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Iron intake and markers of iron status and risk of Barrett's esophagus and esophageal adenocarcinoma

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Abstract

Objective—To investigate the association between iron intake and iron status with Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC).

Methods—220 BE patients, 224 EAC patients, and 256 frequency-matched controls completed a lifestyle and food frequency questionnaire, and provided serum and toenail samples between 2002 and 2005. Using multiple logistic regression, odds ratios (OR) and 95% confidence intervals (95%CI) were calculated within quartiles of intake/status.

Results—Comparing the fourth to the first quartile, ferritin (OR 0.47; 95%CI: 0.23, 0.97) and transferrin saturation (OR 0.41; 95%CI: 0.20, 0.82) were negatively associated with BE; whilst total iron binding capacity was positively associated per 50 µg/dl increment (OR 1.47; 95%CI: 1.12, 1.92). Comparing the fourth to the first quartile, iron intake (OR 0.50; 95%CI: 0.25, 0.98), non-heme iron intake per 10 mg/day increment (OR 0.29; 95%CI: 0.08, 0.99), and toenail iron (OR 0.40; 95%CI: 0.17, 0.93) were negatively associated with EAC; whilst heme iron intake was positively associated (OR 3.11 95%CI: 1.46, 6.61).

Principal conclusion—In contrast to the hypothesis that increased iron intakes and higher iron stores are a risk factor for BE and EAC, this study suggests that higher iron intakes and stores may have a protective association with BE and EAC, with the exception of what was found for heme iron intake.

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Keywords

adenocarcinoma; Barrett's esophagus; epidemiology; ferritin; iron

Introduction

Incidence of esophageal adenocarcinoma (EAC) has dramatically increased in recent decades, and is the most rapidly increasing cancer in the United States and Western Europe [1–4]. Despite improvements in surgery and chemotherapy, the outlook for patients diagnosed with EAC remains poor, with a five year survival rate of less than 20% [5, 6]. Barrett's esophagus (BE) is a metaplastic change of the distal esophagus in which the normal stratified squamous epithelium is replaced by specialized intestinal columnar epithelium; a potential complication of chronic gastro-esophageal reflux (GER). Although BE patients have an overall approximate 30–125 fold increased lifetime risk of developing EAC, their absolute risk is less than 1% per year [7]. Nevertheless, successful BE and EAC prevention depends on the identification of modifiable risk factors.

One proposed modifiable risk factor for both BE and EAC is a high iron intake and/or elevated iron stores. Increased cancer cell division occurs in the presence of iron [8, 9] and it has been demonstrated that rates of EAC were 10-fold higher in rats treated with iron (intraperitoneal injection of iron dextran (~50 mg Fe/kg/month)) than in untreated rats [10–12]. A constant balance between iron uptake, transport, storage, and utilization is required to maintain iron homeostasis, and prevent the potential formation of hydroxyl radicals which may contribute to the development of EAC. However, the human data that exists has been conflicting [13–20]. The aim of the present study was to investigate the association between iron intake and iron status with BE and EAC, using data and samples collected as part of an all-Ireland case control study, the Factors INfluencing the Barrett's Adenocarcinoma Relationship (FINBAR) study.

Materials and Methods

Subjects and design

Study methods have been described in detail elsewhere [21]. Briefly, the FINBAR study commenced in Ireland in March 2002 and continued until July 2005. The study included three groups: (a) patients with EAC, (b) patients with long-segment BE, and (c) normal population controls, recruited from Northern Ireland and the Republic of Ireland.

EAC cases were patients (aged <85 years) with histologically confirmed adenocarcinoma; with verification that the tumor was located in the esophagus. *In situ* cancers were not included. BE cases were included if they had long length BE at endoscopy (> 3 cm) and specialized intestinal metaplasia without dysplasia on histological examination. Both incident and prevalent cases were included. Population controls were aged 35–84 years without a history of esophageal or other gastro-intestinal cancer, or a known diagnosis of BE. They were selected at random from general practitioner lists in Northern Ireland and the Dublin and Cork areas. Controls were frequency matched by sex and five year age band to the distribution of EAC patients, to ensure that the age and sex profile of BE patients and population controls resembled that of the EAC cases.

All subjects underwent a structured computerized interview with trained interviewers using a modified version of the semiquantitative European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaire (FFQ) [22]. Information was collected on nonsteroidal anti-inflammatory drugs (NSAID) use (weekly use for six months or more;

cards with generic and brand names were provided for the subjects to aid memory recall), gastroesophageal reflux (GER) symptoms (ever/never; defined as symptoms of heartburn and/or acid reflux occurring at least once weekly (>50 times per year) more than five years before interview), smoking status (never, former, current; current smokers defined as smokers who had smoked at least one cigarette daily for 6 months five years before interview date. Previous smokers were those smokers who had quit smoking more than five years before interview date. People who had never smoked, who had smoked <100 cigarettes in their lifetime, or who had smoked <1 cigarette daily for 6 months were defined as never smokers), education (years), occupational type (manual/non-manual), and alcohol consumption (g/wk). Anthropometric measures, including height and weight were recorded at the time of interview, and body mass index (BMI) five years before the interview date was calculated by dividing self-reported weight in kilograms by current height in meters squared.

A 30 ml sample of peripheral venous blood (non-fasting) was taken, transported on ice and centrifuged (most within two hours). Serum was stored at -80°C until analysis. Samples were analyzed using a western blot assay (Helico Blot 2.1, Genelabs Diagnostics, Singapore), which is a qualitative assay for the detection of IgG antibodies to different *h.pylori* antigens in human serum or plasma.

Dietary assessment

Dietary information was obtained using the modified EPIC FFQ [22]. Habitual intake of 101 food items relating to a period five years before interview (pre-morbid diet) was collected. Macronutrient and micronutrient intakes were calculated using McCance and Widdowson's food composition tables [23]. Heme iron intake was calculated according to Balder *et al* [24]. Briefly, the FFQ contained 17 meat and fish food items. To derive an individual serving size for each item, standard portion sizes were used [25], and the total iron content (mg/g) of each food item was calculated accordingly [23]. Heme iron content was computed by multiplying the type-specific heme iron percentage by total iron content as follows: 65% for beef/lamb; 39% for pork/ham/bacon/luncheon meats; 26% for chicken/fish; and 21% for liver [24]. Individual mean daily intake of heme iron was assessed by multiplying the estimated heme iron content by the mean daily intake of the relevant food items. Mean daily intake of non-heme iron intake was calculated by subtracting mean daily intake of heme iron from mean daily intake of total iron.

Serological measures of iron status

Iron status (including serum iron, ferritin, and total iron binding capacity (TIBC)) was measured in the BE cases and normal population controls, but not in the EAC cases as status could be affected by the disease process e.g. blood loss. Serum iron and ferritin were determined using a commercially available ferene assay (Instrumentation Laboratory, Warrington, UK) and turbidimetric assay (Biokit, Barcelona, Spain), respectively; both on the ILab 600 Chemistry System (Instrumentation Laboratory, Warrington, UK). TIBC was also measured on the ILab 600 Chemistry System, using a commercially available sample pretreatment kit and a ferene assay (all Instrumentation Laboratory, Warrington, UK). Transferrin saturation (ratio of serum iron to TIBC) was calculated. Serum C-reactive protein (CRP) was measured using an ultra sensitive assay (Biokit, Barcelona, Spain), on the ILab 600 Chemistry System. The intra-assay and inter-assay coefficients of variation for all analyses was under 3% (using supplied and in-house quality control samples).

Toenail analysis of iron

Clippings from the great toe were taken, stored and transported to the University of Missouri Research Reactor Facility, Columbia, USA, where toenail elemental concentrations were

determined by instrumental neutron activation analysis, as described elsewhere [26]. Briefly, toenail samples were sealed in high purity quartz vials, were irradiated for 50 hours, allowed to decay for 6–10 days, and then counted for four hours each using a high purity germanium detector system. The toenail samples were co-analyzed with NIST SRM 1577 Bovine Liver and the measured value for iron (mean $\pm$ standard deviation (SD)) (257 ± 9 mg/kg) agreed well with the accepted value (268 ± 8 mg/kg). Toenail iron analysis was carried out on all three groups (EAC, BE and normal population controls).

The FINBAR study was approved by the Research Ethics Committee of Queen's University Belfast, the Clinical Research Ethics Committee of Cork Teaching Hospitals, and the Research Ethics Committee Board of St. James's Hospital Dublin.

Statistical analysis

Statistical analysis was conducted using STATA version 9.2 (Timberlake Consultants Ltd, London, UK). Dietary intakes were adjusted for total energy intake using the residual method; intakes were regressed on energy intake, residuals collected, and then added to the mean [27]. In bivariable analyses, Table 1 comparison of descriptive statistics between cases and population controls were investigated using student's *t*-tests for continuous variables and Chi-squared tests for categorical variables; the ordinal variable 'smoking status' was tested using the more appropriate Mantel-Haenszel Chi-squared test. Unconditional multiple logistic regression analysis was used to examine the association between measures of iron intake/status in cases compared with population controls using both categorical (quartiles) exposure variables and continuous exposure variables, to attain odds ratios (OR) and 95% confidence intervals (95%CI). Parsimonious models were built using backward stepwise regression, where individual variables were eliminated from the saturated model in an iterative process. The fit of the model was tested using the likelihood ratio test after the elimination of each variable. Potential covariates included total energy intake (kcal/day), alcohol consumption (g/wk), body mass index (BMI) five years prior to the interview date (kg/m^2), education (years), type of occupation (manual/non-manual), nonsteroidal anti-inflammatory drug (NSAIDs) use more than five years prior to the interview (weekly use for six months or more), location (Northern Ireland/Republic of Ireland), *Helicobacter pylori* (*H.pylori*) infection, and energy adjusted vitamin C (mg/day) and fiber intakes (g/day) (confounding variables included in multivariate analysis are listed in the footnotes of Tables 2 and 3). Sex and age (years), the main frequency-matched variables, and smoking status (never, former, current) and GER (gastro-esophageal reflux) symptoms (ever/never), given their known associations with both iron status and the risk of EAC, were included in all the multivariate adjusted models. Backward stepwise regression was chosen due to the large number of potential confounders. There were no substantial differences in the results of analysis from the fully adjusted model (data not shown). In order to test for trend, median quartile values were assigned to each subject. Subjects with raised CRP levels (> 10 mg/l) were excluded from serological analyses (Tables 1 & 2). This CRP level (> 10 mg/l) represents acute infection or inflammatory disease according to guidelines issued by the Centers for Disease Control and the American Heart Association [28]. Ferritin, also an acute phase reactant, can be raised in inflammatory states. Therefore those with elevated CRP levels were excluded from all serological analyses.

Results

FINBAR included 224 BE patients, 227 EAC patients, and 260 population controls. Overall, 355 EAC patients were suitable for inclusion. Of these, 128 either declined to participate or died before contact was made. The participation rate of eligible, alive patients was 74%, and the overall response rate was 64%. The participation rates of BE and control subjects were 82% and 42%, respectively. Participants with BE ($n = 4$), EAC ($n = 3$), and controls ($n = 4$)

who did not provide dietary information were excluded, leaving a maximum of 220 BE patients (98%), 224 EAC patients (99%), and 256 controls (98%) for inclusion in the analysis. Sufficient serum samples were available for a maximum of 197 BE patients (88%) and 239 population controls (92%); individual numbers analyzed for each serological marker can be found in Table 2. Toenail samples were available for 181 BE patients (81%), 134 EAC patients (59%), and 219 population controls (84%). Although a substantial proportion of EAC cases did not provide a toenail sample, student's *t*-tests and Chi-squared tests demonstrated that availability of toenails was random for this group; the age ($p = 0.68$), sex ($p = 0.69$), BMI ($p = 0.15$) and smoking ($p = 0.55$) profiles were similar for those who supplied/did not supply toenails.

The characteristics of BE and EAC cases compared with the controls are summarized in Table 1. BE cases had a higher overall mean energy intake, were more likely to work in manual jobs, and received fewer years of education than controls. EAC cases had a higher BMI, higher energy intake, were more likely to work in manual jobs, were more likely to smoke, received fewer years of education, and consumed less alcohol than the controls. Both case groups were more likely to have experienced GER symptoms than controls. Controls tended to be *H.pylori* positive compared to either BE or EAC cases.

Barrett's esophagus

Fiber, vitamin C, total iron, and non-heme iron intakes were lower among BE cases compared with the population controls, whereas heme iron intake was higher among BE cases, Table 1. As shown in Table 2 BE was not associated with total iron, heme iron, or non-heme iron intakes in the multivariate models. However, a non-significant inverse association for total iron and non-heme iron intakes with BE was suggested, and a potential positive non-significant association for heme iron intake was also seen. BE cases had lower iron stores than population controls, i.e. lower serum ferritin and transferrin saturation levels, but higher TIBC levels, Table 1. The multivariate models, Table 2 indicated little evidence of an association between serum iron and BE, except for a borderline inverse association in the third quartile (OR 0.52; 95%CI: 0.26, 1.03), but there was little evidence of a trend (p for trend = 0.28). Table 2 also highlighted an inverse association for those in the highest versus the lowest quartile of ferritin (OR 0.47; 95%CI: 0.23, 0.97; p for trend = 0.02) and TIBC was positively associated with BE per 50 µg/dl increment (OR 1.47; 95%CI: 1.12, 1.92; p for trend = 0.01). BE was lower for those in the highest versus the lowest quartile of transferrin saturation (OR 0.41; 95%CI: 0.20, 0.82; p for trend = 0.01), and this association was also evident per 10% increment (OR 0.73; 95%CI: 0.58, 0.91). There was no difference in toenail iron concentrations between BE cases and controls, Table 1; also no association in the multivariate model, Table 2.

Esophageal adenocarcinoma

Vitamin C and non-heme iron intakes were lower among EAC cases compared with the controls, whereas heme iron intake was higher among EAC cases, Table 1. Neither fiber nor total iron intake differed between EAC cases and controls, Table 1. We observed a statistically significant inverse association between total iron intake and EAC, with a borderline trend being suggested (p for trend = 0.06). Conversely, a positive association was seen for those in the highest versus the lowest quartile of heme iron intake (OR 3.11; 95%CI: 1.46, 6.61); there was also a direct association per 1 mg/day increment (OR 1.91; 95%CI: 1.18, 3.09; p for trend < 0.01). There was a statistically significant inverse association between non-heme iron intake and EAC, and a significant trend was evident (p for trend = 0.04); also per 10 mg/day increment (OR 0.29; 95%CI: 0.08, 0.99). There was no difference in toenail iron concentrations between EAC cases and controls, Table 1. However, in the multivariate model, Table 3 EAC was significantly lower for those in the

highest versus lowest quartile of toenail iron (OR 0.40; 95%CI: 0.17, 0.93), and borderline inverse associations were observed in the other quartiles also; a borderline inverse association with EAC was shown per 10 ppm increment (OR 0.64; 95%CI: 0.38, 1.05).

Discussion

In this population based case-control study of iron intakes and iron status in relation to BE and EAC, we have shown that serum ferritin and transferrin saturation were inversely associated with BE, whilst TIBC concentrations appeared to be positively associated; indicative that higher iron stores may have a protective association with BE. However, iron intake, regardless of type specific, was not found to be significantly associated with BE in the multivariate models, although there was a suggestion of an inverse association for total iron and non-heme iron intakes with BE, and a positive association for heme iron intake. Similarly, toenail iron concentrations, a more stable measure of iron exposure, was inversely associated with EAC, as was total iron and non-heme iron intakes; heme iron intake was positively associated with EAC.

A carcinogenic role for iron has been outlined in previous studies, with an increased risk of some cancer types being suggested in individuals with higher iron stores [13–16, 29]. Animal studies have also shown increased rates of EAC in rats treated with iron [10–12]; although these models involved esophagoduodenostomy surgery and intraperitoneal injection of iron dextran, which does not fully mimic the same BE or EAC conditions and nutritional iron exposure as in humans. This may partially explain the contrasting results seen between human and animal studies thus far. It has also been shown in previous studies that patients with EAC had lower iron intakes than population controls [18, 19], and a recent study has shown that individuals with BE had lower than average dietary iron intakes and lower iron stores than controls [20]. Individuals with BE in the present study also had lower iron stores than population controls.

Results from prior studies of heme and non-heme iron intakes and cancer risk are conflicting [24, 30–33]. Lee *et al.* [17] reported a greater risk of upper digestive tract cancer (gastric cancer and esophageal cancer) for individuals with the highest heme iron intakes compared to those with the lowest intakes. The results of this study also found similar results with regards to heme iron intakes with both BE and EAC, although the association was most pronounced and statistically significant with EAC in the multivariate model. The opposite was observed with regards to non-heme iron intakes, but again this was only statistically significant with EAC in the multivariate model. However, it is not possible to disentangle whether these associations are due to the iron intakes *per se* or are proxy indicators for certain dietary or lifestyle factors; being the result of diets rich in heme and non-heme iron foods. The main dietary sources of heme iron are meat, fish and poultry, whilst plant based foods such as lentils and beans are good non-heme iron sources. Previous studies have shown beneficial effects of fruits and vegetables [34–37] and a plant based dietary pattern [38]; also detrimental effects of meat consumption [39–41]. Furthermore, in addition to being one of the main sources of heme iron, meat also contributes to saturated fat intake, which has been shown to be positively associated with EAC in previous studies [42, 43]; as well as other mutagens such as N-nitroso compounds and nitrites [44–46] and carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons [16]. Iron fortification of white and brown flour in the UK is also mandatory and is therefore an important dietary source of non-heme iron; non-heme iron intake was highly correlated with energy adjusted fiber intake in the present study ($r = 0.61$, $p < 0.01$). Although there are many hypothesized which may explain the effect of heme and non-heme iron intakes on BE and EAC, additional studies that investigate the association between diet, particularly dietary patterns, and meat/meat cooking methods and risk are needed, using specifically designed

questionnaire modules that are essential to elucidate these findings. Nevertheless, it will always remain difficult to segregate dietary habits from overall lifestyle and health behaviors as they are not fully measured by questionnaires.

In addition, it is well understood and accepted that FFQs are associated with exposure misclassification due to reporting errors, such as recall bias and imprecise portion size estimation [47]. It has been suggested therefore that additional independent markers of intake or status should be incorporated into studies to assess diet-disease associations. Serum or plasma is the most often used source of iron measurement in population studies; with studies reporting that ferritin may be the most accurate serologic estimate of the body's total iron stores [48, 49]. However, use of this method also has limitations as concentrations of iron can vary day-to-day. In contrast to serum or plasma measurements, toenail clippings provide a more stable measure of trace elements, such as iron, by providing a measure of nutritional status 12 months or more prior to the date of collection of the specimen; enabling the assessment of pre-diagnostic nutritional status [50]. It has been demonstrated in a study involving 127 individuals that toenail iron concentrations measured in paired samples collected from the same individual six years apart were significantly correlated ($r = 0.43$, $p < 0.05$), indicating that a single measurement is reflective of long term iron status [26]. Toenail clippings are also more convenient to collect and store than blood. Toenail iron concentrations were inversely associated with EAC in the present study.

Dietary ascorbic acid has been shown to be a strong promoter of non-heme iron absorption [51, 53] whilst fiber intake may inhibit its absorption [54–58]. Therefore, both ascorbic acid and fiber intakes are potential confounders of the association between iron intake with BE and EAC; a recent meta-analysis reported an inverse association between ascorbic acid intake and EAC (OR 0.49 95%CI 0.39–0.62) [59], whilst an inverse association between fiber intake with BE and EAC has been consistently reported [18, 35, 43, 44, 60, 61]. The fact that ascorbic acid and fiber intakes may potentially play a role in the EAC pathway, and they both demonstrate opposing roles on iron absorption, hence iron status, they were included in multivariate models as evaluated by backward stepwise regression (see footnotes of Table 3).

Individuals with BE in this study had lower iron stores than the population controls which may be explained by the presence of GER symptoms. Gastric acid facilitates the dissociation of iron salts from food, and reduces Fe^{3+} to the more soluble Fe^{2+} to facilitate its absorption in the duodenum [62]. Therefore, use of acid suppressing medications, such as proton pump inhibitors (frequently used to combat GER symptoms) may result in decreased iron absorption [20], leading to lower body iron stores. Although this was not clearly demonstrated in the current study since adjustment for GER symptoms in the multivariate models did not considerably alter the associations seen for either BE or EAC; except for those associations between total iron intake, including heme and non-heme iron intakes, and BE. Individuals who suffer from GER symptoms may have altered their diets in response to GER, i.e. by avoiding high-fat foods (such as meat compared to fruits and vegetables) which are known to exacerbate reflux symptoms, increasing transient lower esophageal sphincter relaxation, and increasing esophageal acid exposure [63]. Additionally, heme iron (meat) absorption is 5–10 fold greater than non-heme iron (plant based foods) [64].

H.pylori may protect against EAC [65], and a recent meta-analysis also reported that *H.pylori* infection may result in a decrease in iron stores [66]. Biological mechanisms may have an effect either separately or in combination on iron stores, including gastrointestinal blood loss, decrease in the absorption of dietary iron from the lumen, and enhanced uptake of the iron by the bacterium [66]. However, *H.pylori* was eliminated from many of the multivariate models in the current study as evaluated by backward stepwise regression,

except for total iron intake and TIBC with BE. Therefore, our results would suggest that *H.pylori* had little effect on iron stores within this population.

There are several strengths to this study. The FINBAR study has a relatively large sample size, is population-based and used stringent inclusion criteria throughout. Information was collected on a wide range of potential confounders such as BMI, smoking status, and GER symptoms. Another key strength of this study was the comprehensive panel of markers of iron exposure investigated, including iron intake and status assessed using both serum and toenail iron concentrations, compared to other studies.

There are also some limitations to this study including the potential for recall bias. The inclusion of independent markers of iron exposure unbiased by recall and relating to iron exposure prior to diagnosis (i.e. toenail iron concentrations) unlike previous studies is advantageous. There is also the possibility of residual confounding from unmeasured variables. Within FINBAR, the response rate of the controls was lower compared with cases, which may have introduced selection bias. Nevertheless, the mean daily intake of iron among controls (13.6 mg/day) was similar to intake reported in the North/South Ireland Food Consumption Survey (approximately 14 mg/day) [67], suggesting that our controls are representative of the general population. Additionally, it is widely understood that the incidence of BE and EAC is higher among males than females, and this is reflected in the numbers of female and male cases recruited as part of FINBAR, Table 1. Population controls were frequency matched by sex and five year age band to the distribution of EAC patients to ensure that the age and sex profile of BE patients and population controls resembled that of the EAC cases. Also, when analysis on the continuous variables for each exposure in Tables 2 and 3 was conducted in males only, the results were similar to those reported for males and females combined (data not shown). Finally, iron intake reported in this study does not include use of iron supplementation as the information collected was inadequate to identify specific supplement usage. It is possible that supplement use and dietary intake may be associated with other unaccounted for health behaviors or risk factors for EAC.

In summary, in contrast to the hypothesis that increased iron intakes and higher iron stores are a risk factor for BE and EAC, this study suggests that higher iron intakes and stores may have a protective association with BE and EAC, with the exception of what was found for heme iron intake. Results from cohort studies investigating the association between iron intake (including both heme and non-heme) and its dietary sources, along with iron status are needed to confirm these results.

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References

1. Bosetti C, Levi F, Ferlay J, et al. Trends in oesophageal cancer incidence and mortality in Europe. *Int J Cancer*. 2008; 122:1118–1129. [PubMed: 17990321]

2. Eloubeidi MA, Mason AC, Desmond RA, El-Serag HB. Temporal trends (1973–1997) in survival of patients with esophageal adenocarcinoma in the United States: a glimmer of hope? *Am J Gastroenterol.* 2003; 98:1627–1633. [PubMed: 12873590]
3. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am.* 2002; 11:235–256. [PubMed: 12424848]
4. Blot WJ, McLaughlin JK. The changing epidemiology of esophageal cancer. *Semin Oncol.* 1999; 26:2–8. [PubMed: 10566604]
5. di Pietro M, Peters CJ, Fitzgerald RC. Clinical puzzle: Barrett's oesophagus. *Dis Model Mech.* 2008; 1:26–31. [PubMed: 19048049]
6. Jankowski JA, Harrison RF, Perry I, Balkwill F, Tselepis C. Barrett's metaplasia. *Lancet.* 2000; 356:2079–2085. [PubMed: 11145505]
7. Shaheen NJ, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology.* 2000; 119:333–338. [PubMed: 10930368]
8. Henle ES, Luo Y, Gassmann W, Linn S. Oxidative damage to DNA constituents by iron-mediated fenton reactions. The deoxyguanosine family. *J Biol Chem.* 1996; 271:21177–21186. [PubMed: 8702888]
9. Ullen H, Augustsson K, Gustavsson C, Steineck G. Supplementary iron intake and risk of cancer: reversed causality? *Cancer Lett.* 1997; 114:215–216. [PubMed: 9103295]
10. Chen X, Ding YW, Yang G, Bondoc F, Lee MJ, Yang CS. Oxidative damage in an esophageal adenocarcinoma model with rats. *Carcinogenesis.* 2000; 21:257–263. [PubMed: 10657966]
11. Chen X, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS. An esophagogastrroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. *Carcinogenesis.* 1999; 20:1801–1808. [PubMed: 10469627]
12. Goldstein SR, Yang GY, Chen X, Curtis SK, Yang CS. Studies of iron deposits, inducible nitric oxide synthase and nitrotyrosine in a rat model for esophageal adenocarcinoma. *Carcinogenesis.* 1998; 19:1445–1449. [PubMed: 9744541]
13. Fracanzani AL, Conte D, Fraquelli M, et al. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. *Hepatology.* 2001; 33:647–651. [PubMed: 11230745]
14. ElMBERG M, Hultcrantz R, Ekbom A, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology.* 2003; 125:1733–1741. [PubMed: 14724826]
15. Geier D, Hebert B, Potti A. Risk of primary non-hepatocellular malignancies in hereditary hemochromatosis. *Anticancer Res.* 2002; 22:3797–3799. [PubMed: 12552996]
16. Grant WB. An ecological study of cancer mortality rates including indices for dietary iron and zinc. *Anticancer Res.* 2008; 28:1955–1963. [PubMed: 18630488]
17. 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; 117:643–647. [PubMed: 15929082]
18. Zhang ZF, Kurtz RC, Yu GP, et al. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. *Nutr Cancer.* 1997; 27:298–309. [PubMed: 9101561]
19. Wolfgarten E, Rosendahl U, Nowroth T, et al. Coincidence of nutritional habits and esophageal cancer in Germany. *Onkologie.* 2001; 24:546–551. [PubMed: 11799309]
20. Corley DA, Kubo A, Levin TR, et al. Iron intake and body iron stores as risk factors for Barrett's esophagus: a community-based study. *Am J Gastroenterol.* 2008; 103:2997–3004. [PubMed: 18853987]
21. Anderson LA, Johnston BT, Watson RG, et al. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. *Cancer Res.* 2006; 66:4975–4982. [PubMed: 16651456]
22. Day N, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort. *European Prospective Investigation of Cancer. Br J Cancer.* 1999; 80:95–103. [PubMed: 10466767]
23. Food Standards Agency. McCance and Widdowson's The Composition of Foods. Sixth summary edition. Cambridge, UK: Royal Society of Chemistry; 2002.

24. Balder HF, Vogel J, Jansen MC, et al. Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:717–725. [PubMed: 16614114]
25. Crawley, H. Food Portion Sizes. London, UK: HMSO; 1998.
26. Garland M, Morris JS, Rosner BA, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. *Cancer Epidemiol Biomarkers Prev.* 1993; 2:493–497. [PubMed: 8220096]
27. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyzes. *Am J Epidemiol.* 1986; 124:17–27. [PubMed: 3521261]
28. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003; 107:499–511. [PubMed: 12551878]
29. Hsing AW, McLaughlin JK, Olsen JH, Møller L, Wacholder S, Fraumeni JF Jr. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. *Int J Cancer.* 1995; 60:160–162. [PubMed: 7829208]
30. 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; 96:403–407. [PubMed: 14996862]
31. Kabat GC, Miller AB, Jain M, Rohan TE. Dietary iron and heme iron intake and risk of endometrial cancer: a prospective cohort study. *Br J Cancer.* 2008; 98:194–198. [PubMed: 18059399]
32. Kabat GC, Miller AB, Jain M, Rohan TE. Dietary iron and heme iron intake and risk of breast cancer: a prospective cohort study. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:1306–1308. [PubMed: 17548704]
33. Kabat GC, Miller AB, Jain M, Rohan TE. A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. *Br J Cancer.* 2007; 97:118–122. [PubMed: 17551493]
34. Anderson LA, Watson RG, Murphy SJ, et al. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J Gastroenterol.* 2007; 13:1585–1594. [PubMed: 17461453]
35. Brown LM, Swanson CA, Gridley G, et al. Adenocarcinoma of the esophagus: role of obesity and diet. *J Natl Cancer Inst.* 1995; 87:104–109. [PubMed: 7707381]
36. Kubo A, Levin TR, Block G, et al. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am J Gastroenterol.* 2008; 103:1614–1623. [PubMed: 18494834]
37. Terry P, Lagergren J, Hansen H, Wolk A, Nyren O. Fruit and vegetable consumption in the prevention of oesophageal and cardia cancers. *Eur J Cancer Prev.* 2001; 10:365–369. [PubMed: 11535879]
38. Kubo A, Levin TR, Block G, et al. Dietary patterns and the risk of Barrett's esophagus. *Am J Epidemiol.* 2008; 167:839–846. [PubMed: 18218607]
39. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med.* 2007; 4:e325. [PubMed: 18076279]
40. Gonzalez CA, Jakszyn P, Pera 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; 98:345–354. [PubMed: 16507831]
41. Navarro Silvera SA, Mayne ST, Risch H, et al. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer.* 2008; 123:852–860. [PubMed: 18537156]
42. Lagiou P, Talamini R, Samoli E, et al. Diet and upper-aerodigestive tract cancer in Europe: the ARCAGE study. *Int J Cancer.* 2001; 124:2671–2676. [PubMed: 19230023]
43. Mayne ST, Risch HA, Dubrow R, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev.* 2001; 10:1055–1062. [PubMed: 11588131]
44. Chen H, Tucker KL, Graubard BI, et al. Nutrient intakes and adenocarcinoma of the esophagus and distal stomach. *Nutr Cancer.* 2002; 42:33–40. [PubMed: 12235648]

45. Hughes R, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. *Carcinogenesis*. 2001; 22:199–202. [PubMed: 11159760]
46. Lin K, Shen W, Shen Z, Wu Y, Lu S. Dietary exposure and urinary excretion of total N-nitroso compounds, nitrosamino acids and volatile nitrosamine in inhabitants of high- and low-risk areas for esophageal cancer in southern China. *Int J Cancer*. 2002; 102:207–211. [PubMed: 12397637]
47. Schaefer EJ, Augustin JL, Schaefer MM, et al. Lack of efficacy of a food-frequency questionnaire in assessing dietary macronutrient intakes in subjects consuming diets of known composition. *Am J Clin Nutr*. 2000; 71:746–751. [PubMed: 10702168]
48. Beutler E, Felitti V, Ho NJ, Gelbart T. Relationship of body iron stores to levels of serum ferritin, serum iron, unsaturated iron binding capacity and transferrin saturation in patients with iron storage disease. *Acta Hemeatol*. 2002; 107:145–149.
49. Mei Z, Cogswell ME, Parvanta I, et al. Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. *J Nutr*. 2005; 135:1974–1980. [PubMed: 16046725]
50. Longnecker MP, Stram DO, Taylor PR, et al. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. *Epidemiology*. 1996; 7:384–390. [PubMed: 8793364]
51. Fleming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PW, Wood RJ. Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study. *Am J Clin Nutr*. 1998; 67:722–733. [PubMed: 9537620]
52. Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *Am J Clin Nutr*. 2001; 73:93–98. [PubMed: 11124756]
53. Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP. The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr*. 2003; 78:436–440. [PubMed: 12936926]
54. Cook JD, Noble NL, Morck TA, Lynch SR, Petersburg SJ. Effect of fiber on nonheme iron absorption. *Gastroenterology*. 1983; 85:1354–1358. [PubMed: 6313466]
55. Cook JD. Food iron availability: back to the basics. *Am J Clin Nutr*. 1997; 67:593–594. [PubMed: 9537604]
56. Fleming DJ, Tucker KL, Jacques PF, Dallal GE, Wilson PW, Wood RJ. Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort. *Am J Clin Nutr*. 2002; 76:1375–1384. [PubMed: 12450906]
57. Hurrell RF. Bioavailability of iron. *Eur J Clin Nutr*. 1997; 51:S4–S8. [PubMed: 9023471]
58. Peneau S, Dauchet L, Vergnaud AC, et al. Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. *Am J Clin Nutr*. 2008; 87:1298–1305. [PubMed: 18469253]
59. Kubo A, Corley DA. Meta-analysis of antioxidant intake and the risk of esophageal and gastric cardia adenocarcinoma. *Am J Gastroenterol*. 2007; 102:2323–2330. [PubMed: 17581269]
60. Tzonou A, Lipworth L, Garidou A, et al. Diet and risk of esophageal cancer by histologic type in a low-risk population. *Int J Cancer*. 1996; 68:300–304. [PubMed: 8903470]
61. Mulholland HG, Cantwell MM, Anderson LA, et al. Glycemic index, carbohydrate and fiber intakes and risk of reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. *Cancer Causes Control*. 2009; 20:279–288. [PubMed: 18839322]
62. McColl KE. Effect of proton pump inhibitors on vitamins and iron. *Am J Gastroenterol*. 2009; 104:S5–S9. [PubMed: 19262546]
63. el-Serag HB. The epidemic of esophageal adenocarcinoma. *Gastroenterol Clin North Am*. 2002; 31:421–40 viii. [PubMed: 12134611]
64. Cook JD. Adaptation in iron metabolism. *Am J Clin Nutr*. 1990; 51:301–308. [PubMed: 2407101]
65. Islami F, Kamangar F. Helicobacter pylori and esophageal cancer risk: a meta-analysis. *Cancer Prev Res (Phila Pa)*. 2008; 1:329–338.
66. Muhsen K, Cohen D. Helicobacter pylori infection and iron stores: a systematic review and meta-analysis. *Helicobacter*. 2008; 13:323–340. [PubMed: 19250507]

67. Hannon EM, Kiely M, Harrington KE, Robson PJ, Strain JJ, Flynn A. The North/South Ireland Food Consumption Survey: mineral intakes in 18–64-year-old adults. *Public Health Nutr.* 2001; 4:1081–1088. [PubMed: 11820921]

Table 1

Characteristics, iron intake and status (mean $\pm$ SD) of Barrett's esophagus and esophageal adenocarcinoma subjects and controls

Characteristics	Controls $n_{max} = 256$	Barrett's esophagus $n_{max} = 220$	$P - value^a$	Esophageal adenocarcinoma $n_{max} = 224$	$P-value^a$
Age (years)	63 $\pm$ 13	62 $\pm$ 12	0.50	64 $\pm$ 11	0.25
Sex, n (%)					
Male	216 (84.4)	181 (82.3)		189 (84.4)	
Female	40 (15.6)	39 (17.7)	0.54 ^b	35 (15.6)	1.00 ^b
BMI 5 yrs prior (kg/m ²)	27.1 $\pm$ 3.9	26.9 $\pm$ 4.0	0.74	28.6 $\pm$ 4.9	<0.01
Waist-hip ratio	0.96 $\pm$ 0.08	0.96 $\pm$ 0.08	0.65	0.96 $\pm$ 0.07	0.37
Energy intake (kcal)	2,576 $\pm$ 811	2,721 $\pm$ 770	0.05	2,756 $\pm$ 813	0.02
Energy adjusted fiber (g/d)	18.6 $\pm$ 5.2	17.4 $\pm$ 5.3	0.02	17.8 $\pm$ 5.5	0.14
Energy adjusted vitamin C (mg/d)	144.1 $\pm$ 75.7	129.0 $\pm$ 72.1	0.03	119.4 $\pm$ 68.3	<0.01
Energy adjusted total iron (mg/d)	14.0 $\pm$ 2.1	13.3 $\pm$ 2.1	<0.01	13.7 $\pm$ 2.3	0.10
Energy adjusted heme iron (mg/d)	1.2 $\pm$ 0.5	1.3 $\pm$ 0.5	0.04	1.3 $\pm$ 0.5	<0.01
Energy adjusted non-heme iron (mg/d)	12.8 $\pm$ 2.1	12.0 $\pm$ 2.3	<0.01	12.4 $\pm$ 2.3	0.02
Serum iron (μ g/dl) ^c	93.48 $\pm$ 33.96	88.82 $\pm$ 32.67	0.15	-	-
Ferritin (ng/ml) ^c	125.97 $\pm$ 114.27	92.07 $\pm$ 135.90	<0.01	-	-
TIBC (μ g/dl) ^c	302.54 $\pm$ 42.53	321.77 $\pm$ 51.61	<0.01	-	-
Transferrin saturation (%) ^{cd}	31.7 $\pm$ 12.4	28.2 $\pm$ 10.9	<0.01	-	-
Toenail iron (ppm)	10.36 $\pm$ 36	9.85 $\pm$ 8.69	0.57	8.94 $\pm$ 7.71	0.14
Education (years)	12 $\pm$ 3	11 $\pm$ 3	0.02	11 $\pm$ 3	<0.01
Occupation type, n (%)					
Manual	123 (49.4)	127 (58.5)		131 (60.1)	
Non-manual	126 (50.6)	90 (41.5)	0.05 ^b	87 (39.9)	0.02 ^b
GER symptoms, n (%) ^e					
Ever	49 (19.2)	159 (72.3)		108 (48.2)	
Never	206 (80.8)	61 (27.7)	<0.01 ^b	116 (51.8)	<0.01 ^b
Smoking status, n (%)					
Current	44 (17.7)	50 (22.8)		76 (34.7)	
Previous	105 (42.2)	82 (37.5)		98 (44.8)	
Never	100 (40.1)	87 (39.7)	0.42 ^c	45 (20.5)	<0.01 ^c
Alcohol (g/day)	26.3 $\pm$ 23.3	22.3 $\pm$ 25.4	0.13	19.8 $\pm$ 22.0	0.01
<i>H. pylori</i> positive (%)	58.5	50.5	0.09 ^b	49.0	0.04 ^b
Location, n (%)					
Northern Ireland	119 (46.5)	150 (68.2)		114 (50.9)	
Republic of Ireland	137 (53.5)	70 (31.8)	<0.01 ^b	110 (49.1)	0.34 ^b

Abbreviations: BMI, body mass index; GER, gastro-esophageal reflux; *H. Pylori*, Helicobacter pylori infection; SD, standard deviation; TIBC, total iron binding capacity.

^a cases compared to controls by student's *t*-test, except

^b chi-squared tests ^cMantel-Haenszel chi-squared test.

^c subjects with raised C-reactive protein levels (>10 mg/l) were excluded from analysis.

^d transferrin saturation = total iron/TIBC $\times$ 100.

^e heartburn/acid reflux symptoms experienced at least once weekly or >50 times per year >5 years before interview date.

Table 2

Odds ratios of Barrett's esophagus in relation to iron intake and status

	Controls (n)	Barrett's esophagus (n)	Unadjusted OR (95% CI)	Age and energy adjusted OR (95% CI)	Multivariate adjusted ^a OR (95% CI)
<i>Energy adjusted total iron (mg/d)</i>					
<12.76	64	88	1.00	1.00	1.00 ^b
12.76 - <13.94	65	49	0.55 (0.34-0.90)	0.57 (0.34-0.93)	0.77 (0.42-1.44)
13.94 - <15.44	63	55	0.63 (0.39-1.03)	0.65 (0.40-1.07)	1.15 (0.62-2.10)
15.44	64	28	0.32 (0.18-0.55)	0.31 (0.18-0.54)	0.52 (0.26-1.02)
P-value for trend			<0.01	<0.01	0.15
per 10 mg/d increment	256	220	0.19 (0.08-0.47)	0.20 (0.08-0.49)	0.55 (0.18-1.65)
<i>Energy adjusted heme iron (mg/d)</i>					
<0.74	64	39	1.00	1.00	1.00 ^c
0.74-<1.23	67	47	1.15 (0.67-1.99)	1.14 (0.66-1.97)	1.12 (0.58-2.18)
1.23-<1.39	60	49	1.34 (0.77-2.32)	1.36 (0.78-2.36)	1.05 (0.54-2.04)
1.39	65	79	1.99 (1.19-3.34)	1.93 (1.15-3.24)	1.66 (0.89-3.09)
P-value for trend			0.01	0.01	0.16
per 1 mg/d increment	256	214	1.45 (1.02-2.06)	1.41 (0.99-2.00)	1.42 (0.94-2.15)
<i>Energy adjusted non-heme iron (mg/d)</i>					
<11.56	64	93	1.00	1.00	1.00 ^c
11.56-<12.84	64	47	0.51 (0.31-0.83)	0.52 (0.32-0.85)	0.58 (0.32-1.05)
12.84-<14.10	64	39	0.42 (0.25-0.70)	0.43 (0.26-0.72)	0.67 (0.36-1.25)
14.10	64	35	0.38 (0.22-0.63)	0.37 (0.22-0.63)	0.63 (0.34-1.17)
P-value for trend			<0.01	<0.01	0.15
per 10 mg/d increment	256	214	0.16 (0.07-0.39)	0.17 (0.07-0.42)	0.43 (0.15-1.20)
<i>Serum iron (µg/dl)^f</i>					
<72.99	58	62	1.00	1.00	1.00 ^d
72.99-<90.15	56	49	0.82 (0.48-1.38)	0.80 (0.47-1.37)	0.80 (0.42-1.54)
90.15-<108.49	58	40	0.65 (0.38-1.11)	0.61 (0.35-1.05)	0.52 (0.26-1.03)
108.49	57	43	0.71 (0.41-1.20)	0.61 (0.35-1.06)	0.75 (0.37-1.50)

	Controls (n)	Barrett's esophagus (n)	Unadjusted OR (95% CI)	Age and energy adjusted OR (95% CI)	Multivariate adjusted ^a OR (95% CI)
<i>P</i> -value for trend			0.15	0.05	0.28
per 50 µg/dl increment	229	194	0.81 (0.60–1.08)	0.75 (0.56–1.02)	0.85 (0.57–1.28)
<i>Ferritin (ng/ml)</i> ^f					
<52.60	60	90	1.00	1.00	1.00 ^e
52.60–<96.01	59	53	0.60 (0.37–0.98)	0.57 (0.34–0.95)	0.82 (0.44–1.55)
96.01–<160.03	61	26	0.28 (0.16–0.50)	0.27 (0.15–0.47)	0.33 (0.16–0.67)
160.03	59	28	0.32 (0.18–0.55)	0.31 (0.17–0.54)	0.47 (0.23–0.97)
<i>P</i> -value for trend			<0.01	<0.01	0.02
per 50 ng/ml increment	239	197	0.87 (0.78–0.96)	0.80 (0.71–0.91)	0.89 (0.78–1.02)
<i>TIBC (µg/dl)</i> ^f					
<274.79	49	33	1.00	1.00	1.00 ^b
274.79–<297.37	50	28	0.83 (0.44–1.58)	0.78 (0.41–1.50)	0.48 (0.21–1.09)
297.37–<322.25	50	36	1.07 (0.58–1.98)	1.03 (0.55–1.92)	0.77 (0.35–1.67)
322.25	49	87	2.64 (1.50–4.63)	2.49 (1.40–4.42)	1.93 (0.95–3.93)
<i>P</i> -value for trend			<0.01	<0.01	0.01
per 50 µg/dl increment	198	184	1.55 (1.24–1.94)	1.53 (1.22–1.92)	1.47 (1.12–1.92)
<i>Transferrin saturation (%)</i> ^g					
<24.53	49	78	1.00	1.00	1.00
24.53–<30.76	50	39	0.49 (0.28–0.85)	0.44 (0.25–0.78)	0.43 (0.22–0.84)
30.76–<36.17	50	28	0.35 (0.20–0.63)	0.33 (0.18–0.59)	0.28 (0.14–0.58)
36.17	49	39	0.50 (0.29–0.87)	0.44 (0.25–0.78)	0.41 (0.20–0.82)
<i>P</i> -value for trend			0.01	<0.01	0.01
per 10 % increment	198	184	0.77 (0.64–0.92)	0.74 (0.61–0.89)	0.73 (0.58–0.91)
<i>Toenail iron (ppm)</i>					
<5.48	54	51	1.00	1.00	1.00 ^c
5.48–<7.64	57	48	0.89 (0.52–1.53)	0.86 (0.50–1.49)	0.87 (0.44–1.72)
7.64–<11.52	53	45	0.90 (0.52–1.56)	0.88 (0.51–1.54)	0.68 (0.34–1.37)
11.52	55	37	0.71 (0.40–1.25)	0.69 (0.39–1.23)	0.82 (0.40–1.65)
<i>P</i> -value for trend			0.25	0.22	0.58

	Controls (<i>n</i>)	Barrett's esophagus (<i>n</i>)	Unadjusted OR (95% CI)	Age and energy adjusted OR (95% CI)	Multivariate adjusted ^a OR (95% CI)
per 10 ppm increment	219	181	0.94 (0.75–1.17)	0.94 (0.75–1.18)	1.07 (0.82–1.40)

Abbreviations: CI, confidence interval; OR, odds ratio; TIBC, total iron binding capacity. Bold values refer to statistically significant results.

^a sex, age (years), smoking status (never, former, current) and gastro-esophageal reflux symptoms (ever/never) included in all multivariate adjustments, also

^b helicobacter pylori infection (seropositive/seronegative), location (Northern Ireland/Republic of Ireland);

^c location;

^d location, serum ferritin;

^e location, serum iron.

^f subjects with raised C-reactive protein levels (>10 mg/l) were excluded from analysis.

^g transferrin saturation = serum iron/TIBC × 100.

Table 3

Odds ratios of esophageal adenocarcinoma in relation to iron intake and status

	Controls (n)	Esophageal adenocarcinoma (n)	Unadjusted OR (95% CI)	Age and energy adjusted OR (95% CI)	Multivariate adjusted ^a OR (95% CI)
<i>Energy adjusted total iron (mg/d)</i>					
<12.76	64	83	1.00	1.00	1.00 ^b
12.76 - <13.94	65	51	0.61 (0.37–0.99)	0.59 (0.36–0.97)	0.46 (0.25–0.82)
13.94 - <15.44	63	45	0.55 (0.33–0.91)	0.56 (0.34–0.94)	0.51 (0.28–0.95)
15.44	64	45	0.54 (0.33–0.90)	0.50 (0.30–0.84)	0.50 (0.25–0.98)
P-value for trend			0.01	0.01	0.06
per 10 mg/day increment	256	224	0.50 (0.22–1.15)	0.47 (0.20–1.10)	0.56 (0.16–1.88)
<i>Energy adjusted heme iron (mg/d)</i>					
<0.74	64	30	1.00	1.00	1.00 ^c
0.74–<1.23	67	59	1.88 (1.08–3.28)	1.85 (1.06–3.25)	1.66 (0.75–3.64)
1.23–<1.39	60	48	1.71 (0.96–3.04)	1.76 (0.98–3.14)	1.74 (0.77–3.91)
1.39	65	84	2.76 (1.60–4.74)	2.68 (1.55–4.62)	3.11 (1.46–6.61)
P-value for trend			<0.01	<0.01	<0.01
per 1 mg/day increment	256	221	1.67 (1.17–2.38)	1.66 (1.16–2.39)	1.91 (1.18–3.09)
<i>Energy adjusted non-heme iron (mg/d)</i>					
<11.56	64	84	1.00	1.00	1.00 ^b
11.56–<12.84	64	52	0.62 (0.38–1.01)	0.63 (0.38–1.03)	0.50 (0.28–0.91)
12.84–<14.10	64	37	0.44 (0.26–0.74)	0.45 (0.26–0.75)	0.40 (0.21–0.76)
14.10	64	48	0.57 (0.35–0.94)	0.55 (0.33–0.91)	0.52 (0.26–1.02)
P-value for trend			0.01	0.01	0.04
per 10 mg/day increment	256	221	0.37 (0.16–0.86)	0.35 (0.15–0.82)	0.29 (0.08–0.99)
<i>Toenail iron (ppm)</i>					
<5.48	54	42	1.00	1.00	1.00 ^d
5.48–<7.64	57	37	0.83 (0.47–1.49)	0.85 (0.48–1.53)	0.50 (0.23–1.07)
7.64–<11.52	53	33	0.80 (0.44–1.45)	0.80 (0.44–1.45)	0.44 (0.20–1.01)
11.52	55	22	0.51 (0.27–0.97)	0.54 (0.28–1.03)	0.40 (0.17–0.93)
P-value for trend			0.04	0.05	0.07

	Controls (<i>n</i>)	Esophageal adenocarcinoma (<i>n</i>)	Unadjusted OR (95% CI)	Age and energy adjusted OR (95% CI)	Multivariate adjusted ^a OR (95% CI)
per 10 ppm increment	219	134	0.81 (0.61–1.08)	0.83 (0.62–1.10)	0.64 (0.38–1.05)

Abbreviations: CI, confidence interval; OR, odds ratio. Bold values refer to statistically significant results.

^a sex, age (years), smoking status (never, former, current) and gastro-esophageal reflux symptoms (ever/never) included in all multivariate adjustments, also energy adjusted vitamin C intake (mg/d), energy adjusted fiber intake (g/d), education (years), BMI five years prior (kg/m²);

^b alcohol (g/day), BMI five years prior, energy intake (kcal/day);

^c alcohol, education, BMI five years prior.