Heme and non-heme iron consumption and risk of gallstone disease in men[1–3]

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ABSTRACT

Background: Excessive iron intake can promote biliary cholesterol crystal formation in experimental studies. The absorption of heme iron is more complete than that of non-heme iron in humans; however, the effect of long-term consumption of heme and non-heme iron on the risk of gallstones is unknown.

Objective: The objective of the study was to examine long-term iron intake in relation to the occurrence of gallstone disease.

Design: We prospectively studied intakes of heme and non-heme iron and the risk of gallstone disease in a cohort of 44 758 US men from 1986 to 2002. Iron consumption was assessed by using a validated semiquantitative food-frequency questionnaire. Newly diagnosed gallstone disease was ascertained biennially.

Results: We documented 2468 incident cases of symptomatic gallstones during 597 699 person-years of follow-up. The age-adjusted relative risks (RRs) for men with intakes of heme iron and non-heme iron, when the highest and lowest quintiles were compared, were 1.21 (95% CI: 1.06, 1.37; *P* for trend = 0.0008) and 1.02 (95% CI: 0.90, 1.16; *P* for trend = 0.45), respectively. After adjustment for multiple potential confounding variables, when extreme quintiles were compared, the multivariate RR of heme iron intake was not significantly changed and remained significant with a dose-response relation (RR = 1.21; 95% CI: 1.03, 1.42; *P* for trend = 0.01), and that of non-heme iron intake was not significant (RR = 1.14; 95% CI: 0.99, 1.31; *P* for trend = 0.18).

Conclusion: Our findings suggest that a higher consumption of heme iron is associated with a greater risk of gallstone disease among men. *Am J Clin Nutr* 2007;85:518–22.

KEY WORDS Iron, gallstone, gallbladder, heme iron, non-heme iron, men

INTRODUCTION

Gallstone disease is very common in Western countries and increasingly is a major cause of abdominal morbidity (1). Approximately 80% of gallstones in Western populations are cholesterol stones (2). Many factors have been associated with the risk of cholesterol gallstones, but cholesterol-saturated bile is an important determinant of gallstone formation (2). High plasma triacylglycerol concentrations are associated with a greater risk of cholesterol gallstones (2).

The human body has a considerable capacity to store iron, but there is no regulated iron excretion in dietary iron overload (3). Homeostatic mechanisms increase iron absorption from the intestine in iron deficiency, but its down-regulation in high intakes of iron, particularly intakes of heme iron, is insufficient to prevent the accumulation of high iron stores (3, 4). Iron is the most abundant transition metal in the body because of its roles in oxygen binding and electron transport (5, 6). During adulthood, iron stores gradually increase almost linearly with age in men (3, 7). Dietary iron overload in adults may be of concern in the United States (8). Elevated iron stores, aside from primary and secondary pathologic forms of iron overload, may be harmful because of their associations with several chronic diseases, including the metabolic syndrome (9–13).

In experimental studies, a high iron diet can elevate plasma triacylglycerol concentrations (14). Iron is a prooxidant prone to produce reactive oxygen metabolites that may promote cholesterol crystal formation in the bile (15). The relation between iron intake and the molar percent cholesterol concentration in the bile in human and animal studies, however, is mixed (16, 17). The effect of long-term iron consumption on the incidence of gallstones in humans is unknown. In a large cohort of US men, we examined long-term iron intake in relation to the occurrence of gallstone disease.

SUBJECTS AND METHODS

Study population

The Health Professionals Follow-up Study began in 1986 when 51 529 US male dentists (58%), veterinarians (20%), optometrists (7%), osteopathic physicians (4%), and podiatrists (3%) aged 40–75 y returned a questionnaire by mail regarding diet, medication use, and medical history. The participants in this cohort are mainly white (>91%). Follow-up questionnaires have

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been sent every 2 y to update information on exposures and to ascertain the occurrence of newly diagnosed illnesses, including gallstone disease. Diet was assessed every 4 y. At baseline, we excluded men who reported a cholecystectomy or a diagnosis of gallstone disease, men with a reported daily energy intake outside the range of 800–4200 kcal/d, and men with ≥70 blank food items on the dietary questionnaire. Men with a diagnosis of cancer (other than nonmelanoma skin cancer), which can lead to changes of diet and lifestyle, were also excluded. After exclusions, the study population consisted of 44 758 men who were followed from 1986 to 2002. The average follow-up rate for biennial questionnaires was >94% in each 2-y follow-up cycle. The present study was approved by the institutional review board on the use of human subjects in research of the Brigham and Women's Hospital in Boston.

Assessment of dietary variables

Dietary information was derived from a 131-item semiquantitative food-frequency questionnaire (SFFQ) (18). The participants were asked to indicate the frequency, on average, of consuming a typical serving size of selected foods during the previous year. There were 9 options for respondents to choose from, ranging from never or less than once per month to ≥6 times/d. Nutrient scores were computed by multiplying the frequency of consumption of each unit of food from the SFFQ by the nutrient content of the specified portion according to food-composition tables from the US Department of Agriculture (19) supplemented with manufacturers' data. A full description of the SFFQ and the procedures used for calculating nutrient intake, as well as data on reproducibility and validity in this cohort, were reported previously (18, 20). The validity of the SFFQ was assessed in a random sample of 127 participants living in the Boston area. Nutrient intake from the food-frequency questionnaire was compared with two 1-wk diet records spaced ≈6 mo apart. Pearson correlation coefficients between the diet records and the dietary questionnaire were adjusted for total energy intake and for within-person variability in daily intake. The Pearson correlation coefficient between total iron intake assessed by using the 1986 questionnaire and total iron intake assessed by using two 1-wk dietary records was 0.54. In addition, heme iron consumption based on this SFFQ has been shown to be related to ferritin, a marker of body iron status, in men and women from the Framingham Heart Study (21) and in women from the Nurses' Health Study (22).

Assessment of non-dietary variables

The participants reported their body weight, cigarette smoking status, use of medications, and leisure-time physical activity every 2 y during the follow-up. The correlation coefficient between self-reported weight and measured weight was 0.96 (23). Physical activity was estimated by using the cumulative average number of hours per week on the basis of the reported time spent doing various activities. Each activity was weighted by its intensity level. The validity of self-reported physical activity in this cohort was reported previously (24).

Ascertainment of endpoints

The primary endpoint was incident symptomatic gallstones. In 1986 and on each follow-up questionnaire, the participants were asked whether they had undergone a cholecystectomy or had a gallstone diagnosis performed by a physician. The participants were also asked whether the gallstone diagnosis had been confirmed by radiographic procedures or surgery and whether their gallstones were symptomatic. To verify the self-reported symptomatic gallstone disease, a random sample of 441 self-reported diagnoses of cholecystectomy or gallstones were reviewed against medical records, and all but 1 of the 441 diagnoses was confirmed.

Statistical analysis

For each participant, follow-up time accrued from the month of return of the 1986 questionnaire in the analysis of iron intake and ended at the month of cholecystectomy, diagnosis of symptomatic gallstones, death, or the end of the study period, whichever occurred first. Men with asymptomatic gallstones or those whose gallstone diagnosis was not based on radiology or surgery and men with diagnosed cancer were excluded from subsequent follow-up. Thus, the eligible population at risk comprised only those who did not have gallstone disease and cancer at the beginning of each 2-y follow-up interval. We divided participants into 5 categories (quintiles) according to their cumulative iron intake. Incidence rates were calculated by dividing the number of events by person-years of follow-up in each category. Relative risks (RRs) were calculated as the incidence rate of gallstone disease among men in different categories of iron intake compared with the incidence rate among men in the lowest intake category, with adjustment for age.

We divided total iron intake into heme iron (found in animal products) and non-heme iron (derived from fortified cereals, plant-based food, and supplements) for separate analyses. The incidence of gallstone disease was examined in relation to the cumulative average of exposure variables from all available questionnaires up to the start of each 2-y follow-up interval to reduce within-subject variation and best represent long-term dietary intake (25). For example, the incidence of gallstone disease during the 1986 to 1990 time period was related to iron intake from the 1986 questionnaire, and the incidence of gallstone disease during the 1990 to 1994 time period was related to the average iron intake from the 1986 and 1990 questionnaires. RRs were computed by using the Cox proportional hazards regression model (26). In multivariate analyses, we simultaneously included intake of total energy and potential confounding covariates, including age, body mass index [calculated as weight (in kg)/height^2 (in m)], weight change during the past 2 y, cigarette smoking status, history of diabetes mellitus, physical activity, use of thiazide diuretics and non-steroidal antiinflammatory drugs, and intakes of alcohol, caffeine, dietary fiber, carbohydrate, protein, and saturated, polyunsaturated, and monounsaturated fats. Tests of linear trend across increasing categories were conducted by assigning the median value of exposure for each category and treating these as a single continuous variable. All RRs are presented with 95% CIs, and all reported P values are two-sided. All analyses were performed with Statistical Analysis System software, release 8.2 (SAS Institute, Cary, NC).

RESULTS

At baseline in 1986, men with a higher heme iron intake tended to be heavier, current smokers, and sedentary and consumed less fiber, alcohol, and carbohydrate but had higher intakes of protein, caffeine, saturated fat, and monounsaturated fat than did men

TABLE 1
Baseline characteristics of 44 758 US men by quintile of heme iron and non-heme iron intake in 1986: the Health Professionals Follow-up Study[1]

	Quintiles					
Characteristic	1 (lowest)	2	3	4	5 (highest)	*P* for trend[2]
Heme iron						
No. of participants (*n*)	10 484	7163	10 671	8083	8357	—
Age (y)	53.3 ± 9.7[3]	52.9 ± 9.3	52.6 ± 9.3	52.9 ± 9.3	52.8 ± 9.1	<0.001
BMI (kg/m^2)	24.3 ± 4.5	24.6 ± 4.7	24.9 ± 4.9	25.2 ± 5.0	25.5 ± 5.3	<0.001
Physical activity (METs)[4]	23.8 ± 28.1	21.1 ± 25.1	19.6 ± 24.3	18.8 ± 24.8	16.4 ± 23.0	<0.001
Current smoker (%)	1.4	1.3	2.2	1.9	2.3	<0.001
Mean daily intake						
Carbohydrate (g)	268 ± 42	246 ± 34	234 ± 32	220 ± 30	199 ± 33	<0.001
Protein (g)	81 ± 14	88 ± 13	92 ± 13	97 ± 13	106 ± 16	<0.001
Alcohol (g)	11 ± 16	12 ± 16	11 ± 15	11 ± 14	10 ± 13	<0.001
Caffeine (mg)	208 ± 241	235 ± 244	246 ± 247	257 ± 251	276 ± 273	<0.001
Saturated fat (g)	21 ± 6	23 ± 5	24 ± 5	26 ± 5	28 ± 5	<0.001
Monounsaturated fat (g)	24 ± 6	26 ± 5	27 ± 5	29 ± 4	31 ± 5	<0.001
Dietary fiber (g)	24 ± 8	22 ± 6	21 ± 6	20 ± 5	18 ± 5	<0.001
Non-heme iron						
No. of participants (*n*)	8702	8923	9082	9014	9037	—
Age (y)	52.0 ± 9.3	52.7 ± 9.2	52.7 ± 9.2	53.1 ± 9.5	54.0 ± 9.6	<0.001
BMI (kg/m^2)	25.1 ± 4.8	25.1 ± 5.1	25.0 ± 4.8	24.7 ± 4.9	24.6 ± 4.7	<0.001
Physical activity (METs)[4]	17.5 ± 22.2	19.0 ± 23.7	20.5 ± 24.9	21.3 ± 27.5	21.9 ± 27.4	<0.001
Current smoker (%)	2.7	2.0	1.7	1.2	1.4	<0.001
Mean daily intake						
Carbohydrate (g)	220 ± 43	228 ± 39	235 ± 38	247 ± 40	244 ± 44	<0.001
Protein (g)	87 ± 16	92 ± 15	94 ± 15	94 ± 16	93 ± 17	<0.001
Alcohol (g)	15 ± 19	11 ± 14	10 ± 14	10 ± 13	10 ± 14	<0.001
Caffeine (mg)	267 ± 270	259 ± 259	242 ± 242	222 ± 238	223 ± 245	<0.001
Saturated fat (g)	27 ± 6	26 ± 5	24 ± 5	23 ± 5	23 ± 6	<0.001
Monounsaturated fat (g)	29 ± 6	28 ± 5	27 ± 5	26 ± 5	26 ± 6	<0.001
Dietary fiber (g)	16 ± 4	19 ± 4	22 ± 5	24 ± 7	23 ± 8	<0.001

[1] Values have been standardized for age of the cohort.
[2] Tests for linear trend for all variables except current smoking status were calculated by assigning the median value of each category as the score.
[3] $\bar{x}$ ± SD (all such values).
[4] Metabolic equivalent tasks per week; defined as a multiple of the metabolic equivalent of sitting at rest.

with a lower heme iron intake (**Table 1**). Men who reported a higher non-heme iron intake tended to be more physically active, weighed less, smoked less, and consumed more fiber, carbohydrate, protein, but had lower intakes of alcohol, caffeine, saturated fat, and monounsaturated fat, than did men with a lower non-heme iron intake.

During 597 699 person-years of follow-up from 1986 to 2002, we documented 2468 incident cases of symptomatic gallstones, of which 1453 cases required cholecystectomy. Because iron intake was associated both directly and inversely with several potential risk factors, we analyzed their relations with gallstone disease before and after adjustment for these variables.

The median heme iron intake for the highest and lowest quintiles varied nearly 2.5-fold, and that of non-heme intake varied nearly 3.5-fold (**Table 2**). Heme iron intake was associated with an increased risk of gallstone disease in the age-adjusted and multivariate analyses with a significant trend. The RR for men with heme iron intake in the highest quintile compared with men in the lowest quintile was 1.21 (95% CI: 1.06, 1.37; *P* for trend = 0.0008) in the age-adjusted analysis. The multivariate RR was not significantly changed (1.21; 95% CI: 1.03, 1.42; *P* for trend = 0.01) when extreme quintiles were compared after adjustment for multiple potential confounding variables, including age, body mass index, recent weight change, cigarette smoking status, history of diabetes mellitus, physical activity, thiazide diuretics, non-steroidal antiinflammatory drugs, and intakes of alcohol, caffeine, dietary fiber, carbohydrate, protein, and saturated, polyunsaturated, and monounsaturated fats, and total energy (Table 2). The RR for men with non-heme iron intake in the highest quintile compared with men in the lowest quintile was 1.02 (CI: 0.90, 1.16; *P* for trend = 0.45) in the age-adjusted analysis. In the multivariate analysis, when extreme quintiles were compared, the RR was 1.14 (CI: 0.99, 1.31; *P* for trend = 0.18).

To examine the possibility that latent gallstone symptoms may distort the relation between heme iron intake and the risk of gallstone disease, thereby biasing the results, we conducted an analysis excluding all cases that occurred during the first 4-y follow-up period. Compared with men in the lowest quintile of dietary intake of heme iron, men in the highest quintile had an age-adjusted RR of 1.22 (95% CI: 1.06, 1.41; *P* for trend = 0.007) and a multivariate RR of 1.27 (95% CI: 1.06, 1.53; *P* for trend = 0.02) after excluding the first 4-y follow-up period.

To examine whether the association with heme iron intake was modified by risk factors for gallstone disease, we repeated the multivariate analyses within subgroups of potential confounding variables. We found no important change in effect. The positive

TABLE 2
Adjusted relative risks (95% CIs) of gallstone disease (GSD) by quintile of intakes of heme iron and non-heme iron in US men in the Health Professionals Follow-up Study, 1986–2002

	Quintiles					*P* for trend[1]
	1 (lowest)	2	3	4	5 (highest)	
Heme iron						
Median intake (mg/d)	0.8	1.1	1.3	1.6	2.0	—
Cases of GSD	540	379	606	460	483	—
Person-years	140 026	96 274	143 192	108 176	110 031	—
Model 1: age-adjusted	1.00	1.04 (0.92, 1.19)	1.07 (0.94, 1.21)	1.19 (1.05, 1.35)	1.21 (1.06, 1.37)	0.0008
Model 2: multivariate[2]	1.00	1.05 (0.92, 1.21)	1.08 (0.94, 1.24)	1.20 (1.04, 1.39)	1.21 (1.03, 1.42)	0.01
Non-heme iron						
Median intake (mg/d)	9.4	11.2	12.9	16.4	34.3	—
Cases of GSD	491	467	500	478	532	—
Person-years	116 739	119 596	121 627	120 194	119 544	—
Model 1: age-adjusted	1.00	1.00 (0.88, 1.14)	1.01 (0.89, 1.15)	1.03 (0.90, 1.17)	1.02 (0.90, 1.16)	0.45
Model 2: multivariate[2]	1.00	1.07 (0.93, 1.22)	1.13 (0.98, 1.30)	1.15 (1.00, 1.32)	1.14 (0.99, 1.31)	0.18

[1] Tests for linear trend were calculated by assigning the median value of each category as the score.
[2] Cox proportional hazards model included the following potential compounding covariates: age, BMI, recent weight change during the past 2 y, cigarette smoking, history of diabetes, physical activity, use of thiazide diuretics or nonsteroidal antiinflammatory drugs, and intakes of alcohol, caffeine, dietary fiber, carbohydrate, protein; saturated, polyunsaturated, and monounsaturated fats; and total energy.

associations between heme iron intake and the risk of gallstone disease persisted in all subgroups.

We also addressed the possibility of detection bias by excluding cases with unremoved stones as these were presumably less symptomatic, limiting the analysis to cholecystectomy cases. The age-adjusted RR for men in the highest quintile of dietary intake of heme iron compared with men in the lowest quintile was 1.26 (95% CI: 1.07, 1.48; *P* for trend = 0.002), and the multivariate RR was 1.25 (95% CI: 1.01, 1.53; *P* for trend = 0.02).

DISCUSSION

Body iron stores accumulate by the absorption of dietary iron, including heme iron and non-heme iron. In experimental studies that used controlled meals, the absorption of heme iron was shown to be more complete and less regulated than that of non-heme iron (27). Heme iron, which is mainly present in red meat, fish, and poultry, is highly bioavailable, and its absorption is substantially higher than that of non-heme iron. Non-heme iron absorption is more likely to be influenced by various dietary enhancers and inhibitors, and its bioavailability varies significantly (28). In the present large cohort study, we observed that a higher intake of heme iron was associated with a higher risk of gallstone disease, with a dose-response relation that was not accounted for by other potential risk factors, including other measured dietary variables, and the multivariate adjustment did not change the RR significantly.

Excessive chronic iron ingestion may cause substantial harm to the body, depending on dose and duration of excess iron exposure (4). Excess iron cannot be excreted from the body because there is no regulated iron excretion in situations of dietary overload. Iron is incorporated into heme to serve specific functions or sequestered and stored in ferritin. Experimental studies have shown that a high dietary iron intake can induce lipid peroxidation (29) and stimulate generation of hydroxyl radicals (30), which in turn may stimulate mucous glycoprotein secretion in the gallbladder (31, 32) and promote cholesterol crystal formation in bile (15), both of which may promote the formation of gallstones. An increased iron intake can alter blood lipids and increase the ratio of saturated to unsaturated fatty acids (33) and thus may enhance cholesterol gallstone formation (34, 35). Also, consumption of a high iron diet can elevate plasma triacylglycerol concentrations (14) and thus may increase the risk for gallstones (2).

The prospective design of our study avoided the potential for differential report of iron intake by gallstone cases and noncases, because all data on dietary intake were collected before the diagnosis of gallstone disease. Also, in the present cohort, the consistently high follow-up rates reduced the possibility that our results were biased by men lost to follow up.

Because information on nutrient intake was collected by self-report, the possibility of misclassification may be of concern. Random within-person variation could attenuate any true association of interest, but the SFFQ was designed to minimize this error by assessing average long-term dietary intake during the successive follow-up periods. These repeated measurements took into account possible changes in diet with time and reduced random variation. Because of the prospective design, any measurement errors would be expected to be unrelated to the gallstone disease endpoints. Thus, any nondifferential misclassification would most likely bias the RRs toward the null and lead to an underestimation of the true effect.

To address the possibility of bias due to latent gallstone disease, we incorporated a lag period of 4 y between dietary assessment at baseline and subsequent development of gallstone disease. The positive association persisted after the first 4 y of follow-up were excluded. Additionally, we performed our analyses among men with cholecystectomy and excluded men with unremoved gallstones who may be presumably less symptomatic and more prone to detection bias. The positive association persisted after the exclusion.

In the present large study population, it was not possible to perform diagnostic screening procedures for the presence of gallstones. Because most gallstones are asymptomatic, it is likely that there was underascertainment of gallstones. It was not likely

that the presence of asymptomatic gallstones at baseline was associated with the reporting of iron intake. Because RR estimation in follow-up cohort studies would not be biased by uniform underascertainment (36), our results were not likely biased due to asymptomatic gallstones. In conclusion, our findings suggest that a higher consumption of heme iron was associated with an increased risk of gallstone disease among men.

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WCW and ELG supervised the study. All authors participated in the data analyses and in writing the manuscript. None of the authors have any conflict of interest.

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