Dietary intake of heme iron and risk of gastric cancer in the European prospective investigation into cancer and nutrition study

Paula Jakszyn[1], Antonio Agudo[1], Leila Lujan-Barroso[1], H Bas Bueno-de-Mesquita[2-21], Mazda Jenab[3], Carmen Navarro[4], Domenico Palli[5], Heiner Boeing[6], Jonas Manjer[7], Mattijs E Numans[8], Laszlo Igali[9], Marie-Christine Boutron-Ruault[10], Françoise Clavel-Chapelon[10], Sophie Morois[10], Sara Grioni[11], cSalvatore Panico[12], Rosario Tumino[13], Carlotta Sacerdote[14], J. Ramon Quirós[15], Esther Molina-Montes[16], Jose Ma Huerta Castaño[4], Aurelio Barricarte[17], Pilar Amiano[18], Kay-Tee Khaw[19], Nicholas Wareham[19], Naomi E. Allen[20], Timothy J Key[20], Suzanne M. Jeurnink[2-21], Petra HM Peeters[8], Christina Bamia[22], Elisabeth Valanou[22,23], Antonia Trichopoulou[22,23], Rudolf Kaaks[24], Annekatrin Lukanova[24], Manuela M. Bergmann[6], Björn Lindkvist[25], Roger Stenling[26], Ingegerd Johansson[27], Christina C. Dahm[28,29], Kim Overvad[29], Anja Olsen[30], Anne Tjonneland[30], Guri Skeie[31], Ann Ragnhild Broderstad[31], Eiliv Lund[31], Dominique S. Michaud[32], Traci Mouw[32], Elio Riboli[33] and Carlos A González[1]

[1] Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology, (ICO-IDIBELL) Barcelona, Spain

[2] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[3] International Agency for Research on Cancer (IARC-WHO), Lyon, France

[4] Department of Epidemiology, Murcia Health Council, CIBER Epidemiología y Salud Pública (CIBERESP), Murcia, Spain

[5] Molecular and Nutritional Epidemiology Unit, ISPO (Cancer Prevention and Research Institute), Florence, Italy

[6] Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany

[7] Department of Surgery, Skåne University Hospital Malmö, Lund University, Malmö, Sweden

[8] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht

[9] Norfolk and Norwich University Hospitals, United Kingdom

[10] INSERM, Center for Research in Epidemiology and Population Health, Institut Gustave Roussy, Paris South University, Villejuif, France

[11] Department of Preventive and Predictive Medicine, Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

[12] Department of Clinical And Experimental Medicine, Federico Ii University, Naples, Italy

[13] Cancer Registry and Histopathology Unit, Civile M.P. Arezzo Hospital, Ragusa, Italy

[14] Center for Cancer Prevention (CPO Piedmont), and Human Genetic Foundation (Hugef), Turin, Florence, Italy

[15] Public Health and Participation Directorate, Health and Health Care Services Council, Asturias, Spain

[16] Andalusian School of Public Health, CIBER Epidemiología y Salud Pública (CIBERESP), Granada, Spain

[17] Navarre Public Health Institute, CIBER Epidemiología y Salud Pública (CIBERESP), Pamplona, Spain

[18] Public Health Division of Gipuzkoa and Ciberesp, Basque Regional Health Department, Spain

[19] Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

[20] Cancer Epidemiology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom

[21] Department of Gastroenterology and Hepatology, University Medical Center Utrecht (UMCU), Utrecht, The Netherlands

[22] WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece

[23] Hellenic Health Foundation, Athens Greece

[24] Department of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany

[25] Department of Internal Medicine, Division of Gastroenterology and Hepatology, Sahlgrenska University Hospital, Gothenburg, Sweden

[26] Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden

[27] Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden

[28] Department of Clinical Epidemiology, Aarhus University Hospital, Aalborg, Denmark

[29] Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark

[30] Danish Cancer Society, Institute of Cancer Epidemiology, Diet Cancer and Health, Copenhagen, Denmark

Epidemiology

[31] Institute of Community Medicine, University of Tromsø, Tromsø, Norway
[32] Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, United Kingdom
[33] School of Public Health, Imperial College London, St Mary's Campus, Imperial College, London, United Kingdom

Even though recent studies suggest that a high intake of heme iron is associated with several types of cancer, epidemiological studies in relation to gastric cancer (GC) are lacking. Our previous results show a positive association between red and processed meat and non cardia gastric cancer, especially in *Helicobacter pylori* infected subjects. The aim of the study is to investigate the association between heme iron intake and GC risk in the European prospective investigation into cancer and nutrition (EURGAST-EPIC). Dietary intake was assessed by validated center-specific questionnaires. Heme iron was calculated as a type-specific percentage of the total iron content in meat intake, derived from the literature. Antibodies of *H. pylori* infection and vitamin C levels were measured in a sub-sample of cases and matched controls included in a nested case-control study within the cohort. The study included 481,419 individuals and 444 incident cases of GC that occurred during an average of 8.7 years of followup. We observed a statistically significant association between heme iron intake and GC risk (HR 1.13 95% CI: 1.01–1.26 for a doubling of intake) adjusted by sex, age, BMI, education level, tobacco smoking and energy intake. The positive association between heme iron and the risk of GC was statistically significant in subjects with plasma vitamin C <39 mmol/l only (log2 HR 1.54 95% CI (1.01–2.35). We found a positive association between heme iron intake and gastric cancer risk.

We reported that a high intake of fresh and processed meat was associated with an increased risk of gastric cancer (GC) in the European prospective investigation into cancer and nutrition study (EURGAST-EPIC).[1] Heterocyclic amines (HA), polycyclic aromatic hydrocarbons (PAH) and pre-formed N-nitrosocompounds (NOCs) have been described for many years as possible factors by which meat consumption may be related to GC. However, results on the relationship between these specific compounds and GC risk are limited and inconsistent.[2–4] Moreover, we have shown that dietary NDMA (*N*-nitrodimethlynitrosamine) did not show any association with GC risk.[1,5]

Randomized controlled dietary intervention studies with human volunteers show that consumption of red and processed meat (but not white meat) can increase endogenous gastro-intestinal formation of NOCs,[6] many of which are known carcinogens.[7] In fact, red meat is rich in heme iron, which is the likely responsible agent for the endogenous NOCs formation.[8,9] Our results from the EURGAST study[5] have shown a significant positive association between the estimated endogenous NOC production (from iron in meat) and risk of noncardia GC.

Iron may also have carcinogenic potential owing to its catalytic effect on the formation of hydroxyl radicals, suppression of the activity of host defence cells and promotion of cancer cell multiplication.[10] Free iron also catalyses lipid peroxidation and produces DNA damage.[11,12] The carcinogenicity of iron has been demonstrated in animal models.[12,13]

During the last 5 years, several studies have been published on the effect of heme iron and the risk of several types of cancer (colorectal, breast, endometrial, lung and upper digestive) with mixed results.[14–19] However, the assessment of the relationship between heme iron intake and gastric cancer has received little attention in epidemiological studies,[20] despite its biological plausibility.

It is well established that *Helicobacter pylori* infection increases the risk of developing distal noncardia gastric cancer but is not associated with the cardia site.[21] Infection with *H. pylori* is likely to increase NO production from macrophages in response to bacterial overgrowth, so that there will be a greater availability of NO in *H. pylori*-infected individuals.[22] Haem is readily nitrosated and can then nitrosate other substrates in the presence of NO.

Vitamin C may be protective against *H. pylori*-associated gastric carcinogenesis by enhancing mucosal immune response, neutralizing free radicals, reducing the formation of gastric NOC, inhibiting cell proliferation and directly affecting *H. pylori* growth.[23] Inflammation induced by *H. pylori* infection in the stomach not only causes significantly increased requirements for vitamin C but also reduces secretion of the vitamin C into the gastric lumen.[23]

To further understand our previously reported positive association between red meat intake, endogenous NOCs and GC risk within the EPIC cohort, we assessed the effect of heme iron intake on GC risk, given that this is one of the main hypotheses behind this relationship. We also examined the interaction between dietary heme iron and other factors such as plasma vitamin C and *H. pylori* infection in a nested case-control study.

Material and Methods
Subjects

The methodological details and rationale behind the EPIC study has been described previously.[24,25] In summary, EPIC is a prospective study involving 23 centers from 10 European countries (United Kingdom, France, Denmark, Sweden,

Epidemiology

Germany, Italy, Spain, The Netherlands, Norway and Greece). A total of 521,457 subjects (153,447 men) aged mostly 35–70 years old, were recruited largely between 1992 and 1998. The ethical review boards from the International Agency for Research on Cancer (IARC) and all local participating centers approved the study.

Followup and identification of cancer cases

Case identification during followup was mostly based on population cancer registries.[1] GC includes cancers coded as C16 according to the 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD).[26] They were validated and confirmed by a panel of pathologists who reviewed specimen material and pathology reports from each center.[27] They were also classified according to both anatomic location (cardia and noncardia) and Lauren histological type (intestinal and diffuse). Among incident GC cases, 444 gastric adenocarcinomas were identified during an average of 8.7 years of followup.

Data collection

Dietary data were collected using validated country-specific questionnaires (quantitative or semi-quantitative) recording the usual diet over the previous 12 months.[28,29] The majority of centers used self-administered questionnaires with the number of food items ranging from 88 to 266, except for Spain, Greece and Italy, where questionnaires were administered during a personal interview. A lifestyle questionnaire[24] was used to collect information about socio-demographic characteristics, lifestyles (smoking habits) and medical history. Anthropometric measures as well as blood samples were taken at recruitment.

Assessment of heme iron intake

To estimate heme iron in foods, we used published information on measured values in different types of meat.[30,31] The proportion of heme iron from total iron for each type of meat was 65, 39 and 26% in cooked beef, pork and chicken or fish, respectively. For foods with mixed composition of meat (beef and pork) such as processed meat we applied an average factor. Multiplying the type-specific percentages of heme iron by the total iron content (mg/g) yielded the heme iron amount for each of the meat related products mentioned in the questionnaires. Usual intake of heme iron was assessed by multiplying the estimated heme iron content by the mean daily intake of related food sources (meat and fish) for each subject. Estimation of the amount of meat (fresh and processed) and fish was done as we previously reported[1] Subjects with heme iron intake > 4 mg/day (99th percentile) were considered as extreme outliers and were excluded from the final sample (0.67% of the sample). Five of them were GC cases (1.1% cases).

Calibration of the heme iron data

We used a detailed computerized 24-hr diet recall (24 HR) method,[29] that was performed to obtain a second dietary assessment (between 1995 and 1999) from a random sample of the cohort (7.1% of total cohort; $n = 36,994$ participants) to calibrate dietary measurements across countries and to correct for systematic measurement error of dietary intakes.[32,33]

Country- and gender-specific calibration models were used to obtain individual predicted values of dietary exposure for all cohort participants. The 24 HR heme iron was calculated using the 24 HR values of heme iron. As we need normal distribution of the variables, Box-Cox transformation was used to normalize the 24 HR heme iron and the heme iron from the main dietary questionnaire. This was regressed on to the heme iron from the main dietary questionnaire. Estimated values of heme iron were retransformed to the original scale. Total heme iron was recalculated by using the calibrated components. Consumption values of zero in the main dietary questionnaires (reported by more than 5% of the participants) were excluded from the regression calibration models. Instead, a zero was directly imputed as the corrected value. Total energy, weight, height, age at study recruitment were included as additional covariates.

Statistical analysis

Cox proportional hazards regression models were used to determine the association between heme iron and GC. Age was the primary time variable; entry time was defined as age at recruitment and exit time defined as age of GC diagnosis (for cases), and diagnosis of a cancer other than GC, death or end of followup, whichever came first.

All models were stratified by country to control for differences in followup time and questionnaire design, and by age at study entry (1-year intervals). All models were adjusted for sex, BMI (continuous), education level (no formal education, primary school, secondary school, technical or professional training, university and not specified), smoking habits (a variable combining smoking status and intensity: never, former who smoked <10 years, former who smoked from ≥10 years, current <20 cigarettes/day, current ≥20 cigarettes/day and not specified), and energy intake (Kcal/day). Additional models were performed by adding citrus fruit intake, alcohol intake and cereal fibre intake but since no differences were found, they were not included in the final model.

The cohort was classified into quartiles according to intake of total heme iron. Hazard Ratios (HR) was calculated for each quartile, using the lowest quartile as the reference category. Furthermore, heme iron was also analyzed as continuous (log2 transformation). The natural logarithm is the most common transformation used to normalize right skewed data; however, we used log2 transformation because it produces the same normalizing effect, but the HR is more easily interpretable: it corresponds to the increase of risk of

Table 1. Gastric cancer cases by tumour site and histological subtype in EPIC countries

Country	Cohort sample	Persons-year	Incident cases	Cardia[1]	Noncardia	Intestinal[2]	Diffuse
France[3]	68356	744479.6	17	4	9	6	6
Italy	45078	380774.4	62	11	34	23	23
Spain	39786	392627.3	53	8	32	22	19
United Kingdom	76486	640045.1	62	33	16	23	11
The Netherlands	36796	314562.0	30	9	13	7	14
Greece	25903	183925.3	25	3	10	10	10
Germany	49591	402220.4	53	13	29	18	29
Sweden	48520	498842.5	66	22	32	25	24
Denmark	55375	414583.9	65	27	22	20	13
Norway	35528	212121.0	11	2	5	0	9
Total	481419	4183881.6	444	132	203	154	158

[1]Localization unknown ($n = 109$). [2]Histological type unknown ($n = 132$). [3]Only women.

GC for doubling the intake of heme iron. Additional models were created to assess risk of GC by cardia and noncardia location and diffuse and intestinal types. The Wald Statistic[34] was used to assess homogeneity of risk by location and histological type. Sensitivity analyses were carried out by repeating the analysis excluding subjects with follow up of less than 2 years.

Cox regression models were reconstructed using the calibrated heme iron, calibrated energy intake, and the other adjusting variables used in the noncalibrated model. The standard error of the attenuated coefficient was calculated with bootstrap sampling in the calibration and disease models consecutively.[33]

To further evaluate whether the association between heme iron intake and GC risk was lineal, we created restricted cubic splines (four knots).[35]

Nested case-control study: Plasma vitamin C and *Helicobacter pylori* infection

A nested case-control study within the EPIC cohort was conducted to perform analyses of *H. pylori* infection status[36] as well as plasma vitamin C. Each incident GC case with an available blood sample was matched by sex, age group (±2.5 years), center, and date of blood collection (±45 days) two to four control participants who had a blood sample available and were randomly selected from among participants in the cohort at risk at the time of diagnosis of the index case. For the analyses of heme iron intake stratified by plasma vitamin C the study had 166 cases and 341 matched controls. On the other hand, for the analyses stratified by *H. pylori* infection, the study included 178 non cardia GC cases and 813 matched controls (including 75 new incident cases and 294 controls) from our previous analyses.[36]

Within the nested case-control study, the odds ratio (OR) for association between GC and heme iron in *H. pylori*-infected and noninfected subjects and according to plasma levels of vitamin C, were estimated by multiple unconditional logistic regression, including matching variables in the model (sex, age at recruitment, season of extraction of blood and country). Interaction between heme iron and *H. pylori* infection and heme iron and plasma vitamin C was tested by likelihood ratio tests.[37] All analyses were carried out using SAS statistical software (9.1).

Results

A total of 444 gastric adenocarcinomas were identified during an average followup of 8.7 years. Table 1 summarizes number of cases by country, anatomical site and histological subtype. Of the 444 GC primary incident cases, 132 were classified as cardia cancer and 203 as noncardia, and for 109 cases the site was unknown. In relation to histological subtype, the proportion for intestinal and diffuse type was similar (49 and 51%, respectively) among those that were classified.

Baseline characteristics of the participants according to heme iron levels are reported in Table 2. Median intake of heme iron was 1.02 mg/day and varied between 0.36 mg/day (median 1st quartile) and 1.97 mg/day (median 4th quartile). Subjects with the highest intake of heme iron tended to be older, had a higher mean BMI, had a lower education level, drank more alcohol and smoked more. Consumption of vegetables and fruits was similar between categories. Levels of plasma vitamin C were lower in those with higher heme iron intake levels. Intake of red meat varied between 7 g and 85.1 g/day (lowest vs. highest quartile). Intake of fish varied between 18.6 and 35.2 g/day.

Table 3 presents the HR for dietary heme iron intake and GC risk estimated in a Cox proportional regression model. After adjustments for several potential confounders, heme iron intake was associated with an increased risk of GC (HR for the highest vs. lowest quartile: 1.67 (95% CI: 1.20–2.34, *p* trend 0.002). Using the heme iron intake as a continuous variable (in the log2 scale), the observed HR was 1.15 (95% CI 1.05–1.26), indicating the risk associated with a doubling of the heme iron intake. This association was very similar

Epidemiology

Table 2. Baseline characteristics of EPIC subjects by heme iron intake (mg/day)

Characteristic		Q1, 0.36[1] (0–0.60)[2]	Q2, 0.80 (0.60–1.02)	Q3, 1.25 (1.02–1.53)	Q4, 1.97 (1.53–4.00)
		Heme iron (mg/day)			
Age at recruitment (years)		49.96	51.6	52.1	51.9
Gender (%)	Male	18.9	21.1	29.5	48.4
	Female	81.1	78.9	70.5	51.6
Level school (%)	None	2.7	4.4	4.4	4.7
	Primary school	16.8	23.8	26.1	27.5
	Technical/professional school	22.9	24.7	23.2	20.3
	Secondary school	21.4	23.7	23.6	23.8
	Longer education	30.9	21.0	21.0	22.7
Smoke status (%)	Never	53.8	49.4	47.8	44.0
	Former	27.3	26.5	26.3	26.3
	Current	16.6	21.8	23.8	27.8
BMI (kg/m^2)		23.7	24.7	25.1	25.6
Energy intake (kcal/day)		1710	1845	2056	2420
Red meat intake (g/day)					
	Beef	2.3	12.1	25.3	41.7
	Other (lamb/viscera/pork)	4.7	16.9	24.9	39.8
	Total	7	29	50.2	81.5
Processed meat (g/day)		9.7	24.8	28.5	40.5
Poultry/rabbit (g/day)		6.3	15.2	16.4	20.7
Fish (g/day)		18.6	28.2	28.8	35.2
Fruits and vegetables intake (g/day)		404.3	373.2	396.7	414.9
Alcohol intake (g/day)		3.4	4.24	6.1	10.4
Plasma vitamin C (μmol/l)[3]		41	42	39	37
Plasma *H. pylori* (% of positive controls)[3]		60.3	63.5	60.9	64.5

All values are presented as median.
[1]Median. [2]Range. [3]Nested analysis.

Table 3. Multivariable hazard ratio (HR) of stomach adenocarcinoma (95% confidence intervals) for observed and calibrated intakes of heme iron according to anatomic location and histological type of gastric cancer in the EPIC cohort

Cancer site/type	Categorical analyses				Continuous analysis	
	HR and 95% CI[1]				HR and 95% CI	
	2	3	4	*P* trend	Observed	Calibrated
Stomach (*n* = 444)	1.23 (0.90–1.68)	1.25 (0.91–1.72)	1.67 (1.20–2.34)	0.002	1.15 (1.05–1.26)	1.13 (1.01–1.26)
Anatomic localization[2]						
Cardia (*n* = 132)	1.26 (0.70–2.29)	1.56 (0.87–2.79)	1.81 (0.97–3.39)	0.056	1.07 (0.95–1.22)	1.05 (0.92–1.19)
Noncardia (*n* = 203)	1.34 (0.84–2.14)	1.35 (0.83–2.18)	1.93 (1.18–3.18)	0.008	1.31 (1.09–1.56)	1.21 (0.94–1.55)
Histological sub-type[3]						
Intestinal (*n* = 154)	1.58 (0.89–2.81)	1.65 (0.93–2.95)	2.18 (1.20–3.98)	0.015	1.21 (1.01–1.44)	1.08 (0.94–1.24)
Diffuse (*n* = 158)	1.02 (0.61–1.70)	1.24 (0.75–2.08)	1.62 (0.94–2.79)	0.039	1.20 (1.00–1.44)	1.81 (0.92–3.58)

[1]Reference categories are the lowest quartile. The full-cohort analysis was stratified by country and age at EPIC study entry and adjusted by sex, BMI, education level, tobacco smoking (duration and intensity) and energy intake. [2]Localization unknown (*n* = 109). [3]Histological sub-type unknown (*n* = 132).

and also significant in the calibrated model (calibrated HR (log2) 1.13, 95% CI 1.01–1.26). Sub-group analyses using the categorized variable by tumor localization showed a signifi-

Table 4. Risk of gastric cancer regarding heme iron intake, stratified by plasma vitamin C levels[1] in the EPIC-EURGAST study

	Controls	Cases	Log2 OR and 95% CI[2]
Stratified by plasma vitamin C[1]			
≤39 μmol	172	89	1.54 (1.01–2.35)
>39 μmol	169	77	1.09 (0.77–1.54)
P for interaction			0.317
Stratified by *H. pylori* infection[3,4]			
Infected	526	163	1.30 (1.01–1.68)
Non-infected	287	15	2.20 (0.85–5.69)
P for interactions			0.480

[1]39 μmol/l: median of plasmatic vitamin C. Unmatched analysis: adjusted by sex, age, centre Vitamin C and season of blood extraction, BMI, educational level, tobacco smoking (duration and intensity), *H. pylori* infection and energy intake. [2]For doubling the intake of heme iron. [3]Only no cardia gastric cancer cases. [4]25 GC cases were missing informatiojn for *H. pylori* infection status.

cant effect of heme iron in both noncardia (HR 1.93, 95% CI 1.18–3.18 for the highest *vs.* the lowest quartile; *p* trend 0.008) and in the cardia anatomical sub-site (HR 1.81; 95% CI: 0.97–3.39) (heterogeneity test *p* = 0.182). Sub-group analyses by histological sub-type showed that heme iron was more associated with cancer risk in the intestinal type (HR 2.18; 95% CI 1.20–3.98, *p* trend 0.015) than in the diffuse (HR 1.62; 95% CI 0.94–2.79) (heterogeneity test *p* = 0.303). This pattern was similar using heme iron as a continuous variable, but the differences were not significant after calibration.

Sensitivity analyses excluding the first 2 years of followup time (resulting in the exclusion of 81 cases and 7276 non cases) did not attenuate the association between dietary heme iron and GC (data not shown). Moreover, we analyzed the interaction between heme iron and smoking, and it was not statistically significant (*p* = 0.86) (data not shown).

The risk of GC according to dietary heme iron and plasma vitamin C levels was investigated within the nested case-control study and is shown in Table 4. In relation to plasma vitamin C, the positive association with heme iron was only significant among those with low serum levels of
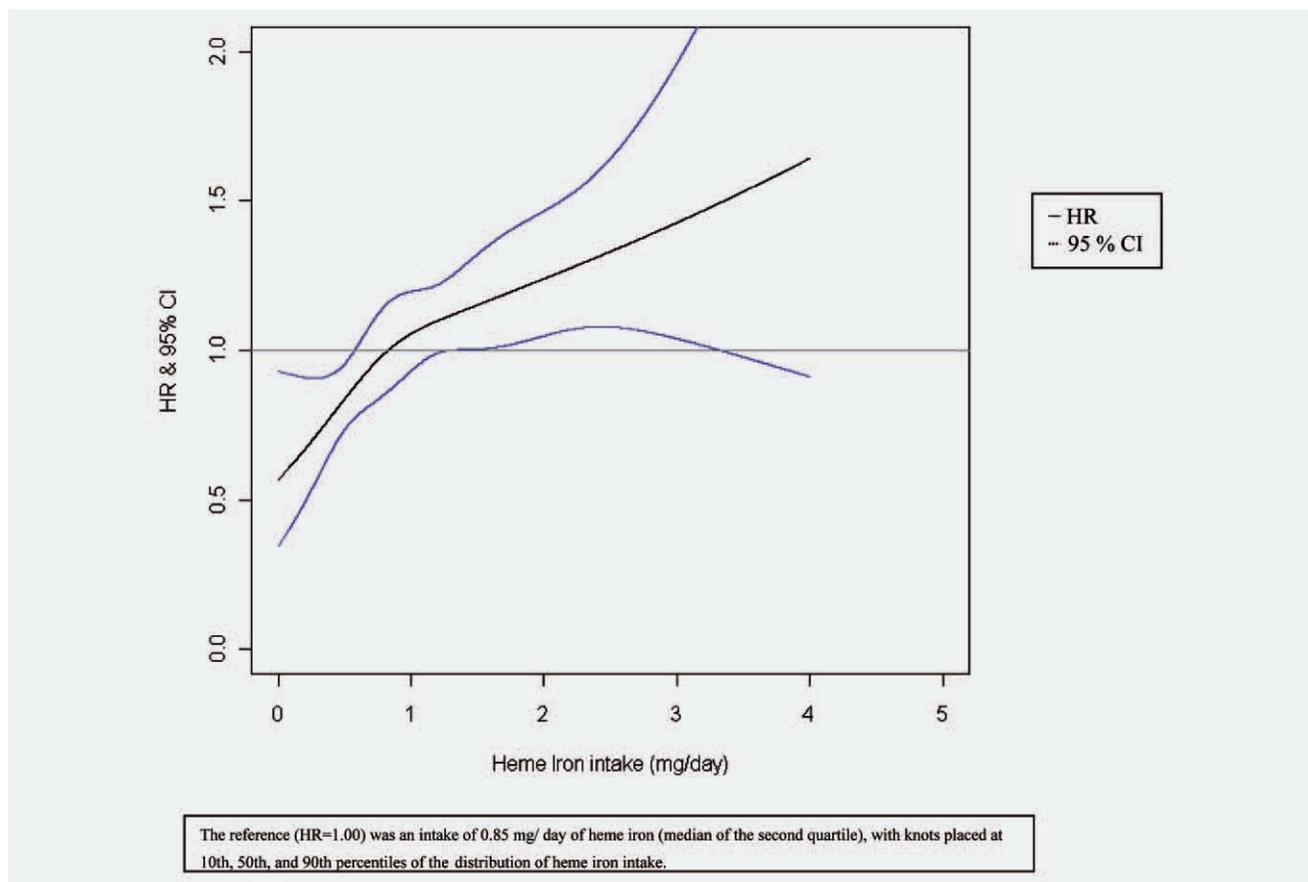

The reference (HR=1.00) was an intake of 0.85 mg/ day of heme iron (median of the second quartile), with knots placed at 10th, 50th, and 90th percentiles of the distribution of heme iron intake.

Figure 1. HR and 95 % CI for the association between heme iron intake and gastric cancer risk using restricted cubic splines. The reference (HR = 1.00) was an intake of 0.85 mg/day of heme iron (median of the second quartile), with knots placed at 10th, 50th and 90th percentiles of the distribution of heme iron intake. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

vitamin C (<39 micromoles/l of plasma vitamin C) (OR, 1.54; 95% CI: 1.01–2.35) (p interaction = 0.317). In relation to *H. pylori* infection, the effect of heme iron only becomes significant within infected subjects (OR: 1.30, 95% CI: 1.01–1.68; p interaction = 0.48).

In Figure 1, we present the shape of the underlying curve association between heme iron intake and GC risk. There is a linear relation at the lower exposures up until an intake of approximately 1.1 mg heme iron/day. Then the increase in risk becomes less pronounced as the exposure increases.

Discussion

Recent studies suggest that the intake of red meat, the most important source of heme iron, was positively associated with GC risk.[1,38–40] However, until now, no study has found has a positive association between dietary heme iron and GC risk in a large scale epidemiological setting. Our results show that subjects in the top quartile of heme iron intake have a 70% higher risk of developing GC than the lowest consumers (categorical analysis). The significant effect of heme seems to be limited to noncardia cancer. This pattern is consistent with the previous reported association in the same population considering other related risk factors (meat intake and endogenous ENOC).[1,5]

Results from the Iowa Women's Health Study (IWHS)[16] including 75 upper digestive tract cancer cases (52 stomach and 25 oesophageal) showed a non significant increased risk of GC in relation to heme iron intake (highest *vs.* lowest quintile 2.83 95% CI 0.84–9.54; p trend 0.06). However, the small number of GC cases could have limited the ability to observe a statistically significant estimate. Moreover, a recent study inclding cardia and non cardia gastric cancer cases has shown no association with heme iron intake.[20] One limitation of this study is the possibility of underestimation of heme iron, because their estimation was based on a database which was limited by the number of meats included on it. Moreover, they included no information on *H. pylori* infection.

Heme iron, an organic form of iron, which represents two-thirds of total body iron, has a greater bioavailability than inorganic iron and could provide a more informative marker of potential iron toxicity.[12] Studies carried out by Bingham *et al.*,[6] have shown that heme iron is the main precursor of endogenous nitrosamines in humans. In fact, in a recent study we have shown a dose response relationship between intake of iron from meat and endogenous formation of NOCs.[5] Moreover, iron can contribute to the formation of free radicals.[10] Many known risk factors for GC such as *H. pylori* infection, inflammation and cigarette smoking are all associated with oxidative stress that may act as a unifying underlying mechanism together with heme iron in the gastric carcinogenesis process.

The amount of heme iron differs greatly between types of meat. Among fresh meats, beef has the highest levels of heme iron per gram, but amounts of heme iron in sheep meat, pork and poultry are also substantial.[31,41] So, on this basis, it is important not only to focus on beef consumption, since people who consume lower amounts of fresh red meat, but consume higher amounts of processed meat, poultry or other meat products, can be exposed to higher levels of heme iron. In our study, subjects in the highest quartile of intake of heme iron consumed on average: 80 g/day of red meat, 40 g/day of processed meat and 55 g/day of white meat (poultry and fish). It seems that intakes below this value do not increase the risk. The public health recommendations provided by the World Cancer recommendations[42] suggest limiting the intake of red meat to 300 g/week. Contribution of heme iron from fish is relatively low and one has to eat large quantities of fish per day to reach similar intakes of heme iron.

Vitamin C may be protective against *H. pylori*-associated gastric carcinogenesis by enhancing mucosal immune response, neutralizing free radicals, reducing the formation of gastric NOC, inhibiting cell proliferation and inhibiting *H. pylori* growth.[23] In our analysis, the effect of heme iron was only significant in subjects with the lowest levels of plasma vitamin C, even though the test for interaction was not statistically significant (p = 0.317). It has been suggested that iron is an essential growth factor for *H. pylori*.[43] However, unexpectedly no interaction between heme iron and *H. pylori* infection was observed (p = 0.48). However, the number of negative *H. pylori* infection among cases was very low and could affect our power to detect statistical significance (15 of 178 cases). Also, we tested the interaction between heme iron and alcohol but it was not statistically significant (data not shown).

HR for GC risk in relation to heme iron meat intake (mg/day) was modeled on a continuous basis using three-knot cubic regression splines. The increase of risk is more marked at the lower intake (but not significant). However, risk only becomes significant with intakes of heme iron around 1.2–1.5 mg/day, which corresponds with the third and fourth quartile (in the categorical analysis).

In recent years, many changes have taken place in the way meat is incorporated into the meal. Some studies have shown[44] that consumption of particular types of meat may be associated with levels of nutrients that are not provided by meat itself, thereby indicating a certain dietary pattern. In fact in our study, subjects with a higher consumption of fish or poultry consumed more vegetables and fruits than those who ate more red meat. Moreover, a higher consumption of red meat was associated with lower intake of fiber or vitamin C,[45] recognized as protective factors for GC.[46,47] Along these lines, we observed that a Mediterranean dietary pattern, characterized by a low intake of red and processed meat and high intake of plant foods and fish, lowered the risk of GC in this population.[48] On the other hand, red meat consumption contributes to higher amounts of zinc, niacin and vitamin B6 and B12 in the diet.[49] When looking at the patterns of consumption within red meat eaters, subjects who eat mainly fresh meat tend to select more healthy food than those eating more processed meats. Although fresh and processed meat

has a high amount of heme iron, processed meat is also higher in saturated fats, and also contains salt and food preservatives (nitrites). These aspects emphasize the need to make dietary recommendations with reference to particular types of meat and to avoid labeling this food group as totally undesirable, since meat consumption has both negative and positive attributes.[49] Programmes to improve dietary quality in terms of adequate selection of the type of meat are as important as dietary recommendations to reduce overall consumption of meat. Moreover, we should encourage increased intakes of fish, fruits, vegetables and whole grain cereals and to include low to moderate amounts of lean meat as a valuable component of a rich and varied diet.

Our study has a number of strengths. Most of the published studies on heme iron intake applied a fix value of 40%[50] to estimate the proportion of heme to total iron in meats. This crude method makes the intakes appear more uniform within study subjects. In our study, heme iron was calculated using specific factors for each type of meat. However, since we did not perform any direct measure of the heme content of the meat we can not overcome the limitation of assigning values using published data. One recent study on heme iron intake and colorrectal adenocarcinoma has shown that heme iron intake estimated using published data and from their own analysis was highly correlated and most individuals were classified in the same quartile of intake. Our results could also be affected by measurement error in dietary intake, a common limitation of epidemiologic studies. However, our results for the association between heme intake and GC risk were calibrated against a more detailed method of dietary assessment (the 24 HR dietary recall). The consistency of our results was reinforced by the close estimated HR provided by the model using the observed (1.15, 95% CI 1.05–1.26) and calibrated data (1.13, 95% CI 1.01–1.26) in the whole sample. However, as the calibration process introduces additional variability, the confidence intervals of the point estimates become wider. This mainly affects the subgroup analyses, where the number of cases is reduced, and the there is a substantial loss of power, leading to nonsignificant estimates. Although our results suggest that the effect of heme iron is more marked, or even that is restricted to noncardia cancer, the limited power precludes drawing a strong conclusion regarding a differential effect of heme iron by tumor localization.

In summary, in this large prospective study, we found that a high dietary intake of heme iron was positive associated with risk of stomach cancer. Considering the biological plausibility of this finding, further investigation is warranted given the limited epidemiological data currently available. Future research may also need to consider the role of individual variation in genes related to iron absorption and metabolism in gastric carcinogenesis.

Acknowledgements

References

1. González CA, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, Ferrari P, Boeing H, del Giudice G, Plebani M, Carneiro F, Nesi G, et al. Meat intake and risk of stomach and esophageal adenocarcinoma within the European prospective investigation into cancer and nutrition (EPIC). *J Natl Cancer Inst* 2006;98:345–54.

2. Terry PD, Lagergren J, Wolk A, Steineck G, Nyrén O Dietary intake of heterocyclic amines and cancers of the esophagus and gastric cardia. *Cancer Epidemiol Biomarkers Prev* 2003;12:940–4.

3. Mirvish SS. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 1995;93:17–48.

4. Jakszyn P, Gonzalez CA Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. *World J Gastroenterol* 2006;12: 4296–303.

5. Jakszyn P, Bingham S, Pera G, Agudo A, Luben R, Welch A, Boeing H, Del Giudice G, Palli D, Saieva C, Krogh V, Sacerdote C, et al. Endogenous versus exogenous exposure to *N*-nitroso compounds and gastric cancer risk in the European prospective investigation into cancer and nutrition (EPIC-EURGAST) study. *Carcinogenesis* 2006;27:1497–501.

6. Lunn JC, Kuhnle G, Mai V, Frankenfeld C, Shuker DE, Glen RC, Goodman JM, Pollock JR, Bingham SA. The effect of haem in red and processed meat on the endogenous formation of *N*-nitroso compounds in the upper gastrointestinal tract. *Carcinogenesis* 2007; 28:685–90.

7. Saffhill R, Margison GP, O'Connor PJ. Mechanisms of carcinogenesis induced by alkylating agents. *Biochim Biophys Acta* 1985;823:111–45.

8. Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal *N*-nitrosation arising from red meat. *Cancer Res* 2003;63:2358–60.

9. Kuhnle GG, Story GW, Reda T, Mani AR, Moore KP, Lunn JC, Bingham SA. Diet-induced endogenous formation of nitroso compounds in the GI tract. *Free Radic Biol Med* 2007;43:1040–7.

10. Babbs CF. Free radicals and the etiology of colon cancer. *Free Radic Biol Med* 1990;8: 191–200.

11. McCord JM. Iron, free radicals, and oxidative injury. *Semin Hematol* 1998;35: 5–12.

12. Huang X. Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. *Mutat Res* 2003;533:153–71.

13. Okada S, Hamazaki S, Ebina Y, Li JL, Midorikawa O. Nephrotoxicity and its prevention by vitamin E in ferric

nitrilotriacetate-promoted lipid peroxidation. *Biochim Biophys Acta* 1987; 922:28–33.

14. Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR, Jr. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa women's health study. *J Natl Cancer Inst* 2004;96:403–7.

15. Lee DH, Anderson KE, Folsom AR, Jacobs DR, Jr. Heme iron, zinc and upper digestive tract cancer: the Iowa women's health study. *Int J Cancer* 2005;117:643–7.

16. Balder HF, Vogel J, Jansen MC, Weijenberg MP, van den Brandt PA, Westenbrink S, van der Meer R, Goldbohm RA. Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev* 2006;15:717–25.

17. Tasevska N, Sinha R, Kipnis V, Subar AF, Leitzmann MF, Hollenbeck AR, Caporaso NE, Schatzkin A, Cross AJ. A prospective study of meat, cooking methods, meat mutagens, heme iron, and lung cancer risks. *Am J Clin Nutr* 2009;89:1884–94.

18. Jakszyn PG, Gonzalez CA, Lujan Barroso L, Ros MM, Bueno-de-Mesquita HB, Roswall N, Tjonneland AM, Buchner FL, Egevad L, Overvad K, Raaschou-Nielsen O, Clavel-Chapelon F, et al. Red meat, dietary nitrosamines and heme iron and risk of bladder cancer in the European prospective investigation into cancer and nutrition (EPIC). *Cancer Epidemiol Biomarkers Prev* 2011;20:555–9.

19. Kabat GC, Cross AJ, Park Y, Schatzkin A, Hollenbeck AR, Rohan TE, Sinha R. Intakes of dietary iron and heme-iron and risk of postmenopausal breast cancer in the National Institutes of Health-AARP diet and health study. *Am J Clin Nutr* 2010;92:1478–83.

20. Cross AJ, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, Sinha R, Abnet CC. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *Am J Gastroenterol* 2011; 106:432–42.

21. Helicobacter and Cancer Collaborative Group. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001;49:347–53.

22. Pignatelli B, Bancel B, Esteve J, Malaveille C, Calmels S, Correa P, Patricot LM, Laval M, Lyandrat N, Ohshima H. Inducible nitric oxide synthase, anti-oxidant enzymes and *Helicobacter pylori* infection in gastritis and gastric precancerous lesions in humans. *Eur J Cancer Prev* 1998;7:439–47.

23. Zhang ZW, Farthing MJ. The roles of vitamin C in *Helicobacter pylori* associated gastric carcinogenesis. *Chin J Dig Dis* 2005; 6:53–8.

24. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondière UR,

Hémon B, Casagrande C, Vignat J, Overvad K, Tjønneland A, et al. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5: 1113–24.

25. Bingham S, Riboli E. Diet and cancer–the European prospective investigation into cancer and nutrition. *Nat Rev Cancer* 2004; 4:206–15.

26. World Health Organization. International classification of diseases, 10th Revision (ICD-10). Geneva: WHO, 1992.

27. Carneiro F, Moutinho C, Pera G, Caldas C, Fenger C, Offerhaus J, Save V, Stenling R, Nesi G, Mahlke U, Bläker H, Torrado J, et al. Pathology findings and validation of gastric and esophageal cancer cases in a European cohort (EPIC/EUR-GAST). *Scand J Gastroenterol* 2007;42:618–27.

28. Margetts BM, Pietinen P. European prospective investigation into cancer and nutrition: validity studies on dietary assessment methods. *Int J Epidemiol* 1997; 26:S1–5.

29. Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, Kroke A, Trichopoulos D, Trichopoulou A, Lauria C, Bellegotti M, Ocké MC, et al. European prospective investigation into cancer and nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr* 2002;5: 1125–45.

30. Carpenter CE, Clark E. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. *J Agric Food Chem* 1995;43:1824–27.

31. Kongkachuichai R, Napatthalung P, Charoensiri R. Heme and non heme iron content in animal producos commonly consumed in Thailand. *J Food Comp Anal* 2002;15:389–98.

32. Ferrari P, Kaaks R, Fahey MT, Slimani N, Day NE, Pera G, Boshuizen HC, Roddam A, Boeing H, Nagel G, Thiebaut A, Orfanos P, et al. Within- and between-cohort variation in measured macronutrient intakes, taking account of measurement errors, in the European prospective investigation into cancer and nutrition study. *Am J Epidemiol* 2004;160: 814–22.

33. Rosner B, Gore R. Measurement error correction in nutritional epidemiology based on individual foods, with application to the relation of diet to breast cancer. *Am J Epidemiol* 2001;154:827–35.

34. Greenland S, Rothman KJ. Introduction to stratified analysis. In: Rothman KJ, Greenland S, eds. Modern epidemiology, 2nd edn. Philadelphia: Lippincott-Raven, 1998. 53–79.

35. Harrell FE. Regression modeling strategies: with applications to linear models, logistic

regression and survival analysis. New York: Springer, 2001.

36. Palli D, Masala G, Del Giudice G, Plebani M, Basso D, Berti D, Numans ME, Ceroti M, Peeters PH, Bueno de Mesquita HB, Buchner FL, Clavel-Chapelon F, et al. CagA+ *Helicobacter pylori* infection and gastric cancer risk in the EPIC-EURGAST study. *Int J Cancer* 2007;120: 859–67.

37. Armitage P, Berry G, Matthews JNS. Statistical methods in medical research, 4th ed. Blackwell Publishers, Oxford, UK 2001.

38. Ito LS, Inoue M, Tajima K, Yamamura Y, Kodera Y, Hirose K, Takezaki T, Hamajima N, Kuroishi T, Tominaga S. Dietary factors and the risk of gastric cancer among Japanese women: a comparison between the differentiated and non-differentiated subtypes. *Ann Epidemiol* 2003;13:24–31.

39. Ngoan LT, Mizoue T, Fujino Y, Tokui N, Yoshimura T. Dietary factors and stomach cancer mortality. *Br J Cancer* 2002;87: 37–42.

40. Kneller RW, McLaughlin JK, Bjelke E, Schuman LM, Blot WJ, Wacholder S, Gridley G, CoChien HT, Fraumeni JF, Jr. A cohort study of stomach cancer in a high-risk American population. *Cancer* 1991;68:672–8.

41. Valenzuela C, López de Romaña D, Olivares M, Morales MS, Pizarro F. Total iron and heme iron content and their distribution in beef meat and viscera. *Biol Trace Elem Res* 2009;132:103–11.

42. World Cancer Research Found/AICR. Food, nutrition, physical activity and the prevention of cancer: a global perspective, Washington, 2007.

43. Perez-Perez C, Israel D. Role of iron in *Helicobacter pylori*: its influence in outer membrane protein expression and in pathogenicity. *Eur J Gastroenterol Hepatol* 2000;12:1263–5.

44. Prynne CJ, Wagemakers JJ, Stephen AM, Wadsworth ME. Meat consumption after disaggregation of meat dishes in a cohort of British adults in 1989 and 1999 in relation to diet quality. *Eur J Clin Nutr* 2009;63:660–6.

45. Elmståhl S, Holmqvist O, Gullberg B, Johansson U, Berglund G. Dietary patterns in high and low consumers of meat in a Swedish cohort study. *Appetite* 1999;32: 191–206.

46. Mendez M, Pera G, Agudo A, Bueno-de-Mesquita HB, Palli D, Boeing H, Carneiro F, Berrino F, Sacerdote C, Tumino R, Panico S, Berglund G, et al. Cereal fiber intake may reduce risk of gastric adenocarcinomas: the EPIC-EURGAST study. *Int J Cancer* 2007; 121:1618–23.

47. Jenab M, Riboli E, Ferrari P, Sabate J, Slimani N, Norat T, Friesen M, Tjønneland A, Olsen A, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, *et al.* Plasma and dietary vitamin C levels and risk of gastric cancer in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). *Carcinogenesis* 2006;27: 2250–7.

48. Buckland G, Agudo A, Luján L, Jakszyn P, Bueno-de-Mesquita HB, Palli D, Boeing H, Carneiro F, Krogh V, Sacerdote C, Tumino R, Panico S, *et al.* Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European prospective investigation into cancer and nutrition (EPIC) cohort study. *Am J Clin Nutr* 2010;91:381–90.

49. Cosgrove M, Flynn A, Kiely M. Consumption of red meat, white meat and processed meat in Irish adults in relation to dietary quality. *Br J Nutr* 2005;93: 933–42.

50. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W, Finch CA. Estimation of available dietary iron. *Am J Clin Nutr* 1978;31:134–41.

Epidemiology