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Dietary Iron and Heme Iron Consumption, Genetic Susceptibility, and Risk of Crohn's Disease and Ulcerative Colitis

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Abstract

Introduction—Dietary iron and heme, likely through their effect on gut commensal bacteria and colonic barrier function, have been shown to modulate colonic inflammation in animal models of colitis. Nonetheless, the link between dietary total and heme iron and risk of Crohn's disease (CD) and ulcerative colitis (UC) has not been previously explored.

Methods—We conducted a prospective cohort study of 165,331 U.S. women enrolled in the Nurses' Health Study (NHS) and NHSII. Dietary information was collected using a validated food frequency questionnaire at baseline (1984) and updated every 2–4 years. Self-reported CD and UC

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Conflicts of Interest: There are no conflicts of interest to declare.

Ethical Approval: The institutional review board at the Partners Healthcare approved this study.

Data sharing: Requests for access to data, statistical code, questionnaires, and technical processes may be made by contacting the corresponding author at hkhalili@mgh.harvard.edu.

Authors Contributions

HK - study concept and design; statistical analysis; interpretation of data; drafting of the manuscript
PDS - study concept and design; interpretation of data; critical revision of the manuscript
ANA - acquisition of data; critical revision of the manuscript
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JS - study concept and design; interpretation of data; critical revision of the manuscript
ATC - interpretation of data; critical revision of the manuscript

diagnoses were confirmed through medical records review. We used Cox proportional hazards models to calculate hazard ratios (HR) and 95% confidence intervals (CIs) while adjusting for potential confounders. In a case-control study nested within these cohorts, we evaluated the interaction between single nucleotide polymorphisms (SNPs) associated with genome-wide susceptibility to CD and UC and dietary total and heme iron intake on risk of CD and UC using logistic regression modeling.

Results—Through 2011, over 3,038,049 person-years of follow up, we documented 261 incident cases of CD and 321 incident cases of UC. Dietary heme iron was non-significantly associated with increased risk of UC (P_{trend} = 0.12). This association appeared to be modified by the UC-susceptibility locus, rs1801274, a coding variant in the *Fcγ RIIA* gene ($P_{interaction}$ = 7.00E-05). In contrast, there was no association between dietary heme iron and risk of CD (P_{trend} = 0.67). We also did not observe an association between total dietary intake of iron and risk of CD or UC (All P_{trend} > 0.35).

Conclusion—In two large prospective cohort studies, dietary total and heme iron were not associated with risk of CD or UC. Our suggestive finding that the association between dietary heme iron intake and risk of UC may be modified by a coding variant in *Fcγ RIIA* gene warrants additional investigation.

Keywords

Inflammatory Bowel Disease; Crohn's Disease; Ulcerative Colitis; Dietary Heme; Iron Intake; Genetic Risk Variants; Nurses' Health Study

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel diseases (IBD), are chronic inflammatory disorders of the gastrointestinal tract in which a barrier normally maintained by adaptive and innate immunity is disrupted. A critical but poorly characterized aspect of innate immune defense relies on the ability to successfully limit the systemic and local availability of iron to invading microbes. In response, commensal bacteria have co-evolved efficient strategies for competing with each other and their hosts for dietary iron. Notably, greater availability of luminal iron induces dysbiosis and increases the abundance of bacterial pathogens in children.[1] In contrast, in a mouse model of spontaneous ileitis, depletion of luminal iron alters gut microbial composition to promote inflammation[2]. Heme, the iron porphyrin pigment, primarily found in red meat, poultry and fish is poorly absorbed in the small intestine. Approximately 90% of dietary heme transits to the colon, and is exploited by colonic bacteria as a growth factor[3]. Nonetheless, despite these compelling data supporting a role of dietary iron in regulating innate immunity and altering the composition of the gut commensal bacteria, the association between dietary total iron and heme iron intake and risk of CD and UC has not been previously studied.

We therefore sought to examine the association between pre-diagnosis total dietary iron and heme iron intake and risk of CD and UC in two large prospective cohorts of US women. Leveraging findings from recent genome-wide association studies[4], we also sought to

characterize the relationship between total iron and heme iron and risk of CD and UC by exploring for presence of gene-environment interaction using established susceptibility loci for CD and UC. Gene-environment interaction between an environmental component and genetic variants in functionally annotated genes has recently been employed to help inference causal associations[5] and provide insight into potential biologic pathways by which an environmental factor such as iron or heme iron may contribute to the etiopathogenesis of IBD.

METHODS

A. Cohort study: Dietary iron and heme iron and risk of UC and CD

A1. Study population—Our study population included participants from NHS and NHSII with available dietary data. These cohorts have been extensively described, previously[10, 11]. Briefly, NHS and NHSII are prospective cohort studies of 121,700 and 116,686 women that have been followed through biennial mailed questionnaires since 1976 and 1989, respectively. The Human Research Committee at Partners Healthcare approved this study.

A2. Assessment of diet—Dietary assessment in both cohorts have been done using 161-item semi-quantitative food frequency questionnaire (SFFQ) every 4 years starting in 1984 in NHS and 1991 in NHSII. Intake of specific dietary factors was computed from the reported frequency of consumption of each specified food item based on US Department of Agriculture data on the content of the relevant nutrient in specified portions. For calculation of total dietary iron, supplemental iron intake was also added to dietary iron. To determine the nutrient composition of the diet independent of the total amount of food consumed, nutrient values were adjusted for total caloric intake by the residual method. The reproducibility and validity (against dietary records) of the FFQs have been extensively documented in NHS and NHSII[6, 7]. Questionnaire items on unprocessed red meat consumption included “beef or lamb as main dish,” “pork as main dish,” “hamburger,” and “beef, pork, or lamb as a sandwich or mixed dish.” Items on processed red meat included “bacon,” “hot dogs,” and “sausage, salami, bologna, and other processed red meats.” The correlation between dietary total iron intake obtained from FFQ and dietary records was 0.55[8]. The major contributors of dietary heme, contributing approximately 60–70% of dietary heme iron, included red meat (beef, pork, lamb), cold breakfast cereal, chicken without skin, skim milk, and hamburgers. The correlation coefficients between the FFQs and the dietary records for these foods ranged from 0.38 for hamburger to 0.81 for skim milk[9].

Dietary data were carried forward from the prior questionnaire cycle when our detailed assessment was not included on a biennial questionnaires (e.g., 1986 data used in 1988–1990 follow-up). We otherwise did not carry forward missing data. Individuals with missing data during a questionnaire cycle in which dietary information was assessed did not contribute person-time to the analyses.

A3. Assessment of other covariates—Information on other lifestyle factors, including ethnicity, physical activity, body weight, smoking status, geographic latitude of residence, use of non-steroidal anti inflammatory drugs (NSAIDs), menopausal hormone therapy, and oral contraceptives were collected from each biennial questionnaire as previously

reported[10, 11]. Participants' self-report of body weight, height, physical activity, and use of oral contraceptives have been previously validated validated[12–14].

A4. Outcome Ascertainment—We have previously detailed our methods for confirming self-reported cases of CD and UC[10, 11, 15]. In brief, since 1976, participants in the NHS have reported diagnoses of UC or CD through an open-ended response on biennial surveys. In addition, biennial questionnaires have specifically queried diagnoses of UC since 1982 and CD since 1992. In the NHSII, we have specifically queried participants about diagnoses of both UC and CD since 1993. When a diagnosis of CD or UC was reported on any biennial questionnaire, a supplementary questionnaire was mailed and related medical records were requested and reviewed by two gastroenterologists blinded to exposure information. We used standardized criteria to confirm cases of CD and UC[16–19].

A5. Statistical analysis—For analysis of dietary total and heme iron intake and food items, person-time for each participant was calculated from the date of return of their baseline questionnaires to the date of the diagnosis of UC or CD, date of last returned questionnaire, or June 1, 2010 for NHS and June 1, 2011 for NHSII, whichever came first. At baseline, we excluded participants with missing dietary data and history of IBD. We used Cox proportional hazards modeling with time-varying covariates to adjust for other known or suspected risk factors prior to each 2 or 4- year interval to calculate adjusted hazard ratios (HR) and 95% confidence interval (CIs). Because weight may be influenced by preclinical disease, we adjusted for BMI using the baseline value, consistent with prior analyses[20, 21]. Dietary total and heme iron were modeled as quintiles while food items were modeled based on average number of servings per day consistent with prior dietary analysis[21, 22]. We observed no heterogeneity in the association of dietary total and heme iron and food items with CD or UC in separate analyses of NHS and NHSII (P for heterogeneity > 0.60 for both UC and CD). Thus, we pooled individual-level data from NHS and NHSII and adjusted for cohort in all analyses. We used SAS version 9.3 (Cary, NC) for all analyses (including analyses in section B3). All *P*-values were 2-sided and < 0.05 was considered statistically significant.

B. Nested Case-Control Study: Dietary iron and heme iron intake, IBD risk variants, and risk of CD and UC

B1. Study population—In 1989–1990, 32,826 NHS participants (aged 43–69 years) returned a blood sample on ice packs by overnight courier and completed a short questionnaire[23]. Between 1996 and 1999, 29,611 NHSII participants (aged 32–54 years) provided blood samples and completed a short questionnaire in a similar protocol[24]. In 2001–2004, 29,684 participants in NHS and 29,859 participants in NHSII, who had not previously provided a blood specimen, mailed in a sample of buccal cells collected using a "swish-and-spit" method. Among participants who provided a blood or saliva specimen, we matched 169 CD cases to 740 controls and 202 UC cases to 740 controls based on age, menopausal status, month of blood collection, and fasting status. Genomic DNA was isolated from buccal cells or blood samples using conventional methods[25].

B2. Genotyping—We used the most recent meta-analysis of genome-wide association studies to identify single nucleotide polymorphisms (SNPs) that have previously been associated with risk of CD and UC[4]. We directly genotyped these SNPs by 5′ nuclease assay (TaqMan®), using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case-control status, and 10% blinded quality control (duplicate) samples were inserted to validate genotyping procedures; concordance for the quality control samples was 100%. Primers, probes, and conditions for the genotyping assay are available upon request. We confirmed that all SNPs were in Hardy-Weinberg equilibrium among the controls using the Chi Square test (All $P > 0.25$).

B3. Statistical Analysis—We used conditional logistic regression for risk of CD or UC with a multiplicative interaction term for dietary total and heme iron and genotypes of common variants defined according to the number of risk alleles while adjusting for other potential risk factors (see section A3). Since CD and UC are rare outcomes, we used odds ratios as estimates of relative risks. To minimize the potential influence of reverse causality, we analyzed prospectively collected data on diet from the questionnaires administered four years prior to diagnosis of CD or UC for cases and their matched controls. Although, there were no significant variations in allele frequency of genotyped risk variants according to European ancestry, we adjusted all models of gene-environment interaction for ancestry (see Section A3). We categorized participants as southern European/Mediterranean or as Scandinavian when that was the only ancestry reported, as other white when a mixture of only white ancestries was reported, and as nonwhite when either African, Asian, or Hispanic ancestry was reported. We used SAS version 9.3 (Cary, NC) for these analyses. Bonferroni-adjusted P value of 2.8E-04 (=0.05/180) was considered statistically significant.

RESULTS

A. Dietary Total Iron and Heme Iron Intake and Risk of UC and CD

Through 2011, among 165,331 women we confirmed 261 incident cases of CD and 321 incident cases of UC over 3,038,049 person-years of follow up. At baseline, compared to women in the lowest quintile of total dietary iron intake, women in the highest quintile were less likely to be current smokers, more likely to be current users of menopausal hormone therapy, and on average, had a higher mean daily consumption of fiber (Table 1). Red meat intake was correlated with intake of heme iron (Spearman $r = 0.58$) but not total iron (Spearman $r = 0.03$).

There was no association between total dietary iron intake and risk of UC ($P_{trend} = 0.35$) (Table 2). However, the risk of UC appeared to be associated with a non-significant increase with higher dietary heme iron intake ($P_{trend} = 0.12$) (Table 2). Compared to women in the lowest quintile of dietary heme iron, the multivariable (MV)-adjusted HR of UC among women in the highest quintile of dietary heme iron was 1.23 (95% CI, 0.85–1.80).

We did not observe an association between total dietary iron and heme iron intake and risk of CD ($P_{trend} = 0.67$ and 0.85, respectively). Compared to women in the lowest quintile of

total dietary iron intake, the MV-adjusted HR of CD among women in the highest quintile of dietary iron was 1.15 (95% CI, 0.76–1.74). Similarly, compared to women in the lowest quintile of dietary heme iron intake, the MV-adjusted HR of CD among women in the highest quintile of dietary heme iron was 0.91 (95% CI, 0.61–1.35) (Table 2).

We explored the possibility that symptoms of undiagnosed CD or UC may lead to changes in total and heme iron intake and therefore performed lagged analyses using dietary data derived from at least 4 years prior to each 2-year follow up and observed no associations between total dietary iron and heme iron intake and risk of CD and UC (All $P_{trend} > 0.19$). Similarly, there were no associations between supplemental iron intake and risk of CD or UC (All $P_{trend} > 0.35$).

B. Red and Processed Meat and Risk of CD and UC

Since we observed a non-significant increased risk of UC with higher heme iron intake, we explored whether red meat, a major contributor to heme iron is also associated with risk of UC. Similar to heme iron, higher intake of red meat was associated with a non-significant increase in risk of UC ($P_{trend} = 0.08$) (Table 3). In lagged analyses accounting for changes in dietary intake as a result of undiagnosed disease, this trend reached statistical significance ($P_{trend} = 0.01$). Specifically, we observed a 14% increase (HR = 1.14, 95% CI 1.01–1.30) in risk of UC for every one serving increase in weekly red meat intake. In contrast, dietary intake of red meat was not associated with risk of CD ($P_{trend} = 0.40$) (Table 3). Similarly, we did not observe an association between processed meat and risk of CD or UC ($P_{trend} = 0.99$ and 0.86, respectively) (Table 3).

C. Interaction Between Dietary Intake of Total and Heme Iron and IBD Susceptibility Variants and Risk of CD and UC

We assessed for presence of gene-environment interaction between dietary intake of total iron and heme iron and known UC and CD susceptibility loci on risk of CD and UC among 149 cases of CD, 172 cases of UC matched to 650 controls. The UC susceptibility locus rs1801274 appeared to significantly modify the association between dietary heme iron and risk of UC after Bonferroni correction of multiple comparisons ($P_{interaction} = 7.00E-05$) (Table 4). Specifically, among women with the GG genotype, each 1 g increase in dietary heme iron intake was associated with a 90% reduction in risk of UC (MV-adjusted OR = 0.11, 95% CI 0.03–0.37). In contrast, among women with AA genotype, each 1 g increase in dietary heme iron intake was associated with a nearly 3-fold increase in risk of UC (MV-adjusted OR = 2.76, 95% CI 1.02–7.48). The effect of dietary heme iron on risk of UC was not modified by other UC susceptibility loci (Supplementary Table 1). We also did not observe any interaction between any of the CD-related susceptibility loci and dietary heme iron on risk of CD (Supplementary Table 1). Similarly, we did not observe any interaction between any of the CD- or UC-related susceptibility loci and total dietary iron intake on risk of CD or UC (Supplementary Table 1).

DISCUSSION

In two large prospective cohorts of US women, we observed an association between dietary heme iron intake and risk of UC that is significantly modified by UC susceptibility locus rs1801274, suggesting a potentially novel pathway that mediates the effect of heme iron intake on development of UC. Rs1801274 is a coding variant located on chromosome 1 within *FcγRIIA* gene (formal HUGO gene nomenclature: *FCGR2A*), a Fcγ receptor family gene. FcγRIIA is a single chain receptor, unique to humans, containing an immunoreceptor tyrosine-based activating motif (ITAM) in its intracellular domain[26]. It is the most broadly distributed FcγR, being found on monocytes, macrophages, platelets, and neutrophils. Activating FcγRIIA plays a key role in the humoral response to infection, mediating many important IgG effector functions that favor pathogen clearance. Interestingly, acute phase proteins such as C-reactive protein (CRP) that are able to opsonize microbial pathogens can also bind and activate FcγRIIA suggesting a significant role for this receptor in the cross-talk between innate and adaptive immunity[27].

The rs1801274 variant represents a G>A missense coding (H131R), which likely alters the binding of FcγRIIA receptor to IgG2 and CRP[28, 29]. Specifically, it has been proposed that FcγRIIA-H131, through its high affinity for IgG2, favors pathogen clearance while also predisposing individuals to autoimmunity (including UC). Conversely, FcγRIIA-R131 has a higher binding affinity for CRP, and lower affinity for IgG2, and therefore is associated with reduced pathogen clearance and a potentially protective effect against the development of autoimmunity. Although there is no population stratification within North America and Europe, significant variations in allele frequency have been noted in Asia and Africa suggesting an adaptation mechanism due to selection pressures within geographically distinct populations[30].

Dietary heme iron directly injures colonic surface epithelium by generating cytotoxic and oxidative stress. Alteration in gut mucosal barrier function related to heme appears to be dependent on the presence of sulfide-producing and mucin-degrading bacteria (e.g. *Akkermansia*)[31]. In mice, increased dietary heme intake is associated with significant changes in gut microbial composition with an increased ratio of Gram-negative to Gram-positive bacteria. This effect is primarily driven by increased abundance of the Gram-negative species, including *Bacteroides* and *Akkermansia* leading to significant increase in lipopolysaccharide production[32]. Interestingly, recent data suggest that pro-inflammatory cytokine production by human macrophages appears to be dependent on the cross talk between toll-like receptors (TLRs) and FcγRIIA in the pro-inflammatory setting of rheumatoid arthritis[33]. TLR receptors play a central role in development of both immune tolerance and autoimmunity through their interaction with the external environment (e.g. commensal bacteria) and immune function (e.g. FcγRIIA). Therefore, it is plausible that dietary heme, through effects on commensal bacteria and intestinal barrier function, may preferentially increase the risk of UC among individuals with genetic predisposition (rs1801274-G/G) while exerting a protective effect on others (rs1801274-A/A) by promoting immune education. Similar mechanisms have recently been proposed for the central role of TLR activation in immunity versus tolerance in diabetes[34].

Lastly, the observed association between red meat intake and risk of UC is likely independent of dietary heme intake as simultaneous adjustment for dietary heme did not significantly alter the effect estimates. In addition, the association of red meat with UC risk was not modified by the UC susceptibility locus rs1801274, suggesting that it is unlikely that the association is fully mediated by heme content. One plausible alternative explanation is the higher content of animal protein in red meat. Previous studies have demonstrated that higher animal protein intake may increase luminal sphingosine-1-phosphate, which is associated with increased risk of UC[35–37]. In addition, animal studies of DSS-induced colitis as well as human observational studies suggest a possible role of red meat intake in worsening colitis[38, 39]. Nevertheless, the exact mechanism by which red meat intake may increase risk and progression of UC remains largely unknown and future studies to better elucidate the potential biologic mechanism are warranted.

Our study has a number of strengths including prospective collection of updated and validated dietary data, long-term follow up, and availability of genetic information in a subset of participants allowing us to examine biologically plausible gene-environment interactions that enhance the likelihood that our findings are causal. We acknowledge several limitations. First, our observed associations may have been influenced by measurement errors arising from FFQs to assess diet. Second, our analyses of gene-environment interaction may have been prone to Type II error based on our stringent Bonferroni-corrected p value. We also acknowledge that statistical interaction does not always translate into biologic effect. However, our correction for multiple testing and the biologically plausibility of our finding minimizes the likelihood of a false positive result. Nevertheless, future large-scale studies examining the interaction between dietary heme iron and variants in *FcγRIIA* gene are needed to confirm our findings. Finally, we emphasize that these epidemiologic findings are insufficient to inform dietary recommendations in patients with established disease. Specifically, whether our findings could also apply to the role of dietary heme iron on UC progression require further investigation and should be the topic of future studies.

In conclusion, we show that dietary heme iron intake is associated with risk of UC. The association is modified by presence of a functional coding variant in *FcγRIIA* (rs1801274) further highlighting the complex and intriguing interaction between diet, host genetics, and immune function on risk of IBD. These results suggest the need for future translational studies focused on the intersection of dietary heme iron, the gut microbiome, host genetics, and immune function to better elucidate biological mechanisms underlying this complex interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Zimmermann MB, Chassard C, Rohner F, et al. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. Am J Clin Nutr. 2010; 92:1406–15. [PubMed: 20962160]
2. Werner T, Wagner SJ, Martinez I, et al. Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. Gut. 2011; 60:325–33. [PubMed: 21076126]
3. Young GP, Rose IS, St John DJ. Haem in the gut. I. Fate of haemoproteins and the absorption of haem. J Gastroenterol Hepatol. 1989; 4:537–45. [PubMed: 2491221]
4. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012; 491:119–24. [PubMed: 23128233]
5. Hunter DJ. Gene-environment interactions in human diseases. Nat Rev Genet. 2005; 6:287–98. [PubMed: 15803198]
6. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. American journal of epidemiology. 1992; 135:1114–26. discussion 1127–36. [PubMed: 1632423]
7. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. American journal of epidemiology. 1985; 122:51–65. [PubMed: 4014201]
8. Willett, W. Nutritional epidemiology. New York: Oxford University Press; 1998.
9. Willett WC, Sampson L, Browne ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. American journal of epidemiology. 1988; 127:188–99. [PubMed: 3337073]
10. Khalili H, Ananthakrishnan AN, Konijeti GG, et al. Physical activity and risk of inflammatory bowel disease: prospective study from the Nurses' Health Study cohorts. BMJ. 2013; 347:f6633. [PubMed: 24231178]
11. Khalili H, Huang ES, Ananthakrishnan AN, et al. Geographical variation and incidence of inflammatory bowel disease among US women. Gut. 2012; 61:1686–92. [PubMed: 22241842]
12. Hunter DJ, Manson JE, Colditz GA, et al. Reproducibility of oral contraceptive histories and validity of hormone composition reported in a cohort of US women. Contraception. 1997; 56:373–8. [PubMed: 9494771]
13. Troy LM, Hunter DJ, Manson JE, et al. The validity of recalled weight among younger women. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1995; 19:570–2.
14. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. International journal of epidemiology. 1994; 23:991–9. [PubMed: 7860180]
15. Khalili H, Higuchi LM, Ananthakrishnan AN, et al. Oral contraceptives, reproductive factors and risk of inflammatory bowel disease. Gut. 2012
16. Loftus EV Jr, Silverstein MD, Sandborn WJ, et al. Crohn's disease in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. Gastroenterology. 1998; 114:1161–8. [PubMed: 9609752]
17. Loftus EV Jr, Silverstein MD, Sandborn WJ, et al. Ulcerative colitis in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. Gut. 2000; 46:336–43. [PubMed: 10673294]
18. Fonager K, Sorensen HT, Rasmussen SN, et al. Assessment of the diagnoses of Crohn's disease and ulcerative colitis in a Danish hospital information system. Scandinavian journal of gastroenterology. 1996; 31:154–9. [PubMed: 8658038]
19. Moum B, Vatn MH, Ekbom A, et al. Incidence of inflammatory bowel disease in southeastern Norway: evaluation of methods after 1 year of registration. Southeastern Norway IBD Study Group of Gastroenterologists. Digestion. 1995; 56:377–81. [PubMed: 8549880]
20. Hu FB, Willett WC, Li T, et al. Adiposity as compared with physical activity in predicting mortality among women. The New England journal of medicine. 2004; 351:2694–703. [PubMed: 15616204]

21. Cho E, Chen WY, Hunter DJ, et al. Red meat intake and risk of breast cancer among premenopausal women. Archives of internal medicine. 2006; 166:2253–9. [PubMed: 17101944]
22. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. Gastroenterology. 2013; 145:970–7. [PubMed: 23912083]
23. Hankinson SE. Circulating levels of sex steroids and prolactin in premenopausal women and risk of breast cancer. Advances in experimental medicine and biology. 2008; 617:161–9. [PubMed: 18497040]
24. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer research. 2006; 66:2476–82. [PubMed: 16489055]
25. Garcia-Albeniz X, Nan H, Valeri L, et al. Phenotypic and tumor molecular characterization of colorectal cancer in relation to a susceptibility SMAD7 variant associated with survival. Carcinogenesis. 2013; 34:292–8. [PubMed: 23104301]
26. Ackerman, ME., Nimmerjahn, F. Antibody Fc : linking adaptive and innate immunity. Amsterdam ; Burlington: Elsevier/Academic Press; 2014.
27. Bharadwaj D, Stein MP, Volzer M, et al. The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med. 1999; 190:585–90. [PubMed: 10449529]
28. Stein MP, Edberg JC, Kimberly RP, et al. C-reactive protein binding to FcgammaRIIa on human monocytes and neutrophils is allele-specific. J Clin Invest. 2000; 105:369–76. [PubMed: 10675363]
29. Salmon JE, Edberg JC, Brogle NL, et al. Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. J Clin Invest. 1992; 89:1274–81. [PubMed: 1532589]
30. van der Pol WL, Jansen MD, Sluiter WJ, et al. Evidence for non-random distribution of Fcgamma receptor genotype combinations. Immunogenetics. 2003; 55:240–6. [PubMed: 12830330]
31. Ijssennagger N, Belzer C, Hooiveld GJ, et al. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. Proc Natl Acad Sci U S A. 2015; 112:10038–43. [PubMed: 26216954]
32. N IJ, Derrien M, van Doorn GM, et al. Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk. PLoS One. 2012; 7:e49868. [PubMed: 23239972]
33. Vogelpoel LT, Hansen IS, Rispens T, et al. Fc gamma receptor-TLR cross-talk elicits pro-inflammatory cytokine production by human M2 macrophages. Nat Commun. 2014; 5:5444. [PubMed: 25392121]
34. Gulden E, Wen L. Toll-Like Receptor Activation in Immunity vs. Tolerance in Autoimmune Diabetes. Front Immunol. 2014; 5:119. [PubMed: 24715890]
35. Degagne E, Pandurangan A, Bandhuvula P, et al. Sphingosine-1-phosphate lyase downregulation promotes colon carcinogenesis through STAT3-activated microRNAs. J Clin Invest. 2014; 124:5368–84. [PubMed: 25347472]
36. Jantchou P, Morois S, Clavel-Chapelon F, et al. Animal protein intake and risk of inflammatory bowel disease: The E3N prospective study. Am J Gastroenterol. 2010; 105:2195–201. [PubMed: 20461067]
37. Liang J, Nagahashi M, Kim EY, et al. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. Cancer Cell. 2013; 23:107–20. [PubMed: 23273921]
38. Jowett SL, Seal CJ, Pearce MS, et al. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut. 2004; 53:1479–84. [PubMed: 15361498]
39. Le Leu RK, Young GP, Hu Y, et al. Dietary red meat aggravates dextran sulfate sodium-induced colitis in mice whereas resistant starch attenuates inflammation. Dig Dis Sci. 2013; 58:3475–82. [PubMed: 23990000]

Table 1

Baseline Characteristics of Participants According to Quintiles of Total Dietary Iron Intake[†]

Mean (std), mg/day	**Q1 N = 29,548 9 (3)**	**Q2 N = 40,438 12 (3)**	**Q3 N = 27,643 14 (4)**	**Q4 N = 34,564 20 (7)**	**Q5 N = 33,136 51 (29)**
Age (yrs), mean (std)	42 (9)	44 (9)	41 (9)	43 (10)	42 (10)
Body mass index (kg/m^2), mean (std)	25 (5)	25 (5)	25 (5)	25 (5)	24 (5)
Smoking					
Never	53	52	59	58	59
Past	23	28	26	28	28
Current	24	20	15	14	13
Latitude of residence, %					
Southern latitude	15	13	13	13	13
Appendectomy, %	18	21	18	20	19
Pre-menopause, %	73	65	76	67	71
Menopausal hormone therapy,%[§]					
Never	54	54	49	50	47
Past	19	20	20	20	20
Current	27	26	31	30	33
Ever use of oral contraceptives, %	71	66	74	67	68
Regular use of NSAIDs, %	13	11	14	13	12
Fiber intake (g/day), mean (std)	14 (4)	16 (4)	19 (5)	19 (5)	19 (6)
Heme intake (g/day), mean (std)	1.0 (0.4)	1.2 (0.5)	1.2 (0.6)	1.2 (0.6)	1.1 (0.5)

[†]Values are means (SD) or percentages and are standardized to the age distribution of the study population. All variables are derived from baseline questionnaires (1984 in NHS and 1991 in NHSII) with the exception of geographic location (1992 in NHS and 1993 in NHSII), and appendectomy (baseline in NHS and 1995 in NHS II).

[§]Percentages among postmenopausal women.

Table 2

Cumulative Average of Dietary Heme and Iron Intake and Risk of Crohn's Disease and Ulcerative Colitis *

	Q1	Q2	Q3	Q4	Q5	P_{trend}
Dietary Heme Intake						
Crohn's disease						
Cases/person-years	53/601,846	48/626,626	53/616,246	55/637,295	52/556,036	
Age-adjusted HR, 95% CI	1.00	0.86 (0.58–1.27)	0.97 (0.66–1.42)	0.98 (0.67–1.43)	1.07 (0.73–1.57)	0.55
MV-adjusted HR, 95% CI§	1.00	0.78 (0.53–1.16)	0.87 (0.59–1.29)	0.87 (0.59–1.28)	0.91 (0.61–1.35)	0.85
Ulcerative colitis						
Cases/person-years	56/601,846	59/626,626	70/616,246	74/637,295	62/556,036	
Age-adjusted HR, 95% CI	1.00	1.01 (0.70–1.46)	1.20 (0.84–1.71)	1.26 (0.89–1.78)	1.22 (0.85–1.75)	0.13
MV-adjusted HR, 95% CI§	1.00	1.01 (0.69–1.45)	1.20 (0.84–1.71)	1.27 (0.88–1.81)	1.23 (0.85–1.80)	0.12
Dietary Iron Intake						
Crohn's disease						
Cases/person-years	51/530,432	53/625,449	45/626,809	51/650,950	61/604,410	
Age-adjusted HR, 95% CI	1.00	0.90 (0.61–1.32)	0.74 (0.49–1.10)	0.82 (0.55–1.21)	1.04 (0.71–1.51)	0.96
MV-adjusted HR, 95% CI§	1.00	0.97 (0.65–1.44)	0.82 (0.54–1.26)	0.92 (0.60–1.39)	1.15 (0.76–1.74)	0.67
Ulcerative colitis						
Cases/person-years	52/530,432	64/625,449	66/626,809	74/650,950	61/604,410	
Age-adjusted HR, 95% CI	1.00	1.07 (0.74–1.55)	1.07 (0.74–1.54)	1.17 (0.82–1.67)	1.11 (0.77–1.61)	0.46
MV-adjusted HR, 95% CI§	1.00	1.12 (0.77–1.63)	1.13 (0.77–1.65)	1.25 (0.85–1.83)	1.18 (0.79–1.76)	0.35

*Abbreviations: confidence interval (CI), quintiles (Q), and standard deviation (std).

§Models adjusted for age (months), smoking (never, past, current), body mass index at baseline (< 20,20–24.9, 25–29.9, ≥ 30 kg/m^2), oral contraceptive use (ever, never), menopausal hormone therapy (never, past, current, premenopause), appendectomy (no, yes), geographic latitude of residence at age 30 (southern, middle, northern, missing/unknown), updated physical activity (quintiles), cohorts (NHS, NHSII), NSAID's use (< 2 tablets/week, ≥ 2 tablets/week), updated fiber intake (quintiles), and total caloric intake.

Table 3

Dietary Red and Processed Meat Intake and Risk of Crohn's Disease and Ulcerative Colitis*

	Q1	Q2	Q3	Q4	Q5	P_{trend}
Red Meat Intake						
Crohn's disease						
Cases/person-years	55/571,487	42/621,342	56/603,157	66/708,647	41/569,491	
Age-adjusted HR, 95% CI	1.00	0.70 (0.47–1.06)	0.95 (0.65–1.38)	0.99 (0.69–1.42)	0.76 (0.50–1.13)	0.69
MV-adjusted HR, 95% CI§	1.00	0.65 (0.43–0.98)	0.86 (0.58–1.26)	0.88 (0.60–1.28)	0.68 (0.44–1.05)	0.40
Ulcerative colitis						
Cases/person-years	52/571,487	58/621,342	55/603,157	102/708,647	52/569,491	
Age-adjusted HR, 95% CI	1.00	1.02 (0.70–1.48)	1.09 (0.74–1.59)	1.54 (1.10–2.16)	1.03 (0.70–1.51)	0.14
MV-adjusted HR, 95% CI§	1.00	1.02 (0.70–1.49)	1.11 (0.75–1.63)	1.58 (1.11–2.25)	1.10 (0.72–1.65)	0.08
Processed Meat Intake						
Crohn's disease						
Cases/person-years	46/627,926	54/588,753	60/699,925	55/601,245	45/556,276	
Age-adjusted HR, 95% CI	1.00	1.17 (0.78–1.74)	1.17 (0.80–1.73)	1.22 (0.82–1.81)	1.08 (0.71–1.63)	0.67
MV-adjusted HR, 95% CI§	1.00	1.10 (0.73–1.64)	1.08 (0.73–1.61)	1.10 (0.73–1.67)	0.99 (0.64–1.54)	0.99
Ulcerative colitis						
Cases/person-years	70/627,926	59/588,753	76/699,925	52/601,245	62/556,276	
Age-adjusted HR, 95% CI	1.00	0.97 (0.70–1.39)	1.00 (0.72–1.38)	0.83 (0.58–1.20)	1.09 (0.77–1.55)	0.95
MV-adjusted HR, 95% CI§	1.00	0.95 (0.66–1.37)	0.98 (0.70–1.38)	0.83 (0.57–1.21)	1.12 (0.77–1.62)	0.86

*Abbreviations: confidence interval (CI), quintiles (Q), and standard deviation (std).

§Models adjusted for age (months), smoking (never, past, current), body mass index at baseline (< 20,20–24.9, 25–29.9, ≥ 30 kg/m^2), oral contraceptive use (ever, never), menopausal hormone therapy (never, past, current, premenopause), appendectomy (no, yes), geographic latitude of residence at age 30 (southern, middle, northern, missing/unknown), updated physical activity (quintiles), cohorts (NHS, NHSII), NSAID's use (< 2 tablets/week, ≥ 2 tablets/week), updated fiber intake (quintiles), and total caloric intake.

Table 4

Association Between Dietary Total and Heme Iron Intake and Risk of Crohn's and Ulcerative Colitis According to Genetic Susceptibility*

		Control (n=693)	Crohn's disease (n=161)	Ulcerative colitis (n= 185)
Entire Cohort (NHS + NHSII)				
Dietary Iron Intake				
RS1801274 (*FcγRIIA*)	GG (n = 205)	1.00	0.98 (0.70–1.38)	0.91 (0.66–1.26)
	GA (n = 391)	1.00	1.01 (0.83–1.22)	1.04 (0.89–1.21)
	AA (n = 227)	1.00	1.06 (0.83–1.33)	1.00 (0.80–1.25)
	$P_{interaction}$§		0.68	0.65
Dietary Heme Iron Intake				
RS1801274 (*FcγRIIA*)	GG (n = 205)	1.00	0.55 (0.17–1.80)	0.11 (0.03–0.37)
	GA (n = 391)	1.00	0.61 (0.28–1.29)	1.27 (0.65–2.50)
	AA (n = 227)	1.00	1.22 (0.45–3.34)	2.76 (1.02–2.78)
	$P_{interaction}$§		0.40	**7.00E-05**

*Odds ratios are calculated for every 1 g increase in dietary total or heme iron intake.

§Models are adjusted for age (years), ancestry (Scandinavian, southern European/Mediterranean, others), smoking (never, past, current), body mass index at baseline (< 20,20–24.9, 25–29.9, ≥ 30 kg/m^2, cumulative average of physical activity (MET-hr/wk), cohorts (NHS, NHSII), cumulative average of fiber intake (g/day), and total caloric intake.