# Dietary Iron and Recurrence of Colorectal Adenomas[1]

**Marilyn Tseng, Robert S. Sandler,[2] E. Robert Greenberg, Jack S. Mandel, Robert W. Haile, and John A. Baron**

Department of Epidemiology [M. T.], Center for Gastrointestinal Biology and Disease [M. T., R. S. S.], Division of Digestive Diseases and Nutrition, Department of Medicine [R. S. S.], University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; Departments of Medicine and Community and Family Medicine and Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, New Hampshire 03755 [E. R. G., J. A. B]; Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455 [J. S. M.]; and Department of Preventive Medicine, School of Medicine, University of Southern California, Los Angeles, California 90033 [R. W. H.]

## Abstract

**Previous research suggests that iron acts as a prooxidant to increase the risk of colorectal neoplasia. This study examined effects of dietary intake of iron on colorectal adenoma recurrence using data from an antioxidant clinical trial. All subjects were free of polyps at study entry but had at least one adenoma removed within the 3 months before enrollment. Follow-up colonoscopies were conducted after 1 and 4 years. Patients who developed one or more adenomatous polyps between years 1 and 4 were classified as cases; all others were controls. Dietary iron intake at baseline and at the end of the study was estimated from self-administered food frequency questionnaires and averaged together for each subject, energy-adjusted, and categorized into quartiles. Odds ratios were adjusted for age, center, sex, calories, treatment group, and alcohol, fiber, folate, and fat intakes in unconditional logistic regression analysis. Dietary iron was inversely associated with adenoma risk, although risk did not decrease monotonically with increasing intake. Odds ratios comparing second, third, and fourth quartiles to the lowest quartile were 0.61 [95% confidence interval (CI), 0.37–1.02], 0.80 (95% CI, 0.45–1.44), and 0.37 (95% CI, 0.19–0.73), respectively. A limited examination showed no clear evidence that use of iron supplements affected risk of recurrence in this study population. This study provides evidence against the hypothesis that recent dietary intake of iron increases risk for colorectal adenomas. However, these results may reflect the presence of other dietary factors found in combination with iron.**

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[2] To whom requests for reprints should be addressed, at Division of Digestive Diseases and Nutrition, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

## Introduction

Considerable evidence exists for a role of oxidation in carcinogenesis, mediated primarily by highly reactive oxygen radicals and free radicals that cause oxidative damage to DNA (1). It is unclear, however, the extent to which dietary factors with presumed anti- and prooxidant activity play a part in human cancer development. Antioxidants are thought to scavenge free radicals by reacting with them to form less reactive products, thus protecting against oxidative damage; some also act to help regenerate other reduced antioxidants (1). Prooxidants, on the other hand, generate free radicals. Dietary prooxidants have received less attention than antioxidants in epidemiological studies, but iron has been suggested as a harmful prooxidant. The prooxidant activity of iron stems from its catalysis of the Haber-Weiss cycle of reactions in which ferric iron is reduced but subsequently regenerated, accompanied by the formation of hydroxy radicals (2).

Numerous studies have examined the association of iron with colorectal neoplasia, with varying results. Indirect support for the hypothesized role of prooxidant iron on colorectal neoplasia has come from studies showing higher risk associated with intake of iron-rich red meat (3, 4, 5, 6). Studies on iron intake and neoplasia (7, 8, 9, 10, 11, 12, 13), however, have not been consistent, although several have found an increased risk of colorectal cancer (14, 15, 16, 17) or adenoma (18, 19) with greater iron stores and/or intake. This report describes the association of recent dietary iron intake with colorectal adenoma recurrence using data from a multicenter antioxidant clinical trial.

## Materials and Methods

**Study Population.** Subjects for these analyses were participants in the Antioxidant Polyp Prevention Study, a multicenter clinical trial of antioxidant supplementation. Patients were identified between December 1984 and June 1988 from colonoscopy reports and pathology logs at six centers: Cleveland Clinic (Cleveland, OH); Dartmouth-Hitchcock Medical Center (Lebanon, NH); Lahey Clinic Medical Center (Burlington, MA); UCLA/Kaiser Sunset (Los Angeles, CA); the University of Iowa (Iowa City, IA); and the University of Minnesota (Minneapolis, MN).

All eligible patients had at least one histologically confirmed adenoma removed within the 3 months before study entry and were judged to be free of further polyps based on complete colonoscopy prior to enrollment. Subjects also had to be in good health and under 80 years old. Patients were excluded if they had familial polyposis, a history of invasive colorectal cancer, any malabsorption syndrome, or any conditions that might be worsened by vitamin C or E supplementation.

Participants in the clinical trial gave informed consent and were randomly assigned using a two-by-two factorial design to four treatment groups: β-carotene plus placebo, vitamins C and E plus placebo, β-carotene plus vitamins C and E, and placebo only. Two complete follow-up colonoscopies were conducted

approximately 1 and 4 years after the qualifying colonoscopy. At these follow-up exams, the size and location of any raised mucosal lesions were recorded, all lesions were excised, and microscopical slides were sent for review to the study pathologist for classification as either neoplastic (adenoma) or nonneoplastic (hyperplastic polyp, lymphoid follicle, or other type of lesion). For these analyses, patients found to have one or more adenomatous polyps during the interval after the 1-year exam up to and including the 4-year exam were classified as cases, whereas those without adenomatous polyps were classified as controls.

**Data Collection.** Information on diet was collected using the Health Habits and History Questionnaire, a semiquantitative food frequency questionnaire developed at the National Cancer Institute (20). The questionnaire was self-administered and was given to patients twice: once before randomization, then again at the end of the study. Each time, subjects reported their intake of over 100 foods believed to contribute importantly to the intake of calories and 17 macro- and micronutrients in the national diet (21, 22). For each food item, subjects estimated their frequency of intake over the previous year and whether their usual portion size was small, medium, or large. Average nutrient intakes per day at baseline and at the end of the study were then calculated based on portion size, nutrient content, and frequency of consumption of each food using the Dietary Analysis System developed for use with the Health Habits and History Questionnaire. Patients were excluded from analysis if their responses to the questionnaire were deemed unreliable: specifically, if they reported eating fewer than three foods a day, skipped more than 50 foods in the questionnaire, or reported daily caloric intake less than 500 or greater than 5000 kcals. These calculations included intake from dietary sources only and did not include nutrients derived from supplements. In addition, because red meat may be a proxy for bioavailable iron and has been associated with increased risk in previous studies, a variable representing weekly frequency of red meat consumption was created based on reported consumption of hamburger, beef, beef stew, pork, veal/lamb, chili con carne, and mixed dishes with meat.

Iron intake during the period of the study was estimated by averaging together mean daily intake at baseline and at the end of the study. We chose to focus our analyses on average rather than on baseline intake because we felt that the average would better represent intake during the exposure period for development of polyps. However, analyses based on baseline intake produced results that were similar to those for the average intake.

Interval questionnaires were sent to patients every 6 months during the trial and elicited information on their compliance with the study treatment, hospitalizations, and symptoms, and use of aspirin and various nutritional supplements. Other detailed information on supplement intake was obtained from a self-administered questionnaire given at enrollment, in which patients were asked whether they had ever taken various vitamin or mineral supplements, how long they had taken them, and their last year of use.

**Data Analysis.** Average nutrient intakes were energy-adjusted using the linear regression techniques of Willett and Stampfer (23) and were categorized into quartiles; ORs[3] were calculated relative to the lowest quartile. We used unconditional logistic regression to estimate ORs and 95% CI, adjusting for potential confounders. Covariates included age, sex, center, treatment group, caloric intake, and energy-adjusted intakes of alcohol, fiber, folate, and total fat, with alcohol, fiber, folate, and fat intakes categorized into quartiles. We also considered number of months between the 1-year and 4-year follow-up colonoscopies, body mass index, aspirin, any vitamin or mineral supplement use during the study, and family history of colorectal cancer as potential confounders. However, because removing them from the model did not substantially change parameter estimates for any of the nutrients of interest, results are presented for models not including these covariates. We classified individuals as users of aspirin during the study if they reported taking aspirin on at least 50% of their returned interval questionnaires. Use of any vitamin or mineral supplements during the study was defined similarly. Average number of servings of red meat per week was also categorized into quartiles, with ORs calculated relative to the lowest quartile and adjusted for the same covariates.

To test for linear trend, we included a variable representing the scaled median value for each quartile of residual intake into the multivariate model, including the same covariates listed above. The effects of dietary iron did not appear to differ between those receiving either study treatment (either vitamin C and E supplementation or $\beta$-carotene supplementation). Therefore, results are presented for all study participants combined.

To examine the association between iron supplement use and adenoma recurrence, individuals were classified as users of iron supplements if they reported taking them at baseline and if their reported last year of use was the same year that they completed the questionnaire. Individuals were also classified based on length of use of iron supplements at any time before the study. Because of the small number of iron supplement users, ORs were estimated relative to nonusers by including the variable in a logistic regression model adjusted only for age and center.

## Results

Data were reviewed for 2029 potentially eligible patients, of whom 387 were found to be ineligible for the trial, 502 refused participation, 152 could not be contacted, and 7 did not enroll in the clinical trial for unknown reasons. The remaining 981 agreed to participate, and 864 were actually randomized. Ultimately, 751 of these underwent both follow-up colonoscopies, and 666 provided satisfactory data on both dietary questionnaires.

Characteristics of the 247 cases and 419 controls were generally comparable with respect to age, race, education, body mass index, current smoking status, and family history of colon cancer (Table 1). A slightly larger proportion of cases were males, whereas controls tended to have fewer prior adenomas and were more likely to use aspirin and to take vitamin or mineral supplements during the period of study.

Contrary to expectation, dietary iron had a strong inverse association with adenoma recurrence, although ORs did not decrease monotonically with increasing iron intake (Table 2). OR comparing second and third quartiles to the lowest quartile were 0.58 (95% CI, 0.35–0.95) and 0.77 (95% CI, 0.44–1.33), respectively. Patients in the highest quartile of energy-adjusted iron intake were about one-third as likely to develop adenomas as those in the lowest quartile (OR, 0.35; 95% CI, 0.19–0.66). Red meat intake, on the other hand, showed little association with adenoma recurrence; after adjustment for other covariates,

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

*Table 1* Characteristics of study population by case-control status

| | Cases ($n$ = 247) | Controls ($n$ = 419) |
|---|---|---|
| Age (mean ± SD) | 61.9 ± 8.3 | 60.9 ± 8.3 |
| Sex (% male) | 81.8 | 76.4 |
| Race (% white) | 84.1 | 85.9 |
| Highest grade attended (% ≥12) | 41.1 | 44.6 |
| Body mass index (mean ± SD) | 27.2 ± 4.5 | 26.7 ± 3.7 |
| Center (%) | | |
| Cleveland Clinic | 18.6 | 16.7 |
| Dartmouth-Hitchcock Medical Center | 23.1 | 14.8 |
| University of Iowa | 16.6 | 16.5 |
| Lahey Clinic | 13.0 | 11.7 |
| UCLA | 19.0 | 21.2 |
| University of Minnesota | 9.7 | 19.1 |
| Current smoker (%) | 17.4 | 18.9 |
| Family history of colon cancer (%) | 19.4 | 22.2 |
| Number of prior adenomas | | |
| 1 | 33.5 | 50.2 |
| 2 | 21.1 | 22.8 |
| ≥3 | 45.5 | 26.9 |
| Aspirin use during study period | 17.4 | 24.1 |
| Any supplement use during study period | 17.4 | 24.1 |

the OR comparing highest to lowest quartiles of weekly red meat intake was 1.26 (95% CI, 0.72–2.21).

We also examined the association between adenoma recurrence and use of iron supplements prior to the study. The OR for adenoma recurrence for use of iron supplements at baseline relative to nonuse was 1.65 (95% CI, 0.68–4.03). However, the estimate was based on only 21 individuals reporting iron supplement use at baseline. Furthermore, 8 of the 21 reported using iron supplements for only 1 year. When patients were categorized based on their length of use of iron supplements, the OR for using iron supplements regularly for 2 years or more at any time before the study was 1.25 (95% CI, 0.58–2.68).

## Discussion

Results from these analyses provide no support for the hypothesis that dietary iron increases risk of adenoma recurrence. Indeed, dietary iron was inversely associated with risk. The use of iron supplements prior to the study or at baseline appeared to be positively associated with recurrence of adenomas, although estimates were based on small numbers and did not reach conventional levels of statistical significance.

Several case-control studies have also found no association or even a slightly inverse association between dietary iron and colorectal adenomas (7, 8, 9, 10). In contrast, most studies of invasive colorectal cancer risk (11, 14, 15, 17), but not all (13), have suggested a positive association with iron intake. Research based on biochemical indicators of iron status, however, have consistently found a positive association between elevated iron status and either colorectal adenomas (18, 19) or cancer (14, 15, 16).

The discrepancy between findings based on biochemical, as opposed to dietary, measures of iron status suggest that the two are not equivalent indicators of iron exposure. For example, a high intake of iron may not increase risk if the consumed iron is either not absorbed or not bioavailable in the lumen. Iron in foods may have low bioavailability, limiting its ability to catalyze free radical-generating reactions. Graf and Eaton (24) has suggested that compounds in dietary fiber, such as phytic acid, can chelate iron, thus inhibiting the production of hydroxy radicals.

*Table 2* Unadjusted and multivariate-adjusted OR and 95% CI by quartile of energy-adjusted iron intake

| Quartile | Median (mg) | Unadjusted OR (95% CI) | Multivariate-adjusted OR[a] (95% CI) |
|---|---|---|---|
| 1 | 11.0 | 1.00 | 1.00 |
| 2 | 12.3 | 0.55 (0.35–0.85) | 0.61 (0.37–1.02) |
| 3 | 14.2 | 0.64 (0.41–0.99) | 0.80 (0.45–1.44) |
| 4 | 20.9 | 0.32 (0.20–0.51) | 0.37 (0.19–0.73) |
| $P$ for trend | | 0.0001 | 0.005 |

[a] Data adjusted for age, sex, center, treatment group, caloric intake, and energy-adjusted intakes of alcohol, fiber, folate, and total fat.

In our study population, iron intake was strongly correlated with intake of dietary fiber (Pearson's $r$ = 0.70) but not with intake of red meat (Pearson's $r$ = 0.24). This suggests that the major contributors to total iron intake were plant sources such as cereals and grains, rather than red meat, and that iron bioavailability in our study population was low. Indeed, the level of red meat intake in this population was fairly low overall; mean frequency of red meat intake ranged from 0.2 servings per week in the lowest quartile to 1.4 servings per week in the highest quartile. The generally low intake of red meat in this population may explain why we found little association between red meat and adenoma recurrence, whereas others have found an association of red meat with colorectal adenomas (3–5) or colorectal cancer (6).

In a case-control study on iron and colorectal adenomas conducted by Bird *et al.* (19), total iron intake, including iron from both the diet and supplements, was positively associated with adenomas. However, the positive association disappeared when the analyses were focused on iron from food only, leading the authors of the study to suggest that iron increases risk primarily when consumed in supplements: apart from the regular diet and in large doses. In contrast, Tseng *et al.* (11) found no association between total iron intake and colorectal adenomas after adjustment for other micronutrients. Our data indicated that use of iron supplements at baseline, or use for at least two years before enrollment, was slightly (and nonsignificantly) associated with adenoma recurrence. However, very few patients reported using iron supplements at enrollment, and almost one-half of all ever-users of iron supplements had taken them for only 1 year. Also, no information was available on the regularity or doses of supplement use. Nevertheless, in light of our findings and others' (19), diet- and supplement-derived iron may most usefully be analyzed separately in future research.

The association between dietary iron and recurrence of adenomas in this study may have been confounded by other dietary or lifestyle factors. For example, the high correlation with fiber intake suggests that iron may have been consumed with other components in the diet that exerted a protective effect in this population. Certainly, the effects of individual nutrients are not easily isolated in any observational analysis, especially in the presence of numerous other correlated dietary factors. Iron may also interact with other dietary factors, exerting different effects depending on the other nutrients with which it is consumed.

An alternative explanation for our findings is that symptoms caused some patients to change their dietary habits during the study, for example, by limiting their meat consumption. However, adenomas are generally asymptomatic. Also, comparison of changes in iron intake between baseline and at the end of the study indicated that mean iron intake increased similarly in cases and controls, from 13.7 to 14.8 mg/days in

cases and from 15.0 to 15.7 mg/days in controls. In addition, when analyses were based on intake measured at baseline only, results were similar.

Although we chose to examine the effects of usual dietary intake during the study period, it is also possible that earlier dietary habits are of greater etiological interest than recent diet, and that recent intake has little or no effect on polyp development or growth. If individuals at higher risk for adenoma recurrence were more likely to change their diets in response to their initial adenoma diagnosis, this could have led to lower intake of iron in the case population during the period of study, resulting in a misleading association between iron and adenoma recurrence. However, when we stratified on number of prior adenomas, we found no evidence that the observed association between iron and adenoma recurrence was the result of bias due to dietary change in those at presumably higher risk of recurrence, *i.e.*, those with a larger number of prior adenomas. Furthermore, for the majority of patients, even those with more than one prior adenoma, the qualifying exam was their first adenoma experience, thus also reducing the potential for bias. It should also be noted that the most appropriate time period for which to collect information on dietary intake is not known; at the very least, these analyses offer findings regarding short-term, recent intake of iron; the effects of long-term or more distant past intake warrant further investigation.

Findings from this study may be of limited generalizability, because participants in the intervention trial represent a highly selected group of individuals. For example, because only individuals with prior adenomas were eligible for participation in the trial, findings from the study relate only to risk of adenoma recurrence; risk factors for a first adenoma may be different. However, a strength of this study is that complete colonoscopies were performed for all study participants according to a uniform schedule, confirming the absence of adenomas in the control population and reducing misclassification of disease status. Using a uniform schedule for follow-up exams for all patients, thus avoiding incidental diagnoses, helped minimize the potential for biased detection of polyps.

Results from these analyses show an inverse association between recent dietary intake of iron and recurrence of adenomas. A limited examination showed no clear evidence that use of iron supplements affected risk of recurrence in this study population. Possible differences in effect between dietary and supplemental iron, measurement of iron intake incorporating information on bioavailability, and the effect of dietary iron intake from the more distant past are all issues that warrant attention in future studies. Food-level analyses and examination of dietary patterns may also prove to be more informative and more appropriate than nutrient-level analyses for purposes of intervention and prevention. Whatever approach is used, however, providing information that can be used practically for intervention remains an important objective for continued work in this area.

## References


1. Machlin, L. J., and Bendich, A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J., *1:* 441–445, 1987.

2. Nelson, R. L. Dietary iron and colorectal cancer risk. Free Radical Biol. Med., *12:* 161–168, 1992.

3. Kune, G. A., Kune, S., Read, A., MacGowan, K., Penfold, C., and Watson, L. F. Colorectal polyps, diet, alcohol, and family history of colorectal cancer: a case-control study. Nutr. Cancer, *16:* 25–30, 1991.

4. Giovannucci, E., Stampfer, M. J., Colditz, G., Rimm, E. B., and Willett, W. C. Relationship of diet to risk of colorectal adenoma in men. J. Natl. Cancer Inst., *84:* 91–98, 1992.

5. Neugut, A. I., Garbowski, G., Lee, W. C., Murray, T., Nieves, J. W., Forde, K. A., Treat, M. R., Waye, J. D., and Fenoglio-Preiser, C. Dietary risk factors for the incidence and recurrence of colorectal adenomatous polyps: a case-control study. Ann. Intern. Med., *118:* 91–95, 1993.

6. Giovannucci, E., Rimm, E. B., Stampfer, M. J., Colditz, G. A., Ascherio, A., and Willett, W. C. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res., *54:* 2390–2397, 1994.

7. Hoff, G., Moen, I. E., Trygg, K., Frolich, W., Sauar, J., Vatn, M., Gjone, E., and Larsen, S. Epidemiology of polyps in the rectum and sigmoid colon. Scand. J. Gastroenterol., *21:* 199–204, 1986.

8. Macquart-Moulin, G., Riboli, E., Cornee, J., Kaaks, R., and Berthezene, P. Colorectal polyps and diet: a case-control study in Marseilles. Int. J. Epidemiol., *40:* 179–188, 1987.

9. Little, J., Logan, R. F. A., Hawtin, P. G., Hardcastle, J. D., and Turner, I. D. Colorectal adenomas and diet: a case-control study of subjects participating in the Nottinham faecal occult blood screening programme. Br. J. Cancer, *67:* 177–184, 1993.

10. Benito, E., Cabeza, E., Moreno, V., Obrador, A., and Bosch, F. X. Diet and colorectal adenomas: a case-control study in Majorca. Int. J. Cancer, *55:* 213–219, 1993.

11. Tseng, M., Murray, S. C., Kupper, L. L., and Sandler, R. S. Micronutrients and risk of colorectal adenomas. Am. J. Epidemiol., *144:* 1005–1014, 1996.

12. Freudenheim, J. L., Graham, S., Marshall, J. R., Haughey, B. P., and Wilkinson, G. A case-control study of diet and rectal cancer in western New York. Am. J. Epidemiol., *131:* 612–624, 1990.

13. Benito, E., Stiggelbout, A., Bosch, F. X., Obrador, A., Kaldor, J., Mulet, M., and Munoz, N. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int. J. Cancer, *49:* 161–167, 1991.

14. Stevens, R. G., Jones, D. Y., Micozzi, M. S., and Taylor, P. R. Body iron stores and the risk of cancer. N. Engl. J. Med., *319:* 1047–1052, 1988.

15. Stevens, R. G., Graubard, B. I., Micozzi, M. S., Neriishi, K., and Blumberg, B. S. Moderate elevation of body iron level and increased risk of cancer occurrence and death. Int. J. Cancer, *56:* 364–369, 1994.

16. Knekt, P., Reunanen, A., Takkunen, H., Aromaa, A., Heliovaara, M., and Hakulinen, T. Body iron stores and risk of cancer. Int. J. Cancer, *56:* 379–382, 1994.

17. Wurzelmann, J. I., Silver, A., Schreinemachers, D. M., Sandler, R. S., and Everson, R. B. Iron intake and the risk of colorectal cancer. Cancer Epidemiol. Biomarkers Prev., *5:* 503–507, 1996.

18. Nelson, R. L., Davis, F. G., Sutter, E., Sobin, L. H., Kikendall, J. W., and Bowen, P. Body iron stores and risk of colonic neoplasia. J. Natl. Cancer Inst., *86:* 455–460, 1994.

19. Bird, C. L., Witte, J. S., Swendseid, M. E., Shikany, J. M., Hunt, I. F., Frankl, H. D., Lee, E. R., Longnecker, M. P., and Haile, R. W. Plasma ferritin, iron intake, and the risk of colorectal polyps. Am. J. Epidemiol., *144:* 34–41, 1996.

20. Block, G., Hartman, A. M., Dresser, C. M., Carroll, M. D., Gannon, J., and Gardner, L. A data-based approach to diet questionnaire design and testing. Am. J. Epidemiol., *124:* 453–469, 1986.

21. Block, G., Dresser, C. M., Hartman, A. M., and Carroll, M. D. Nutrient sources in the American diet: quantitative data from the NHANES II survey. I. Vitamins and minerals. Am. J. Epidemiol., *122:* 13–26, 1985.

22. Block, G., Dresser, C. M., Hartman, A. M., and Carroll, M. D. Nutrient sources in the American diet: quantitative data from the NHANES II survey. II. Macronutrients and fats. Am. J. Epidemiol., *122:* 27–40, 1985.

23. Willett, W., and Stampfer, M. Total energy intake: implications for epidemiologic analyses. Am. J. Epidemiol., *124:* 17–27, 1986.

24. Graf, E., and Eaton, J. W. Suppression of colonic cancer by dietary phytic acid. Nutr. Cancer, *19:* 11–19, 1993.