potential interaction between CAG and ferritin levels on the risk of gastric cancer. We indirectly assessed the potential effect of gastritis and atrophy using the plasmatic levels of pepsinogen 1 as a proxy, but adjusting for pepsinogen 1 levels did not materially affect the association of ferritin (and other markers) with gastric cancer risk. Therefore, our results do not support the hypothesis that ferritin is a marker of CAG, the actual factor associated with gastric cancer risk as was reported in the above mentioned Japanese study.[15] Another possible explanation for the inverse association between ferritin and gastric cancer is that low serum prediagnosis ferritin is due to bleeding from early undetected lesions of gastric cancer. If this was the case, one may expect that the effect is higher when measurement of ferritin and the diagnosis of gastric cancer are close in time than when serum ferritin was measured in samples collected long before the occurrence of clinical cancer. According to our results in Table 4, the association is weakened for cases with >5 years between blood collection and diagnosis, and is stronger for the subsets of cases diagnosed within 2 years after blood collection, supporting the hypothesis that undetected blood loss could contribute, at least in part, to the inverse association between ferritin and gastric cancer risk. However, such an effect was not observed in previous studies in Japanese cohorts[15,16] where the association was found in subjects whose serum collection preceded gastric cancer by up to 15 years.

In our study, four biological markers were used for the assessment of body iron status. Furthermore, we also used available information from the EPIC cohort study concerning iron intake and meat consumption. We found no correlations between iron indices and dietary iron or meat, except for a weak correlation of ferritin with processed meat. Furthermore, this correlation does not take into account important factors such as age and sex which could be altering the magnitude of the correlation. The lack of correlations could be explained by iron's tightly regulated absorption which is affected by its form and other nutrients; hence, higher iron intake is not necessarily linked to high body iron stores[25,26] and this could explain the contrast between the associations of studies on body iron status and iron intake with gastric cancer risk. One could argue about the precision of laboratory measurements and the quality of the dietary questionnaires used in the study; however, we have used reliable techniques in our determinations and validated food frequency questionnaires.

Hp infection is capable of inducing hemorrhagic gastritis leading to iron loss; furthermore, *Hp* infection may also induce gastric atrophy, which reduces gastric acidity and ascorbic acid levels leading to poor absorption of iron, and sequester iron from the host for growth.[15] Very few cases were negative for *Hp* serology and therefore the interaction between iron markers and *Hp* infection was not formally addressed; however, it must be noted that results did not change significantly when analyses were restricted to infected individuals (data not shown). Moreover, in recent analysis, we have shown that eventually all GNCC cases have been previously infected, suggesting that *Hp* infection is a necessary cause of GNCC.[27] Therefore, it would be very difficult to examine whether there is an interaction of body iron status with *Hp* infection on risk of GNCC.

Our study has several strengths. It is the largest study researching biomarkers of iron status and gastric cancer risk, and it is nested in a prospective study with long-term follow-up which allowed the use of plasma retrieved previous to diagnosis, which reduces the possibility that cancer affected the associations we observed in this study. Also, it enabled us to assess diet prior to diagnoses using data from food frequency questionnaires. Iron measurements performed by our laboratory experts were determined by reliable techniques.

The major limitation of our study is that the determination of body iron status was based on a single set of measurements. Preferably, we should have had several measurements at regular intervals up to the development of cancer, as timing of blood collection and specific individual conditions could alter some of these measurements. Other limitations were the few subjects with information on pepsinogen 1 levels, which limited further research on the possible link between gastric atrophy and gastric cancer.

In summary, our results do not support the hypothesis that increased body iron status is associated with increased risk of gastric cancer. Instead, it appears that higher risk of cancer of the stomach, mainly in the noncardia region, is associated with lower body iron status. However, alternative explanations for this association cannot be ruled out. It is possible that the inverse association of serum ferritin (and other markers) with gastric cancer risk is an indirect one: lower ferritin levels could be a consequence of bleeding associated with early gastric cancer lesions, and probably also of achlorhydria or hypochlorhydria induced by chronic gastritis, a risk factor of gastric cancer. Further investigation is needed to clarify the interplay of these factors and iron status in gastric carcinogenesis.

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