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ORIGINAL CONTRIBUTION

Is heme iron intake associated with risk of coronary heart disease? A meta-analysis of prospective studies

Wei Yang · Bin Li · Xiao Dong · Xiao-Qiang Zhang · Yuan Zeng · Jian-Liang Zhou · Yan-Hua Tang · Jian-Jun Xu

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Abstract
Purpose Heme iron may contribute to the development of atherosclerosis by catalyzing production of hydroxyl-free radicals and promoting low-density lipoprotein oxidation. However, epidemiologic findings regarding the association between heme iron intake and risk of coronary heart disease (CHD) are inconsistent. We aimed to investigate the association by carrying out a meta-analysis of prospective studies.
Methods Relevant studies were identified by using PubMed and EMBASE databases between January 1966 and April 2013 and also by manually reviewing the reference lists of retrieved publications. Summary relative risks (RRs) with corresponding 95 % confidence intervals (CIs) were computed using a random-effects model.
Results Six prospective studies, which contained a total of 131,553 participants and 2,459 CHD cases, met the inclusion criteria. Combined results indicated that participants with higher heme iron intake had a 31 % increased risk of CHD, compared with those with lower intake (RR = 1.31, 95 % CI 1.04–1.67), with significant heterogeneity ($P^{\text{heterogeneity}} = 0.05$, $I^2 = 55.0$ %). Excluding the only study from Japan (limiting to Western studies) yielded a RR of 1.46 (95 % CI 1.21–1.76), with no study heterogeneity ($P^{\text{heterogeneity}} = 0.44$, $I^2 = 0.0$ %). The dose–response RR of CHD for an increase in heme iron intake of 1 mg/day was 1.27 (95 % CI 1.10–1.47), with low heterogeneity ($P^{\text{heterogeneity}} = 0.25$, $I^2 = 25.8$ %). We observed no significant publication bias.
Conclusions This meta-analysis suggests that heme iron intake was associated with an increased risk of CHD.

Keywords Heme iron · Prospective studies · Coronary heart disease · Meta-analysis

W. Yang · B. Li · X. Dong (✉) · X.-Q. Zhang · Y. Zeng · J.-L. Zhou · Y.-H. Tang · J.-J. Xu
Department of Cardiothoracic Surgery, Second Affiliated Hospital of Nanchang University, Nanchang 330000, China
e-mail: Dongxiao0791@126.com

Introduction

Iron is a potential pro-oxidant and has been shown to contribute to the development of atherosclerosis by means of catalyzing production of hydroxyl-free radicals and promoting low-density lipoprotein (LDL) oxidation [1]. In 1981, Sullivan proposed the 'iron hypothesis' by which he tried to explain the sex difference in heart disease risk by differences in stored iron levels [2]. Over the past three decades, many epidemiologic studies have investigated the relation between iron and coronary heart disease (CHD), but the findings are equivocal [3–11]. Several factors may explain the inconsistent results, one of which was the lack of discrimination between heme iron and non-heme iron. The absorption of heme iron is more complete and less well regulated than that of non-heme iron [12]. Among Western populations, the majority of total dietary iron is the non-heme iron, whereas the majority of stored body iron arises from the heme iron [13]. Heme iron is found mainly in meat, whereas non-heme iron primarily originates from plant foods [14]. Therefore, the observed association between total iron and CHD may mostly reflect other nutrients contained in plant foods.

To date, several epidemiologic studies have been conducted to investigate the relationship between heme iron and risk for CHD, but the results remain inconsistent [6–11]. We therefore carried out this meta-analysis of prospective studies to summarize the current epidemiologic

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evidence on the association of heme iron intake with CHD risk and to examine whether there was a dose–response relationship.

Materials and methods

Literature search and selection

We conducted a systematic search of PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and EMBASE (http://www.embase.com) databases between January 1966 and April 2013 using the search terms *heme iron* or *haem iron* combined with *cardiovascular disease* (CVD) or *coronary heart disease* or *coronary artery disease* (CAD) or *coronary disease* or *myocardial infarction* or *ischemic heart disease* (IHD) or *CHD* or *CVD* or *CAD* or *IHD*, with no language restrictions.

The titles and abstracts were scanned to exclude any studies that were clearly irrelevant. The full texts of the remaining publications were assessed, and their reference lists were also manually reviewed for any further studies. The inclusion criteria are as follows: (1) the study design was prospective; (2) the exposure of interest was intake of heme iron; (3) the outcome of interest was CHD; and (4) the relative risks (RRs) with corresponding 95 % confidence intervals (CIs) of CHD were reported (or these data could be calculated in the studies). According to these inclusion criteria, we excluded one study [5] because it was a case–control study, and two studies [3, 4] because the exposure of interest in both studies was total iron intake, and two studies [15, 16] because the outcome of interest in both studies was total CVD (not CHD alone).

Data extraction

Two authors (WY and BL) independently conducted the data extraction using a standardized data collection form, with any disagreements resolved by consensus. For each study, the following characteristics were collected: the first author's last name, publication year, country of origin, length of follow-up, age, sex and number of participants, number of cases, CHD outcome (fatal, non-fatal), the highest and the lowest levels of heme iron intake, diet assessment method, adjustments for potential confounding factors, and the most fully adjusted RRs of CHD with corresponding 95 % CIs for each category of heme iron intake.

Data synthesis and analyses

The summary risk estimates were calculated with the method of DerSimonian and Laird using the assumptions of a random-effects model that considers both within-study variations and between-study variations [17]. Statistical heterogeneity among studies included in the meta-analysis was assessed using Q and I^2 statistics [18]. For the Q statistic, a P value of less than 0.1 was considered statistically significant heterogeneity. Potential publication bias was evaluated using Begg's funnel plots and Egger's regression asymmetry test [19]. If the included studies presented results for total heme iron and heme iron by different food sources (e.g., red meat, fish), the results for total heme iron were used. If the results were reported for men and women separately, but not overall risk estimates, a fixed-effects model was used to pool the risk estimates, and the combined results were included in the analysis [11].

Because the levels of heme iron intake in the higher and lower categories differed substantially between primary studies, we also conducted a dose–response meta-analysis using the method proposed by Greenland and Longnecker [20] and Orsini et al. [21]. This method requires the number of cases and person-years and the risk estimates with their variance estimates for at least three quantitative exposure categories. We estimated the distribution of cases/person-years in studies that did not present these data, but reported the total number of cases/person-years. For every study, the median or mean level of heme iron for each category was assigned to each corresponding RR estimate. When the RRs with 95 % CI of CHD for heme iron intake was provided as a continuous variable, the reported data were used in this analysis [10].

Stata version 12.0 software (StataCorp, College Station, TX, USA) was used for these statistical analyses, and all statistical tests were two-tailed.

Results

Study characteristics

The details of literature search are shown in Fig. 1. Six publications [6–11] containing six independent prospective studies that evaluated the relationship between heme iron intake and risk of CHD were considered to be eligible and included in the final analysis. The characteristics of these studies are shown in Table 1. The six studies were published between 1994 and 2012, and contained a total of 131,553 participants and 2,459 CHD cases. Two studies were carried out in the United States, two studies were conducted in Netherlands, and the remaining two studies were from Italy and Japan, respectively. Total number of participants (ranged between 906 and 58,615) and events (ranged between 92 and 884), and the mean duration of follow-up (ranged between 4 and 14.7 years) varied widely between original studies. Most of included studies reported

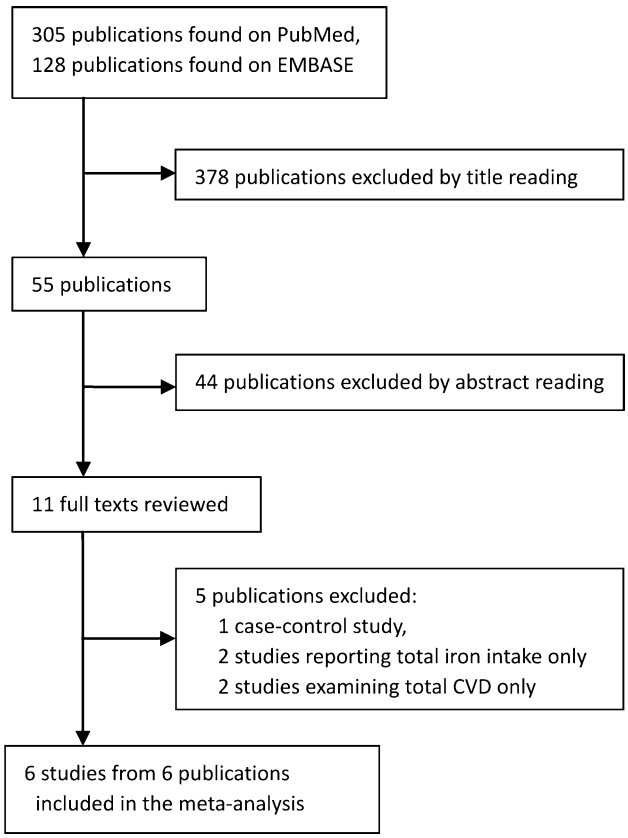

Fig. 1 Search strategy and selection of studies for inclusion in the meta-analysis

RRs (95 % CIs) of CHD that were adjusted for multiple covariates.

Main analysis

Relative risks with 95 % CI of CHD for high versus low category of heme iron intake for individual studies and all studies combined are shown in Fig. 2. Four out of the six studies suggested a statistically significant positive association between heme iron intake and risk of CHD. A combined analysis of all studies suggested that participants with higher heme iron intake had a 31 % increase in risk of CHD, when comparing those with lower intake (RR = 1.31, 95 % CI 1.04–1.67). There was statistically significant heterogeneity ($P^{\text{heterogeneity}} = 0.05$, $I^2 = 55.0$ %). We observed no significant publication bias ($P^{\text{Egger}} = 0.23$).

Sensitivity and stratified analyses

To explore the heterogeneity among studies of heme iron and CHD, we performed sensitivity and stratified analyses. In a sensitivity analysis in which one study at a time was omitted and the rest were combined, we found that the only study [11] from Japan contributed substantially to the heterogeneity among studies. After excluding this study, the summary RR of CHD was 1.46 (95 % CI 1.21–1.76), and there was no study heterogeneity ($P^{\text{heterogeneity}} = 0.44$, $I^2 = 0.0$ %). Further excluding one study [9] in which all participants were type 2 diabetes patients yielded a similar result (RR = 1.47, 95 % CI 1.14–1.89; $P^{\text{heterogeneity}} = 0.29$; $I^2 = 19.4$ %).

Stratifying the analysis by geographic area, the pooled RR was 1.45 (95 % CI 1.13–1.87) for two studies conducted in the United States ($P^{\text{heterogeneity}} = 0.90$, $I^2 = 0.0$ %) and 1.45 (95 % CI 1.00–2.12) for three studies carried out in Europe ($P^{\text{heterogeneity}} = 0.16$, $I^2 = 46.2$ %), respectively. Limiting the analysis to the studies with comparable categories of heme iron intake [6, 8–10], the summary RR of CHD was 1.55 (95 % CI 1.27–1.90, $P^{\text{heterogeneity}} = 0.84$, $I^2 = 0.0$ %).

Adjustment for dietary fats and cholesterol in the analysis appeared to be important because of their potential correlation with heme iron intake [6, 8]. Repeating the analysis by excluding two studies [7, 11] that did not control for these nutrients obtained a summary RR of 1.55 (95 %CI 1.27–1.90), with no heterogeneity ($P^{\text{heterogeneity}} = 0.84$, $I^2 = 0.0$ %).

Dose–response analysis

One study [7] was not eligible for dose–response because it had no data on the level of heme iron intake. The analysis of the remaining studies found that a 1 mg/day increment in heme iron intake was associated with a 27 % increase in risk of CHD (RR = 1.27, 95 % CI 1.10–1.47), with low heterogeneity ($P^{\text{heterogeneity}} = 0.25$, $I^2 = 25.8$ %).

Conclusion

In this meta-analysis of prospective studies, which involved large number of CHD cases, a statistically positive association was observed between heme intake and risk of CHD. Higher heme iron intake appeared to be significantly associated with a 31 % (95 % CI 4–67 %) elevated risk of developing CHD, compared with lower intake, and the observed hazardous effect of heme iron on CHD became more evident when restricting the analysis to the Western populations (RR = 1.46; 95 % CI 1.21–1.76). In the dose–response analysis, an increase in heme iron intake of 1 mg/day appeared to be significantly associated with a 27 % increase in risk of CHD.

Statistically significant heterogeneity was observed in the primary analysis. After excluding the Japan Collaborative Cohort (JACC) Study [11], there was little evidence of heterogeneity. Of note, the JACC Study differed from

Table 1 Characteristics of the included six prospective studies that investigated the relationship between heme iron intake and risk of CHD

Study	Country Duration	Participants	Cases	Heme iron intake, mg/day	RR (95 % CI), high vs. low	Diet assessment method	Adjustment
Ascherio [6]	USA 4 years	44,933 men aged 40–75 years	884 CHD (fatal and nonfatal)	5th quintile: 2.1 (median) 1th quintile: 0.7 (median)	1.48 (1.01–2.16)	Validated 131-item FFQ	Age, BMI, smoking, history of hypertension, diabetes and hypercholesterolemia, family history of MI, profession, and total energy, vitamin E, total iron, saturated fat and cholesterol and alcohol intakes
Klipstein-Grobusch [8]	Netherlands 4 years	4,802 men and women aged ≥55 years	124 MI (fatal and nonfatal)	3th tertile: 1.48 (mean) 1th tertile: 0.36 (mean)	1.86 (1.14–3.09)	Validated 170-item FFQ	Age, sex, BMI, smoking, equivalent household income, education, intakes of alcohol, beta-carotene, vitamin C, vitamin E, fat, saturated fat and cholesterol, and use of antioxidative vitamin supplements
Van der A [10]	Netherlands 4.3 years	16,136 men aged 49–70 years	252 CHD (fatal and non-fatal)	4th quartile: >2.27 1th quartile: <1.28	1.65 (1.07–2.53)	Validated 178-item FFQ	Age, total energy intake, BMI, smoking, physical activity, hypertension, diabetes, hypercholesterolemia, and saturated fat, carbohydrate, fiber, alcohol, beta-carotene, vitamin E and vitamin C intakes
Qi [9]	USA 8.8 years	6,161 women aged 30–55 years	550 CHD (fatal and nonfatal)	5th quintile: 2.83 (median) 1th quintile: 1.70 (median)	1.43 (1.01–2.01)	Validated FFQ	Age, BMI, smoking, alcohol, physical activity, aspirin use, duration of diabetes, history of hypertension and hypercholesterolemia, postmenopausal hormone use, family history of CHD, cereal fiber, glycemic load, polyunsaturated fat–to–saturated fat ratio, multivitamin use, and vitamin C and trans fat intakes
Casiglia [7]	Italy 10 years	906 women aged 61 years	92 CHD (fatal and nonfatal)	3th tertile: N.A. 1th tertile: N.A.	0.96 (0.57–1.61)	Validated 138-item FFQ	N.A.
Zhang et al. [11]	Japan 14.7 years	23,083 men and 35,532 women aged 40–79 years	Men 311 CHD (fatal) Women 246 CHD (fatal)	Men: 5th quintile: 0.44 (median) 1th quintile: 0.07 (median) Women: 5th quintile: 0.48 (median) 1th quintile: 0.06 (median)	Men: 0.74 (0.51–1.07) Women: 1.18 (0.80–1.72)	Validated FFQ	BMI, smoking, ethanol intake, history of hypertension and diabetes, sports time, walking time, educational, perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy

BMI body mass index, *CHD* coronary heart disease, *MI* myocardial infarction, *FFQ* food-frequency questionnaire, *N.A.* not available, *RR* relative risk, *CI* confidence interval

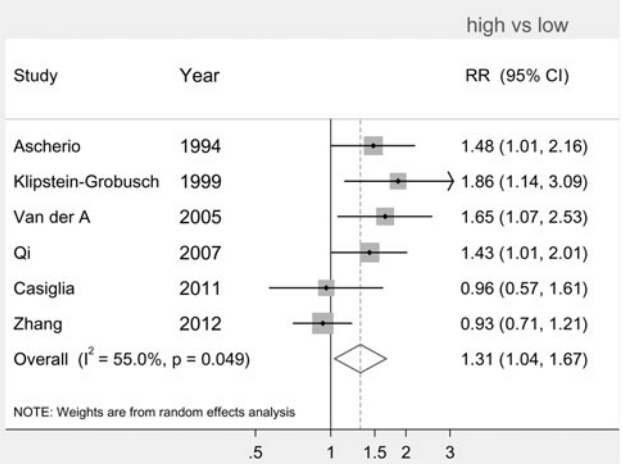

Fig. 2 Relative risks (RRs) with 95 % confidence intervals (CIs) of coronary heart disease comparing the higher with lower heme iron intake. *Squares* represent study-specific RR estimates (the size of the *square* reflects the study-specific statistical weight, *horizontal lines* represent 95 % CIs), and the *diamond* represents the combined RR estimate with corresponding 95 % CI

other studies in various aspects. On one hand, dietary heme iron intake among Japanese is much lower than that among Western populations (Table). Therefore, it is possible that this level of heme iron may not be high enough to develop body iron stores and increase CHD risk. On the other hand, unlike Western populations primarily getting heme iron from red meat, the Japanese populations mainly obtain their heme iron from fish and shellfish that contain some nutrients that may be protective against CHD, such as vitamin D and *n*-3 fatty acids [22–24]. Therefore, failing to control for these nutrients may obscure any true relationship between heme iron and CHD.

Several mechanisms whereby heme iron increases the risk of CHD have been proposed. Heme iron may promote low-density lipoprotein cholesterol oxidation by catalyzing the formation of highly reactive hydroxyl-free radicals [1, 25]; dietary heme iron intake has also been found to be associated with markers of inflammation [26]. All of these may contribute to the development of atherosclerosis. Moreover, epidemiologic evidence has also suggested that greater heme iron intake was positively related to blood pressure and incident diabetes mellitus [27, 28], both of which are risk factors for CHD.

Strengths of this meta-analysis include the prospective design of all included studies that minimizes recall and selection biases that are common in retrospective case–control studies, and the large number of events that enhances the statistical power of this meta-analysis.

A number of limitations should also be acknowledged when interpreting the results from this meta-analysis. First, most included studies measured heme iron intake only once at baseline rather than updating diet information, which may have led to 'regression dilution bias' [29]; in addition, all studies used a FFQ in diet assessment; although some of FFQs have been validated before application, misclassification of exposure (generally non-differential in cohort studies) was still inevitable. Both 'regression dilution bias' and non-differential misclassification would result in an underestimation of summary risk estimates. Second, several subgroup analyses such as those stratified by sex and duration of follow-up were not carried out because the number of studies was relatively small. Third, potential confounding factors would also be of concern, as this meta-analysis was on the basis of observational studies. Considering that meat is a rich source of heme iron, it is possible that some constituents other than heme iron in meat such as saturated fat and cholesterol are responsible for the observed findings. However, the results of our sensitivity analysis excluding two studies [7, 11] that did not control for these nutrients should have minimized this possibility, and two of the included studies also reported that risk estimates of CHD were not materially altered according to adjustment or not for saturated fat and cholesterol intakes [6, 8]; furthermore, some other meat-related factors such as smoking, body mass index, and alcohol drinking have also been controlled for in most of the included studies. Fourth, two studies [15, 16] that examined the relationship between heme iron intake and risk of total CVD, of which one [15] suggested a positive but the other [16] reported a null association, were excluded from this meta-analysis because they did not present results for CHD. Finally, publication bias always merits consideration because this meta-analysis was based on the published literature. Despite that Egger's regression asymmetry test indicated low evidence of such bias, it may nevertheless be present given the weakness of the testing method [30], and the relatively small number of studies included in the test [31].

In conclusion, findings of this meta-analysis suggest that heme intake is associated with an increased risk of CHD, and the observed relation appears to be independent of potential confounding factors, including saturated fat and cholesterol intakes.

Conflict of interest The authors declare that they have no conflict of interest.

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