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Dietary haem iron and coronary heart disease in women

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Aims A role for iron in the risk of ischaemic heart disease has been supported by *in vitro* and *in vivo* studies. We investigated whether dietary haem iron intake is associated with coronary heart disease (CHD) risk in a large population-based cohort of middle-aged women.
Methods and results We used data of 16 136 women aged 49–70 years at recruitment between 1993 and 1997. Follow-up was complete until 1 January 2000 and 252 newly diagnosed CHD cases were documented. Cox proportional hazards analysis was used to estimate hazard ratios of CHD for quartiles of haem iron intake, adjusted for cardiovascular and nutritional risk factors. We stratified by the presence of additional cardiovascular risk factors, menstrual periods, and antioxidant intake to investigate the possibility of effect modification. High dietary haem iron intake was associated with a 65% increase in CHD risk [hazard ratio (HR) = 1.65; 95% confidence interval (CI): 1.07–2.53], after adjustment for cardiovascular and nutritional risk factors. This risk was not modified by additional risk factors, menstruation, or antioxidant intake.
Conclusion The results indicate that middle-aged women with a relatively high haem iron intake have an increased risk of CHD.

Introduction

In 1981, Sullivan proposed the 'iron hypothesis' by which he tried to explain the difference in incidence rates of heart disease between men and women by differences in stored iron levels.[1] *In vivo* and *in vitro* studies have indicated several plausible mechanisms for a detrimental role of iron, primarily based on the ability to increase oxidative stress as a result of its catalytic properties in free radical formation.[2]

Apart from iron supplements, dietary iron is the most important source of iron. The extent to which it is absorbed from the diet strongly depends on the composition of the diet, the form in which iron is present, and the iron status of a subject. Importantly, no dedicated excretory system exists and iron is lost only by means of natural losses of iron-containing cells such as erythrocytes and enterocytes. Dietary iron may be classified as haem iron, originating from animal foods, and non-haem iron, originating primarily from plant foods. Unlike haem iron absorption, non-haem iron absorption is strongly influenced by various foods and nutrients, as well as body iron content,[3–5] therefore, dietary iron

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intake does not directly reflect body iron stores. Once inside the enterocyte, haem iron is cleaved by haem oxygenase, and the released iron joins the common non-haem iron pool. Although in absolute quantity, haem iron intake is lower than non-haem iron intake, the bio-availability of haem iron is much higher since it has the ability to circumvent the down-regulation mechanisms of non-haem iron absorption in iron overload.[6]

Although only a few epidemiological studies have focussed on haem iron in particular, their results were more univocal than the studies on total iron intake.[7–15] Ascherio *et al.*[7] and Klipstein-Grobusch *et al.*[8] both demonstrated increased risks of myocardial infarction or coronary heart disease (CHD) with haem iron intake.

The objective of this study was to investigate whether dietary iron intake, especially haem iron, is associated with CHD risk in middle-aged women. Additionally, we examined whether dietary haem iron intake has a larger effect on CHD in non-menstruating women and women with low antioxidant intake or an unfavourable cardiovascular risk profile.

Methods

Population

The study population consists of participants of the Prospect-EPIC cohort, which is one of the two Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC).[16] Participants were recruited between 1993 and 1997 among women living in Utrecht and its vicinity and who attended the regional population-based breast cancer screening programme. A total of 17 357 women aged 49–70 years were included. Women received two detailed questionnaires by mail. One was a general questionnaire relating to non-dietary factors, the other a food frequency questionnaire. From the initial 17 357 women we excluded 362 women who did not consent to linkage with vital status registries or who were not traceable, 202 women because of missing questionnaires, and 16 women who reported implausibly low ($<$500 kcal/day) or high ($>$6000 kcal/day) energy intake. In addition, we excluded 647 women who reported a history of CHD (ICD-9; 410–414, 427.5) or cerebrovascular disease (ICD-9; 430–438) at baseline. For some women, multiple reasons for exclusion applied, resulting in a total number of 16 136 women that remained in the analyses.

All women signed an informed consent prior to study inclusion. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht.

Food frequency questionnaire

The validated food frequency questionnaire (FFQ) estimates the usual frequency of consumption of 79 main food items over the preceding 12 months.[17,18] Moreover, the FFQ comprises questions regarding nutritional habits, preparation methods, and additions. Colour photographs of 28 dishes were used to estimate habitual portion sizes. Food consumption data were converted into macro- and micronutrients using an updated version of the computerized Dutch food composition table 1996.[19] Use of iron supplements over the preceding 12 months was coded as a bivariate yes/no variable. Overall, the questionnaire enabled the estimation of the average daily consumption of 178 food items. All nutrients, including iron, were adjusted for total energy intake using the regression residual method.[20]

Baseline measurements

The general questionnaire contained questions on demographic characteristics, previous and current illnesses, and risk factors for chronic diseases, such as reproductive history, smoking habits, alcohol consumption, physical activity, and family history.

Systolic and diastolic blood pressure were measured in duplicate, and the mean value was calculated. Furthermore, height and weight were measured without shoes in light indoor clothing to compute body mass index (BMI) defined as weight divided by height squared (kg/m^2). Hypercholesterolaemia and diabetes mellitus were defined as present when women reported that a physician diagnosed this. The same criterion applied for the presence of hypertension in addition to a measured systolic blood pressure $>$ 160 mmHg and/or a diastolic blood pressure $>$95 mmHg. Women were classified according to their smoking habits as current, past, or never smokers. Physical activity was assessed on the basis of the Voorrips questionnaire, which generates a physical activity score based on daily domestic, occupational, and leisure time activity. The questionnaire has been validated in an elderly population.[21]

When women reported the absence of menstrual periods in the previous 12 months they were classified as non-menstruating. The corresponding age at last menstruation was self-reported.

Morbidity and mortality

Data on morbidity were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. Admission files, completely covering all diagnoses, have been filed continuously from all general and university hospitals in The Netherlands since 1990. Whenever a patient is discharged from a hospital, data on sex, date of birth, dates of admission and discharge, one mandatory principal diagnosis, and up to nine optional additional diagnoses are recorded. All diagnoses were coded according to the International Classification of Diseases, ninth Revision (ICD-9). The endpoint of interest was CHD denoted by codes 410–414 and 427.5. Whenever multiple events occurred, CHD or cerebrovascular disease, morbidity or mortality, the first diagnosis was taken as endpoint. Follow-up was complete until 1 January 2000. The database was linked to the cohort on the basis of birth date, gender, postal code, and general practitioner with a validated probabilistic method.[22]

Information on vital status was obtained through linkage with the municipal administration registries. Causes of death were obtained from the women's general practitioners.

Data analysis

First events of CHD were the endpoint of interest. Through to 1 January 2000, a total of 252 women were newly diagnosed with CHD. For these women, follow-up ended at the date of diagnosis or, when this information was absent, at the date of death. Cases of first cerebrovascular disease denoted by codes 430–438 ($n = 92$) were censored at date of diagnosis or date of death. Moving out of The Netherlands ($n = 24$), or death due to causes other than CHD or cerebrovascular disease ($n = 196$), were considered censoring events. For all others ($n = 15\,572$), 1 January 2000 was considered as the censoring date.

Baseline characteristics were expressed for the total study population. Means and standard deviations (SDs) were computed for continuous baseline variables, and frequency distributions for categorical variables. Since the distributions of alcohol intake and physical activity were skewed, both variables are expressed by median and interquartile range (IQR).

To assess any association between iron intake and risk of CHD, Cox proportional hazards regression[23] was used to compute hazard ratios (HRs) and 95% confidence intervals (CI). In each analysis, the lowest quartile (Q) of iron intake was the reference group. Models were adjusted for classical cardiovascular risk factors, i.e. age, BMI, smoking, physical activity, hypertension, diabetes, and hypercholesterolaemia. Further adjustments were made for daily intake of energy, saturated fat, carbohydrate, fibre, alcohol, β-carotene, vitamin E, and vitamin C. Additionally, iron intakes were analysed as continuous variables. The fit of the proportional hazards model was evaluated by examining the log minus log plots. The proportional hazards assumptions were satisfied. The linearity assumption was assessed by comparing the estimates of iron intake in models including the continuous variables as such and models in which the percentile dummies of the continuous variables were included. The linearity assumptions were satisfied. Tests for linear trends were conducted by adding the quartiles of iron intake as a continuous variable in the model.

Considering low dietary antioxidant intake as a proxy for low antioxidant status, a more pronounced association between dietary haem iron intake and CHD is expected in these women. A high antioxidant status could counterbalance the oxidative stress catalysed by iron. Additionally, it is hypothesized that iron may adversely affect CHD risk only in the presence of other cardiovascular risk factors, such as hypertension, hypercholesterolaemia, diabetes, and smoking. We therefore assessed the possibility of effect modification by entering the product term of iron intake and the modifier into the model. The significance of the interaction term was determined by Wald's test.

An anti-oxidative score was constructed based on the combined intake of vitamin E, vitamin C, and β-carotene. Energy-adjusted intakes were computed for each anti-oxidant and subsequently divided into tertiles. From low to high, tertiles were scored 1 to 3 for each antioxidant. The three separate scores were summed to obtain an overall anti-oxidative score (range 3–9). Next, the anti-oxidative score was divided into two groups, representing low antioxidant intake (score 3–5) and medium to high antioxidant intake (score 6–9). Presence or absence of additional risk factors was based on four classical risk factors for cardiovascular disease, i.e. diabetes, hypertension, hypercholesterolaemia and, current smoking.

To assess whether the relationship between iron intake and CHD risk differed between menstruating and non-menstruating women, we performed stratified analyses. Since menstruating women lose iron through menses, their dietary iron intake may be insufficiently large to develop high body iron stores, this is in contrast to non-menstruating women who lack this natural protective effect. At the outset of the study, we decided not to adjust for multiple comparisons.[24,25] All statistical procedures were performed using the statistical package SPSS (SPSS for Windows, Release 11.0.1. 2001. SPSS Inc., Chicago, IL, USA). *P*-values were two-sided and $P < 0.05$ was considered statistically significant.

Results

The 16 136 women in the study were followed for a total of 69 600 person-years. Median follow-up was 4.3 years.

Table 1 Baseline characteristics of the total study population ($n = 16\,136$)

	Mean (SD)
Age at intake, years[a]	56 (52–62)
BMI, kg/m^2	26.0 (4.0)
Physical activity score[a,b]	5.3 (2.7–9.4)
Total energy intake, kcal/day	1798 (435)
Total dietary iron intake, mg/day[c]	10.52 (1.45)
Haem iron, mg/day[c]	1.81 (0.76)
Non-haem iron, mg/day[c]	8.74 (1.30)
Saturated fat intake, g/day[c]	29.6 (5.4)
Carbohydrate intake, g/day[c]	195.3 (27.6)
Fibre intake, g/day[c]	22.4 (4.4)
Alcohol intake, g/day[a,c]	3.7 (0.5–12.8)
	n (%)
Hypertension	4337 (26.9)
Smoking status	
Current	3541 (21.9)
Past	5557 (34.4)
Never	7034 (43.6)
Diagnosis of hypercholesterolaemia	784 (4.9)
Diagnosis of diabetes	430 (2.7)
Use of iron supplements	536 (4.2)

[a]Median (IQR).
[b]Higher scores indicate higher levels of physical activity.
[c]Energy-adjusted.

The mean (SD) daily intake in milligrams was 10.52 (1.45) for total iron, 1.81 (0.76) for haem iron, and 8.74 (1.30) for non-haem iron.

Table 1 shows the baseline characteristics of the study population. The median age of the total group was 56 years (interquartile range: 52–62). Around 27% of the cohort members were hypertensive and 22% were current smokers. The prevalence of diabetes and hypercholesterolaemia was 4.9 and 2.7%, respectively. Most anthropometric results resembled those found in similar cohorts.[16]

Table 2 presents risk estimates for iron intake and CHD. According to the basic model, in which we adjusted for classical risk factors for cardiovascular disease, women in the highest quartile of haem iron intake were at increased risk of developing CHD compared with women in the lowest quartile (HR = 1.52, 95% CI: 1.06–2.19). When this model was further adjusted for nutritional factors, the HR increased slightly to 1.65 (95% CI: 1.07–2.53). Restricting our analyses to women who did not use iron supplements did not alter our results (HR Q4 vs. Q1 = 1.65; 95% CI: 1.07–2.54). When analysed as a linear continuous variable, the HR for haem iron intake per mg/day was 1.15 (95% CI: 0.95–1.40). The estimate for the continuous relationship and its 95% CI are clearly compatible with a linear association, although it is borderline significant. Categorization decreases the power to detect deviations from linearity by reducing the amount of information; therefore it is common that regression results for categorical and continuous variables are slightly different. Total dietary iron intake and non-haem iron intake were not significantly related

Table 2 Iron intake and CHD risk ($n = 16\,136$)

	Range (mg/day)	Cases/person-years	Crude model		Basic model[a]		Multivariate model[b]	
			HR	95% CI	HR	95% CI	HR	95% CI
Total iron intake[c]								
Quartile 1	<9.56	66/17 498	1.0	–	1.0	–	1.0	–
Quartile 2	9.57–10.50	66/17 291	1.01	0.72–1.43	1.15	0.80–1.65	1.11	0.76–1.62
Quartile 3	10.50–11.43	63/17 406	0.96	0.68–1.36	1.10	0.76–1.59	1.05	0.70–1.59
Quartile 4	>11.43	57/17 405	0.87	0.61–1.24	1.08	0.75–1.57	0.98	0.61–1.58
P for trend				0.412		0.735		0.878
Continuous per mg/day			0.89	0.81–0.98	0.98	0.89–1.08	0.92	0.79–1.06
Haem iron intake[c]								
Quartile 1	<1.28	54/17 413	1.0	–	1.0	–	1.0	–
Quartile 2	1.28–1.76	53/17 384	0.98	0.67–1.44	1.01	0.68–1.51	1.06	0.71–1.59
Quartile 3	1.76–2.27	57/17 334	1.06	0.73–1.54	1.05	0.71–1.56	1.12	0.74–1.71
Quartile 4	>2.27	88/17 469	1.62	1.16–2.28	1.52	1.06–2.19	1.65	1.07–2.53
P for trend				0.003		0.019		0.019
Continuous per mg/day			1.21	1.03–1.41	1.14	0.97–1.34	1.15	0.95–1.40
Non-haem iron intake[c]								
Quartile 1	<7.88	83/17 430	1.0	–	1.0	–	1.0	–
Quartile 2	7.88–8.73	59/17 465	0.71	0.51–0.99	0.82	0.58–1.16	0.74	0.51–1.08
Quartile 3	8.73–9.56	56/17 351	0.68	0.48–0.95	0.85	0.60–1.22	0.74	0.49–1.12
Quartile 4	>9.56	54/17 353	0.66	0.47–0.92	0.87	0.61–1.25	0.68	0.42–1.12
P for trend				0.013		0.469		0.150
Continuous per mg/day			0.96	0.88–1.05	1.02	0.93–1.11	0.99	0.88–1.12

[a]Adjusted for age at intake (continuous), BMI (continuous), smoking (current/past/never), physical activity (continuous), hypertension (yes/no), diabetes (yes/no), hypercholesterolaemia (yes/no).

[b]Adjusted for age at intake (continuous), total energy intake (continuous), BMI (continuous), smoking (current/past/never), physical activity (continuous), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), energy-adjusted saturated fat intake (continuous), energy-adjusted carbohydrate intake (continuous), energy-adjusted fibre intake (continuous), energy-adjusted alcohol intake (quintiles), energy-adjusted β-carotene intake (continuous), energy-adjusted vitamin E intake (continuous), energy-adjusted vitamin C intake (continuous).

[c]Energy-adjusted.

to CHD. Multivariable adjusted hazard ratios for the highest vs. the lowest quartile were 0.98 (95% CI: 0.61–1.58) and 0.68 (95% CI: 0.42–1.12) for total iron and non-haem iron, respectively.

Antioxidant intake, the presence of other cardiovascular risk factors, or menstrual periods did not modify the association between iron intake and CHD risk (data not shown).

Discussion

The results of the present study of 16 136 women aged 49–70 show that a relatively high dietary haem iron intake is associated with increased risk of CHD. We examined whether certain factors such as the presence of other cardiovascular risk factors, menstruation, or antioxidant intake modified this association, but no major effects were observed.

An advantage of a prospective over a retrospective study design is that exposure data are collected at the beginning of the study and that subjects are followed over time for occurrence of cardiovascular diseases. The possibility that estimates are biased by the fact that subjects may change their diets due to emerging diseases is hereby reduced to a minimum. Additionally, prevalent cases of CHD and cerebrovascular disease were excluded from the analyses to prevent information on dietary intake being biased by the known presence of disease. Finally, the FFQ proves a valid and precise way to assess dietary habits and nutrient intakes over longer time periods.[26] Although the FFQ was not specifically validated for iron intake, the relative validity for the estimate of meat intake, the most important source of haem iron, was rather high (Spearman rank correlation coefficient of 0.7 comparing the FFQ with 12 24 h recalls).[17] The mean iron intake (10.7 mg/day) of 50- to 65-year-old women found in the Dutch National Food Consumption Survey 1998,[27] was quite similar to the mean intake in our population (10.5 mg/day). The main sources of haem iron in this population of middle-aged women were fresh and processed meat, whereas cereals or cereal products and non-alcoholic beverages were the most important sources of non-haem iron.

Iron supplementation use was relatively rare in our population. Only 4% of the women reported use of iron supplements, which is substantially lower than reported in other studies.[28–30] In our study, the use of iron supplements by itself was not associated with an increased risk of CHD. Women who show signs of iron deficiency and whose body iron levels cannot be restored to normal levels by diet alone are usually those taking iron supplements. Therefore it is more plausible that

iron supplements compensate for low body iron stores, rather than contribute to iron overload in these women.

One of the limitations of our study is that the presence of diabetes and hypercholesterolaemia was based solely on a self-reported physician's diagnosis. In multivariable models, mis-classification of co-variates may result in inadequate confounder correction. However, biochemical analyses were performed for a random sample of 1700 women of the baseline cohort so we had some indication of the prevalence of undiagnosed diabetes and hypercholesterolaemia in our population. Based on a self-reported physician's diagnosis, 2.8% of the women had diabetes and 5.2% had hypercholesterolaemia. When blood measurements were taken into account, these percentages were 3.3% and 6.6%, respectively.

Because women of reproductive age lose iron by menstruation, we evaluated the hypothesis that these women are relatively protected from developing iron overload and thereby from CHD. In this hypothesis we would expect the relation between high levels of iron intake and CHD to be strongest in non-menstruating women. We stratified by menstrual periods and observed that only non-menstruating women have an increased risk of CHD (HR Q4 vs. Q1–3 = 1.58, 95% CI: 1.14–2.19), and that the effect of high dietary haem on CHD is virtually absent in menstruating women (HR Q4 vs. Q1–3 = 1.10, 95% CI: 0.44–2.81). But because the number of CHD cases in menstruating women is very small, caution should be taken when interpreting these risk estimates.

Apart from the relationship between dietary haem iron and CHD, the effects of total iron intake and non-haem iron intake on CHD were also examined. Both total iron and non-haem iron showed no association with CHD risk, which confirms that haem iron is probably more biologically relevant than non-haem iron. Because most total iron intake is non-haem, this too may not be an adequate reflection of iron exposure.

The mechanism by which excess body iron may affect cardiovascular disease risk is still unclear. Shortly after reperfusion following a myocardial infarction, superoxide radicals are generated.[31] These superoxide radicals act as a source of hydrogen peroxide, which in turn reacts with iron to form more highly reactive hydroxyl radicals. This latter reaction is also known as the Fenton reaction, and the hydroxyl radicals may aggravate myocardial damage.[32,33] The number of fatal cases of CHD in our study was too small to perform any meaningful separate analyses for fatal and non-fatal events. Moreover, hydroxyl radicals also play an important role in one of the crucial steps in early atherosclerosis, the oxidation of low-density lipoprotein (LDL) cholesterol.[34–36] In addition to these processes, iron might affect atherosclerosis in other, yet undefined, ways.

Most previous studies that have investigated the association between dietary iron intake and cardiovascular disease risk were conducted in populations of both men and women. Therefore, the hazardous effect of high dietary iron intake and the protective effect of menstruation on body iron stores and thereby cardiovascular disease risk could not be studied. High intake of haem iron was associated with increased risk of CHD or myocardial infarction in two previous reports.[7,8] Both Ascherio *et al.*[7] and Klipstein-Grobusch *et al.*[8] found risk estimates similar to ours for the highest levels of haem iron intake compared with the lowest. Additionally, two other papers have reported an association between high total iron intake and myocardial infarction or CHD.[9,10] In contrast, there are several others that do not support an association between iron intake and cardiovascular endpoints.[11–15] The discrepancy between these studies is probably due to large variations in measurements of iron intake (e.g. FFQ, 24 h recalls, 4-day food records), cardiovascular endpoints (e.g. CHD, myocardial infarction, carotid atherosclerosis) and the degree of adjustment for confounders. Furthermore, a large variation in average total iron intake may also explain why some studies have shown a positive association with CHD risk and others have not. The variation in iron intake between populations may be partially explained by differences in meat intake and frequency and amount in which iron supplements are taken, whereas differences in the assumptions used for computing the iron content of products may be an important factor as well.

In conclusion, our study supports the hypothesis that a relatively high haem iron intake increases the risk of CHD.

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