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META-ANALYSIS

Dietary intake of heme iron and risk of cardiovascular disease: A dose–response meta-analysis of prospective cohort studies

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Abstract *Background and aims:* Iron is thought to play a fundamentally important role in the development of cardiovascular disease (CVD). This meta-analysis was performed to investigate the dose–response association between dietary intake of iron (including heme and non-heme iron) and the risk of CVD.
Methods and results: We performed a search of the PubMed and Embase databases for prospective cohort studies of the association between dietary iron intake and CVD risk. Thirteen articles comprising 252,164 participants and 15,040 CVD cases were eligible for inclusion. Heme iron intake was associated significantly with increased risk of cardiovascular disease, and the pooled relative risk (RR) for each 1 mg/day increment was 1.07 (95% confidence interval: 1.01 to 1.14, I^2 = 59.7%). We also found evidence of a curvilinear association ($P < 0.05$ for non-linearity). In contrast, we found no association between CVD risk and dietary non-heme (0.98, 0.96 to 1.01, I^2 = 15.8%) or total iron (1.00, 0.94 to 1.06, I^2 = 30.4%). Subgroup analyses revealed that the association between heme iron intake and CVD risk was stronger among non-fatal cases (1.19, 1.07–1.33) and American patients (1.31, 1.11–1.56).
Conclusions: Higher dietary intake of heme iron is associated with an increased risk of cardiovascular disease, whereas no association was found between CVD and non-heme iron intake or total iron intake. These findings may have important public health implications with respect to preventing cardiovascular disease.
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Introduction

Cardiovascular disease (CVD) has long been a significant public health problem. It causes maximum disability-adjusted life-years (DALYs) as reported by the Global Burden of Disease Study [1]. Although the prevalence of CVD has declined in developed countries (e.g. the United Kingdom and the United States) [2,3], the prevalence has increased considerably in developing countries. Indeed, a

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recent study reported that in the coming ten years, CVD is projected to be a major cause of morbidity and mortality in many developing countries [4].

Dietary factors are thought to play a fundamentally important role in CVD development [5]. Recently, several successive meta-analyses suggested that the consumption of red meat might be associated with the risk of CVD [6–8]. This positive correlation has been attributed – at least partly – to heme iron, which is abundant in red meat. Generally speaking, iron is a "double-edged sword" for living systems. Iron is essential for a wide range of key biological functions, including oxygen transport and cellular respiration. On the other hand, excessive levels of iron – particularly heme iron – can increase oxidative stress, leading to enhanced lipid peroxidation [9,10], a typical protein modifications, and DNA damage [11]. Several meta-analyses of prospective cohort studies revealed a positive correlation between heme iron intake and the risk of type 2 diabetes mellitus and some types of cancer [12–14].

Several prospective cohort studies examined the association between heme iron intake and the risk of various forms of CVD or total CVD outcome. However, the overall conclusion remains controversial, as the results of these studies were not consistent. One recent meta-analysis by Hunnicutt et al. investigated the correlation between dietary iron intake and the risk of coronary heart disease (ICD-9 codes 410–414 and ICD-10 codes $I^2$0– $I^2$5); however, this study did not categorically cover all cardiovascular diseases (i.e. codes 390–459 and I01–I99)[15]. Thus, to the best of our knowledge, no meta-analysis has been performed to examine the specific association between dietary heme iron intake and CVD risk.

To investigate this association, we performed a systematic review and dose–response meta-analysis of all available prospective cohort studies in order to examine the dose–response association between long-term intake of iron and total CVD outcome.

Methods

The design, implementation, analysis, and reporting of our meta-analysis were performed in accordance with the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) protocol [16].

Search strategy

We performed a search of the PubMed and Embase databases for studies published through July, 2014. We searched for prospective cohort studies that examined the association between dietary intake of heme iron and the risk of CVD. The following terms were used for the PubMed search (similar terms were used to search Embase): ("dietary iron" OR "heme iron" OR "haem iron" OR "iron intake" OR "iron consumption") AND ("cardiovascular disease" OR "coronary heart disease" OR "myocardial infarction" OR "heart failure" OR "stroke" OR "cerebrovascular disease" OR "hypertension" OR "hypertensive disease" OR "ischemic heart disease" OR "ischaemic heart disease"). The references included in the relevant articles retrieved were also reviewed to identify additional potential publications. We restricted our search to human studies. Finally, we applied no language restriction to the search. Two authors (XF and PA) conducted the literature search independently.

Selection of articles

Studies that satisfied the following four criteria were included in our meta-analysis: 1) published prospective cohort studies; 2) the exposure of interest was dietary iron intake, including heme iron, non-heme iron, and total iron; 3) the outcome was risk of CVD, including morbidity of coronary heart disease (CHD), stroke, hypertensive disease, heart failure, and/or CVD-related mortality; 4) the authors reported the relative risk estimates, including 95% confidence intervals, for at least three quantitative categories of dietary iron intake. The study selection process is depicted diagrammatically in Fig. 1.

During screening, we excluded retrospective and/or cross-sectional studies, non-human studies, and non-original studies (e.g. reviews, editorials, and commentaries). Studies of other exposures and endpoints were also excluded.

Data extraction

Two authors (XF and PA) extracted detailed information from each included article independently. Disagreements were resolved through group discussion with the third author (FW). For each study that met the selection criteria, we extracted the following information: basic information (e.g. author, publication year, age and gender of the cohort, cohort size, and number of cases); study characteristics (e.g. the name and geographic location of the study, and

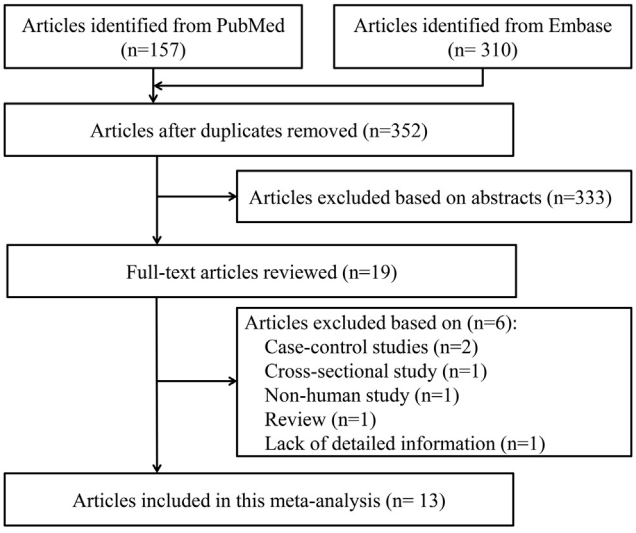


Figure 1 Flow diagram of literature search and study selection.

the follow-up duration); assessment of iron intake; CVD assessment; the parameters that were compared; and risk estimates with 95% confidence intervals. If the risk estimates were reported in several multivariate-adjusted models in the original study, the most thoroughly adjusted model (and its covariates) was extracted.

The quality of each study was assessed by two authors (XW and HW) independently in accordance with the Newcastle-Ottawa Scale (NOS) for non-randomized studies [17]. This scale assigns a maximum of nine points to each study: four points for selection, two points for comparability, and three points for the assessment of outcomes and adequacy of follow-up. Scores of 0–3, 4–6, and 7–9 were regards as reflecting low, moderate, and high quality, respectively.

Statistical analysis

In this meta-analysis, we selected relative risks (RRs) and corresponding 95% confidence intervals (CIs) as the effective risk estimates for all included studies, and hazard ratios (HRs) were treated as RRs directly. Any results stratified by gender were treated as two independent reports. To begin with, we pooled these RRs subsequently by using a random effects model (DerSimonianand-and-Laird method) [18], which combines within-study variations with between-study heterogeneity.

Due to the relatively wide range of definitions for the exposure categories in the included articles, we performed a dose–response analysis, using the method recommended by Greenland and Longnecker and the publicly available Stata code written by Orsini et al. [19,20]. For this method, the doses of iron intake, distributions of cases and person-years, and RRs with 95% confidence intervals were required for at least three quantitative exposure categories. If the total number of cases or person-years was reported (but not the distribution), we estimated the distribution based on the definitions of the quantiles. When neither medians nor means were reported, we used the categorical midpoint. If the highest or lowest category was open-ended, the midpoint of the category was set by assuming that the width of the category is the same as the next adjacent category. To estimate the potential curvilinear association between heme iron, total iron, and CVD risk, we used a restricted cubic splines model with three knots at fixed percentiles (10%, 50%, and 90%) of the distribution [21].

We also conducted subgroup analyses by gender, study location (America or non-America), number of participants ($<$10,000 or $\geq$10,000), follow-up duration ($<$10 years or $\geq$10 years), and study quality (score $\leq$7 or $>$7) to explore the significance of the difference in RRs and the influence of potential residual confounding factors.

Heterogeneity among the studies was estimated using the I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high degrees of heterogeneity, respectively [22]. Publication bias was evaluated by visual inspection of a funnel plot and Egger's regression

Table 1 Characteristics of included prospective cohort studies.

Author, year	Study name	Location	Sex	Age at baseline (years)	No of participants	Follow-up (years)	Exposure assessment	Endpoints (No of cases)
Ascherio et al., 1994 [24]	HPFS	USA	Male	40–75	44,933	4	133-item FFQ	MI (844)
Liao et al., 1994 [25]	NHANES I	USA	Male and female	40–74	4237	13	24HDR	CHD (1151)
Klipstein-Grobusch et al., 1999 [26]	Rotterdam Study	Netherlands	Male and female	55+	4802	4	170-item FFQ	MI (124)
Malaviarachchi et al., 2002 [27]	NSNS	Canada	Male and female	18–74	2198	8	24HDR + FFQ	MI (94);
Lee et al., 2005 [28]	IWHS	USA	Female	55–69	34,492	15	127-item FFQ	CVD mortality (1767)
van der A et al., 2005 [29]	Prospect-EPIC	Netherlands	Female	49–70	16,136	4.3	178-item FFQ	CHD (252)
Qi et al., 2007 [30]	NHS	USA	Female	30–55	6.161	8.8	FFQ	CHD (550)
Galan et al.,2010 [31]	SU.VI.MAX	France	Male and female	35–60	1636	5.4	24HDR	Hypertension (697)
Casiglia et al., 2011 [32]	LEOGRA	Italy	Female	61.1 ± 17.1	906	10	138-item FFQ	CVD (220)
de Oliveira Otto et al., 2012 [33]	MESA	USA	Male and female	45–84	5285	6.2	120-item FFQ	CVD (279)
Zhang et al., 2012 [34]	JACC	Japan	Male and female	40–79	58,615	14.7	33-item FFQ	CVD mortality (2690)
Kaluza et al., 2013 [35]	COSM	Sweden	Male	45–79	38,859	11.7	96-item FFQ	Stroke (3097)
Kaluza et al., 2014 [36]	COSM	Sweden	Male	45–79	36,882	11.7	96-item FFQ	MI (3271)

COSM=Cohort of Swedish Men; EPIC = European Prospective Investigation into Cancer and Nutrition; HPFS=Health Professionals Follow-up Study; IWHS=Iowa Women's Health Study; LEOGRA = Last Evidences of Genetic Risk factors in the Aged; JACC = Japan Collaborative Cohort; NHANES-I=First National Healthand Nutrition Examination Survey; NHS= Nurses' Health Study; NSNS=Nova Scotia Nutrition Survey; MESA = Multi-Ethnic Study of Atherosclerosis; SU.VI.MAX = Supplémentation en Vitamines et Minéraux Antioxydants; CHD = coronary heart disease; CVD = cardiovascular disease; FFQ = food frequency questionnaire; 24HDR = 24-h dietary record.

Table 2 Outcomes and covariates of included studies.

Study	Case ascertainment	Comparison	RRs or HRs (95%CI)	Covariates in fully adjusted model
Ascherio et al., 1994 [24]	Confirmed by medical records or autopsy, and interviews with family members	Dietary total iron intake: highest (median 37 mg/d) *v* lowest (median 11 mg/d) quintile	0.72 (0.51−1.00)	Age
		Dietary heme iron intake: highest (median 2.1 mg/d) *v* lowest (median 0.7 mg/d) quintile	1.48 (1.01−2.16)	Age; body mass; smoking habits; alcohol consumption; history of hypertension, diabetes, hypercholesterolemia; family history of MI; profession; and intake of total energy, vitamin E, saturated fat and cholesterol
Liao et al., 1994 [25]	Confirmed by death certificates, and hospital (or nursing home) records	Dietary total iron intake: highest (NA) *v* lowest (NA) quintile (Men)	0.96 (0.86−1.08)	Baseline age, systolic blood pressure, serum cholesterol, education, and cigarette smoking
		Dietary total iron intake: highest (NA) *v* lowest (NA) quintile (Women)	0.99 (0.84−1.17)	
Klipstein-Grobusch et al., 1999 [26]	Confirmed by research physicians using the records of participating general practitioners and medical specialists	Dietary total iron intake: highest (>12.4 mg/d) *v* lowest (<10.6 mg/d) quintile	1.11 (0.67−1.87)	Age; sex; BMI; pack-year of smoking; equivalent household intake; education; alcohol intake; energy-adjusted β-carotene, vitamin C, vitamin E, fat, saturated fat and cholesterol; and use of antioxidative vitamin supplements
		Dietary heme iron intake: highest (>1.0 mg/d) *v* lowest (<0.7 mg/d) quintile	1.86 (1.14−3.09)	
Malaviarachchi et al., 2002 [27]	Confirmed by hospital discharge records, physician visits, and vital statistics	Dietary total iron intake: highest (median 9.82 mg/d) *v* lowest (median 4.69 mg/d) quintile	0.59 (0.34−1.05)	Age, gender, smoking, Body Mass Index, diabetes, income, dietary lipids, saturated fat, and cholesterol
		Dietary heme iron intake: highest (median 1.33 mg/d) *v* lowest (median 0.15 mg/d) quintile	0.65 (0.37−1.14)	
Lee et al., 2005 [28]	Confirmed by the State Health Registry of Iowa	Dietary non-heme iron intake: highest (median 24.4 mg/d) *v* lowest (median 6.9 mg/d) quintile	Alcohol consumption 0−9 g/d: 1.05 (0.81−1.35) Alcohol consumption≥10 g/d: 1.20 (0.57−2.51) Alcohol consumption≤30 g/d: 1.67 (0.53−5.23)	Age, energy intake, BMI, waist-hip ratio, physical activity, cigarette smoking, alcohol consumption, hormone replacement therapy, high blood pressure, saturated fat, , polyunsaturated fat, folate, -trans fatcarotene, vitamin E, and vitamin C
		Dietary heme iron intake: highest (median 2.43 mg/d) *v* lowest (median 0.57 mg/d) quintile	Alcohol consumption 0−9 g/d: 0.94 (0.71−1.26) Alcohol consumption≥10 g/d: 2.47 (1.10−5.55) Alcohol consumption≤30 g/d: 4.11 (1.14−14.87)	
van der A et al., 2005 [29]	Obtained from the Dutch Centre for Health Care Information	Dietary total iron intake: highest (>11.43 mg/d) *v* lowest (<9.59 mg/d) quintile	0.98 (0.61−1.58)	Age, total energy intake, BMI, smoking, physical activity, hypertension, diabetes, hypercholesterolemia, energy-adjusted saturated fat intake, energy-adjusted carbohydrate
		Dietary non-heme iron intake: highest (>9.56 mg/d) *v* lowest (<7.88 mg/d) quintile	0.68 (0.42−1.12)	

(*continued on next page*)

Table 2 (*continued*)

Study	Case ascertainment	Comparison	RRs or HRs (95%CI)	Covariates in fully adjusted model
		Dietary heme iron intake: highest (>2.27 mg/d) *v* lowest (<1.28 mg/d) quintile	1.65 (1.07−2.53)	intake, energy-adjusted fiber intake, energy-adjusted alcohol intake, energy-adjusted β-carotene intake, energy-adjusted vitamin E intake, energy-adjusted vitamin C intake
Qi et al., 2007 [30]	Confirmed by review of medical records or autopsy reports	Dietary total iron intake: highest (NA) *v* lowest (NA) quintile	1.32 (0.95−1.84)	Age, BMI, smoking, alcohol, physical activity, aspirin use, duration of
		Dietary heme iron intake: highest (median 2.83 mg/d) *v* lowest (median 1.70 mg/d) quintile	1.43 (1.01−2.01)	diabetes, history of hypertension and hypercholesterolemia, postmenopausal hormone use, family history of CHD, cereal fiber, glycemic load, polyunsaturated fat−to−saturated fat ratio, trans fat intakes, multivitamin use, and vitamin C
Galan et al.,2010 [31]	Measured by a trained investigator during clinical examinations	Dietary non-heme iron intake: highest (median 13.7 mg/d) *v* lowest (median 7.7 mg/d) quintile	0.61 (0.40−0.92)	Age, sex, smoking status, antioxidant supplementation group, baseline BMI, baseline energy intake (kJ/d), baseline physical activity, sodium, potassium,
		Dietary heme iron intake: highest (median 1.9 mg/d) *v* lowest (median 0.7 mg/d) quintile	0.89 (0.67−1.20)	vitamin C, alcohol cholesterol, SFA, and PUFA intakes
Casiglia et al., 2011 [32]	Monitored through the Italian Register's Office and confirmed by hospitals, retirement homes, and physicians' files	Dietary total iron intake: highest (median 8.7 mg/d) *v* lowest (median 3.9 mg/d) quintile	0.97 (0.62−1.53)	Serum iron, prevalence of anemia, intake of fibers and caffeine, and total daily energy intake
		Dietary non-heme iron intake: highest (NA) *v* lowest (NA) quintile	1.32 (0.87−2.00)	
		Dietary heme iron intake: highest (NA) *v* lowest (NA) quintile	1.39 (0.75−1.72)	
de Oliveira Otto et al., 2012 [33]	Confirmed by cohort clinic visits, telephone calls to participants, medical record abstractions, or obituaries	Dietary non-heme iron intake: highest (14.8 mg/d) *v* lowest (6.4 mg/d) quintile	0.95 (0.70−1.2 9)	Energy intake, age, sex, race-ethnicity, education, study center, alcohol intake, physical activity, BMI, fiber intake,
		Dietary heme iron intake: highest (1.2 mg/d) *v* lowest (0.3 mg/d) quintile	1.06 (0.81−1.40)	cigarette smoking, dietary supplement use, the ratio of polyunsaturated fat intake:saturated fat intake, Mg, and antioxidant intake
Zhang et al., 2012 [34]	Confirmed by death certificates	Dietary total iron intake: highest (median 10.58 mg/d) *v* lowest (median 5.12 mg/d) quintile (Man)	1.27 (1.01−1.58)	BMI, smoking, ethanol intake, history of hypertension and diabetes, sports time, walking time, educational,
		Dietary non-heme iron intake: highest (median 10.19 mg/d) *v* lowest (median 3.84 mg/d) quintile (Man)	1.04 (0.86−1.26)	perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy
		Dietary heme iron intake: highest (median 0.44 mg/d) *v* lowest (median 0.07 mg/d) quintile (Man)	0.96 (0.81−1.14)	
			0.94 (0.77−1.15)	

		Dietary total iron intake: highest (median 9.81 mg/d) *v* lowest (median 5.14 mg/d) quintile (Women)		
		Dietary non-heme iron intake: highest (median 9.46 mg/d) *v* lowest (median 3.81 mg/d) quintile (Women)	0.99 (0.83−1.19)	
		Dietary heme iron intake: highest (median 0.48 mg/d) *v* lowest (median 0.06 mg/d) quintile (Women)	0.94 (0.79−1.12)	
Kaluza et al., 2013 [35]	Confirmed by linkage with the Swedish Hospital Discharge Registry	Dietary non-heme iron intake: highest (median 16.9 mg/d) *v* lowest (median 9.4 mg/d) quintile	0.99 (0.85−1.14)	Age, education, smoking status and pack-years of smoking, body mass index, total physical activity, history of hypertension, high blood cholesterol level, diabetes mellitus, aspirin use, family history of myocardial infarction, alcohol intake and intake of dietary fiber, vitamin E, β-carotene, vitamin C, and calcium
		Dietary heme iron intake: highest (median 2.69 mg/d) *v* lowest (median 1.04 mg/d) quintile	1.16 (1.03−1.31)	
Kaluza et al., 2014 [36]	Confirmed by linkage with the Swedish Hospital Discharge Registry	Dietary non-heme iron intake: highest (median 16.8 mg/d) v lowest (median 9.4 mg/d) quintile (Fatal)	0.93 (0.67−1.30)	Age, education, smoking status, total physical activity, history of hypertension, high blood cholesterol level, ever aspirin use, regular supplement use, family history of myocardial infarction before age of 60 years, alcohol consumption and quintiles of energy-adjusted intakes of protein, saturated fat, PUFA, cholesterol, fiber, vitamin E, β-carotene, vitamin C, potassium, sodium, calcium, magnesium, and zinc
		Dietary non-heme iron intake: highest (median 16.8 mg/d) v lowest (median 9.4 mg/d) quintile (Non-Fatal)	0.93 (0.78−1.11)	
		Dietary heme iron intake: highest (median 2.68 mg/d) v lowest (median 1.04 mg/d) quintile (Fatal)	1.51 (1.07−2.13)	
		Dietary heme iron intake: highest (median 2.68 mg/d) v lowest (median 1.04 mg/d) quintile (Non-Fatal)	0.95 (0.7 9−1.13)	

test (with significance set to $P < 0.1$) [23]. All statistical analyses were performed with Stata version 11 (StataCorp LP, College Station, TX, USA). All tests were two-tailed, and differences were considered to be significant if $P < 0.05$.

Results

Search results

Our search yielded 157 articles from PubMed and 310 articles from Embase that were published prior to July 31, 2014. After duplicate studies and studies that did not satisfy the inclusion criteria were excluded, 13 prospective cohort studies were ultimately included in the meta-analysis [24–36].

Nine of these articles reported the association between dietary iron intake and the risk of CHD [24–27,29,30,32,34,36]; three described the association between dietary iron intake and the risk of stroke [32,34,35]; one described the association between dietary iron intake and the risk of hypertension [31]; and four described the association between dietary iron intake and the risk of total CVD [28,32–34]. The detailed steps used in selecting the studies are shown in Fig. 1.

Study characteristics

Tables 1 and 2 summarize the information extracted from the studies, all of which had a prospective cohort design and participants who had no prior diagnosis of CVD at baseline. These studies regarding heme iron were published from 1994 through 2014 and included a total of 252,164 participants – including 15,040 CVD cases – with mean follow-up durations that ranged from 4 to 15 years. Five studies were performed in the United States [24,25,28,30,33], six studies were performed in Europe [26,29,31,32,35,36], and the remaining two studies were performed in Canada [27] and Japan [34]. With the exception of the study performed in France [31], heme iron intake was assessed by administering a food frequency questionnaire (FFQ). The average score was 7.7, and the scores for all of the studies were 6 or higher (moderate or high quality) (Table S1).

Association between dietary intake of heme iron and the risk of CVD

Eight papers [24,26–36] with twelve reports reported an association between dietary heme iron intake and the risk of CVD. The total risk estimate in the Iowa Women's Health Study [28] was not directly reported, and thus this was pooled from the results stratified by alcohol consumption. Overall, heme iron intake for the highest *vs.* lowest category was positively correlated with risk (RR: 1.19, 95% CI: 1.06–1.34) (Fig. 2). There was moderate study heterogeneity ($I^2 = 57.3\%$). No publication bias was observed based on either the funnel plot (Fig. S1) or Egger's test ($P = 0.90$).

One study [32] was not eligible for the dose–response analysis because it did not report specific data regarding the level of heme iron intake. Using a dose–response analysis, we found that each 1 mg/day increment in dietary heme iron intake was associated with a 6% higher risk of CVD (incremental RR: 1.07, 95% CI: 1.01 to 1.14; Fig. 3), with moderate heterogeneity ($I^2 = 59.7\%$) and a significant trend ($P < 0.05$) towards linearity. In addition, a significant curvilinear association was also revealed using the restricted cubic splines model ($P < 0.05$ for non-linearity; Fig. 4).

Association between dietary intake of non-heme iron and the risk of CVD

Seven papers [28,29,31,33–36] with eight reports were included in the analysis of non-heme iron and CVD risk. The pooled relative risk of CVD for the highest *vs.* lowest category of dietary non-heme iron intake was 0.99 (95% CI: 0.92 to 1.06; Fig. S2), with no heterogeneity ($I^2 = 0\%$). No publication bias was observed based on either the funnel plot (Fig. S3) or Egger's test ($P = 0.70$).

All six of these studies were eligible for inclusion in the trend estimate. A dose–response analysis revealed no association between CVD risk per 5 mg/day increment and non-heme iron intake (RR: 0.98, 95% CI: 0.96 to 1.01; Fig. S4), with low heterogeneity ($I^2 = 15.8\%$). We found no curvilinear association between non-heme iron intake and risk ($P = 0.26$ for non-linearity; Fig. 5).

Association between dietary intake of total iron and the risk of CVD

Eight papers [24–27,29,30,32,34] with ten reports were included in the analysis of total iron and CVD risk. The combined relative risk of CVD for the highest *vs.* lowest category of dietary total iron intake was 0.99 (95% CI: 0.89 to 1.11; Fig. S5), with moderate heterogeneity ($I^2 = 40.3\%$). No publication bias was observed based on either the funnel plot (Fig. S6) or Egger's test ($P = 0.90$).

Similar to the dose–response curves for non-heme iron, a higher intake of total iron (an increment of 5 mg/day) was not associated with an increase in CVD risk (RR: 1.00, 95% CI: 0.94 to 1.06; Fig. S7), with low heterogeneity ($I^2 = 30.4\%$). Using a restricted cubic splines model, we found no evidence of a curvilinear association between total iron intake and risk ($P = 0.88$ for non-linearity; Fig. 6).

Subgroup analyses

We performed subgroup analyses for both heme iron intake and total iron intake in order to identify potential sources of heterogeneity (Table 3). With respect to total iron intake, the associations were similar – and not significant – in the various subgroups, which were based on gender, study location, number of participants, duration of follow-up, and whether CVD cases were fatal.

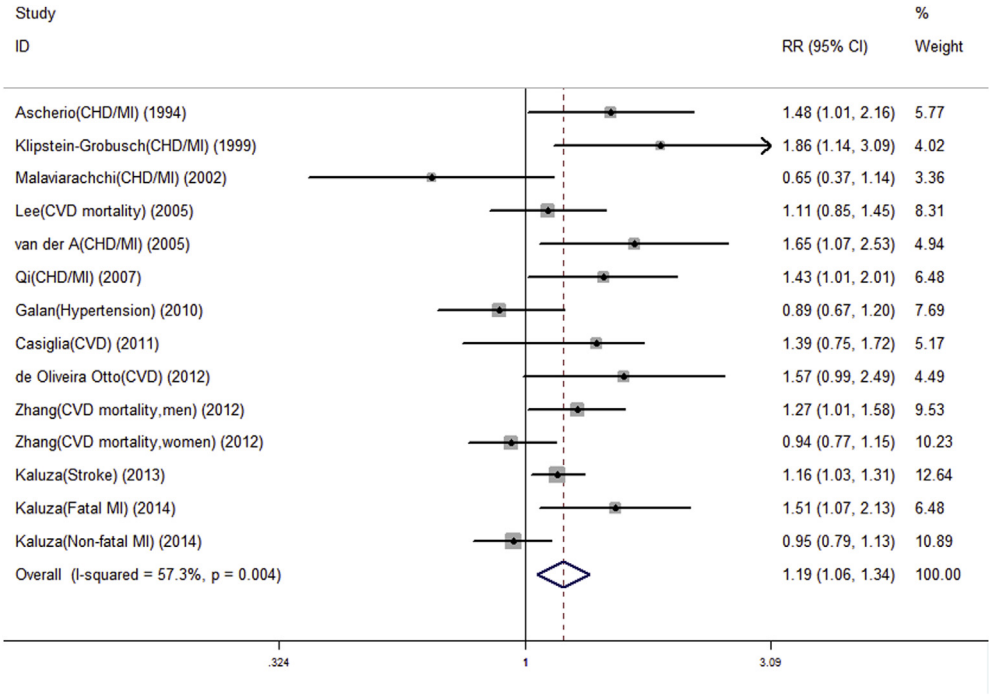


Figure 2 Forest plot of CVD for the highest *vs.* lowest category of dietary heme iron intake.

With respect to heme iron intake, we found a significant association only in the studies that were based on American cohorts (RR: 1.31, 95% CI: 1.11–1.56), with no heterogeneity ($I^2 = 0$). Moreover, the association between heme iron intake and CVD risk was more robust among non-fatal CVD cases (RR: 1.19, 95% CI: 1.07–1.33) than fatal CVD cases (RR: 1.10, 95% CI: 0.95–1.28), with no heterogeneity ($I^2 = 0$).

Discussion

Findings

Our meta-analysis revealed that dietary intake of heme iron – but not non-heme or total iron intake – was found to be significantly associated with a greater risk of

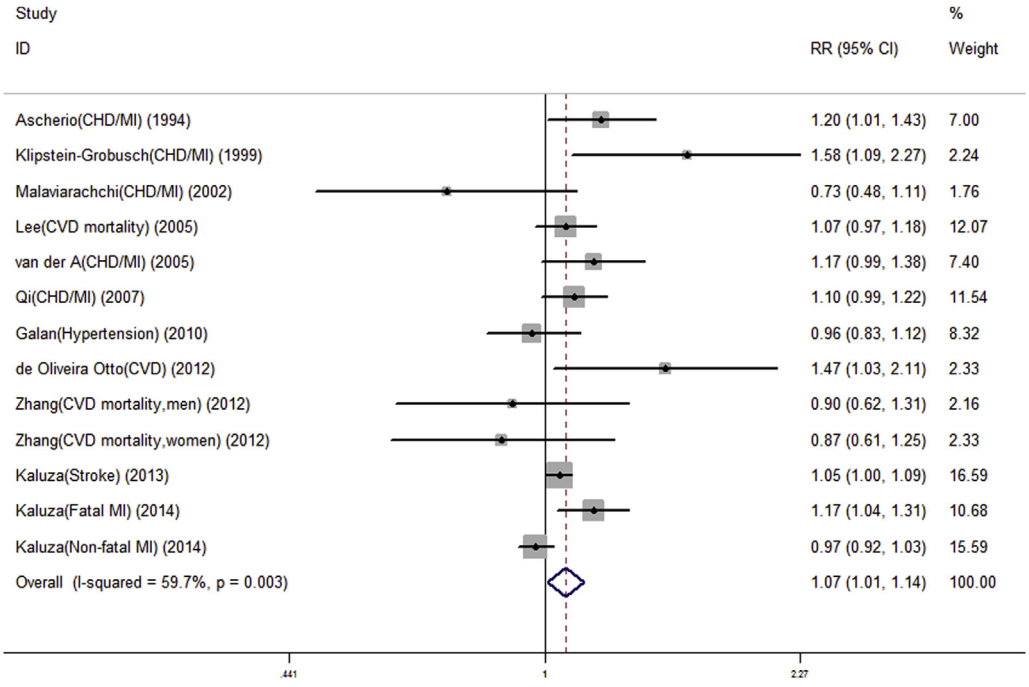


Figure 3 Dose–response meta-analysis of each 1 mg/day increment in dietary heme iron intake and the risk of CVD.

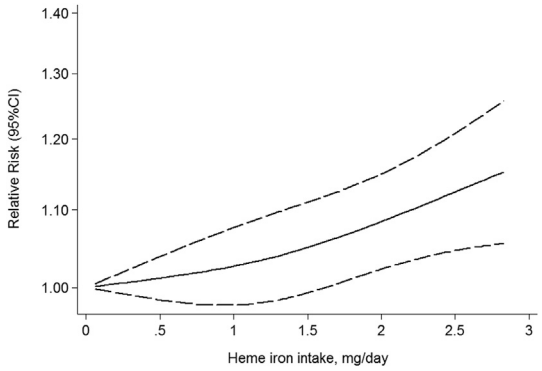


Figure 4 Dose–response analysis of the curvilinear association between dietary heme iron intake and the risk of CVD.

cardiovascular disease (CVD). The dose–response analyses revealed a 7% increase in the risk of cardiovascular disease for each 1 mg/day increase in dietary heme iron; no significant trend was identified for either non-heme intake or total iron intake. Subgroup analyses revealed that the association between heme iron intake and CVD risk was more robust in the United States, and this might be attributed to a greater intake of red meat in this population [37].

Ever since the relationship between iron and cardiovascular disease was proposed by Jerome Sullivan in 1981 [38], many studies have attempted to confirm this hypothesis. Ascherio et al. were the first group to analyze the association between iron intake and the risk of coronary disease by performing a large prospective study of nearly 45,000 American men 40–75 years of age [24]. After adjusting for other risk factors, the authors found an increased risk of myocardial infarction (MI) among men with higher intake of heme iron compared to total iron intake. Five years later, Klipstein-Grobusch et al. performed the Rotterdam Study and arrived at a similar conclusion, noting that high dietary heme iron intake may specifically affect MI-related mortality [26]. The results of a case–control study in Greece also support the hypothesis that excess dietary iron intake increases the risk of CVD [39]. Data from the Nurses' Health Study (NHS) and the Iowa Women's Health Study (IWHS) indicate that higher

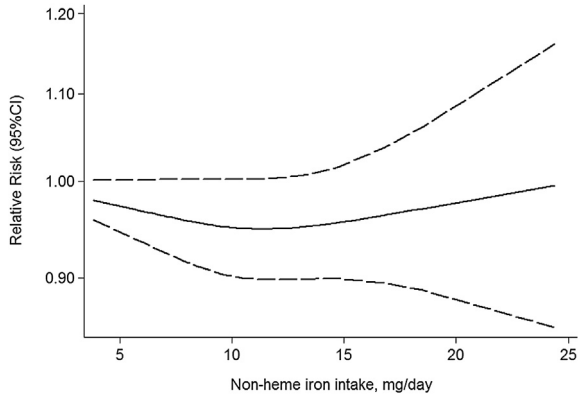


Figure 5 Dose–response analysis of the curvilinear association between dietary non-heme iron intake and the risk of CVD.

heme iron intake might increase CVD risk among women with type 2 diabetes and alcoholics [28,30]. Zhang et al. reported that dietary intake of total iron was positively correlated with mortality due to stroke and total CVD among Japanese men [34]. Moreover, a recent large cohort study in Sweden suggested that high heme iron intake might increase the risk of stroke [38]. Finally, the clinical effects of dietary iron intake on cardiovascular pathophysiology are supported by experiments in rats. Castellanos et al. found that infarct volume after permanent middle cerebral artery occlusion was 66% greater in the iron fed rats than in the control group [40]. Using Dahl salt-sensitive rats, Naito et al. indicated that dietary iron restriction could be an effective strategy for prevention of hypertensive cardiovascular remodeling [41].

In contrast, however, several studies found no evidence to support a significant association between dietary iron intake and the risk of CVD. For example, Reunanen et al. found no association between dietary iron intake and coronary mortality [42]. A study from India also found that there was no difference between dietary iron intakes in CHD cases and healthy controls [43]. In addition, two studies reported that dietary iron intake may serve as a protective factor against the development of hypertension and MI [44,45].

To the best of our knowledge, this is the first dose–response meta-analysis to examine the effect of dietary iron intake on total cardiovascular disease morbidity and mortality. Iron is an essential trace element in the human body and an integral component of cytochromes, oxygen-binding molecules, and many enzymes. Because of iron's central – and essential – roles in physiology, maintaining iron homeostasis requires 1–3 mg of absorbed iron per day to offset losses from desquamated cells [46]. On the other hand, excess iron can also injure organs by catalyzing the production of reactive oxygen species (ROS), which cause lipid peroxidation, oxidation of amino acids, protein fragmentation, and DNA damage [9–11].

In a typical human diet, the two forms of iron - heme and non-heme - have different dietary sources, absorption mechanisms, and metabolic pathways [47]. Heme iron, which constitutes approximately 15% of our total iron intake, is present exclusively in the hemoglobin and myoglobin of red meat, poultry, and fish. For example, a three-ounce portion of lean beef contains 1.1 mg of absorbable heme iron [48]. The remaining dietary iron fraction is comprised of non-heme iron, which is present in cereals, fruits, and vegetables. Because heme iron is protected within the porphyrin complex, its absorption rate is 5–10-fold higher than non-heme iron [49].

In addition to its peroxidative properties and its high absorption rate in the duodenum, dietary heme iron is also associated with C-reactive protein, a classic biomarker of inflammation; inflammation contributes to both the onset and progression of atherosclerosis [50]. Furthermore, a meta-analysis of prospective studies revealed that increased heme iron intake and higher body iron stores are significantly associated with a higher risk of type 2 diabetes mellitus, a known risk factor for CVD [51].

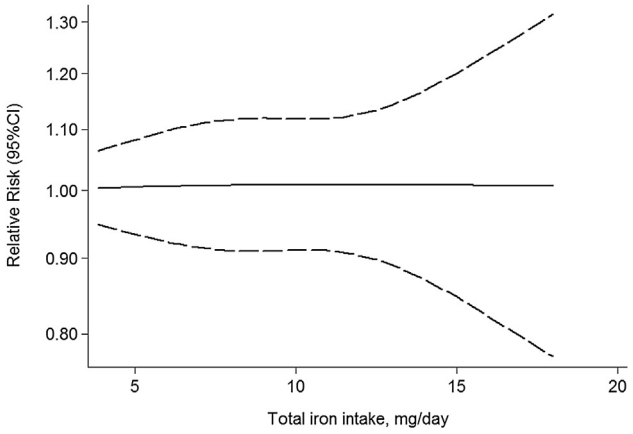


Figure 6 Dose–response analysis of the curvilinear association between dietary total iron intake and the risk of CVD.

Strengths and limitations

A noteworthy strength of this meta-analysis is the prospective design of the included studies, which eliminated any possible selection bias or recall bias, which can occur in retrospective case–control studies. Moreover, many of the studies in this meta-analysis had large sample sizes and long follow-up durations, which provided high statistical power to our quantitative assessment of the correlation between dietary iron intake and the risk of CVD. In comparison to the similar study by Hunnicutt et al., the quality of our meta-analysis was increased by generating dose–response curves in addition to the comparisons of the highest and lowest categories [15]. Finally, the relatively large number of studies regarding heme iron intake and total iron intake enabled us to perform subgroup analyses based on gender, study location, number of participants, and follow-up duration.

Despite some heterogeneity, the findings were consistent and statistically significant. We also systematically reviewed and assessed the summarized association between CVD and various forms of dietary iron.

Our meta-analysis has several potential limitations as well. First, most of the included studies measured dietary iron intake at baseline only, rather than updating the dietary information; thus, the intake of iron may have changed during the follow-up period, resulting in possible misclassification and regression dilution bias [52]. Another potential limitation is that the majority of dietary data were collected using food frequency questionnaires (FFQs). Although most of these questionnaires were validated and were shown to have reasonable reproducibility, a potential misclassification of exposure was unavoidable [53].

Moreover, because of the observational nature of the included studies, the possibility of residual confounding due to other risk factors cannot be excluded, although most investigators had adjusted for major potential confounders (for example, cholesterol level, alcohol intake, and smoking status) in their original studies. In addition, the available data regarding dietary intake of iron – particularly non-heme iron – is relatively limited. Therefore, additional prospective studies are needed in order to obtain a more detailed analysis.

Conclusions

In summary, the results of our meta-analysis suggest that heme iron intake is a risk factor for CVD; in contrast, we found no evidence that either non-heme intake or total iron intake is correlated with CVD risk. Our use of linear and nonlinear models, as well as dose–response analyses with detailed information, supports these results. Our findings may have important public health implications with respect to developing guidelines to prevent cardiovascular disease by reducing the daily consumption of foods that are high in heme iron.

Table 3 Subgroup analyses of dietary intake of heme and total iron and risk of CVD.

Subgroup	Heme iron intake			Total iron intake		
	No of reports	Relative risk (95% CI)	I^2 (%)	No of reports	Relative risk (95% CI)	I^2 (%)
CVD cases						
Fatal	8	1.10 (0.95–1.28)	52.9	3	1.11 (0.60–2.05)	66.2
Non-fatal	5	1.19 (1.07–1.33)	0	3	1.00 (0.61–1.63)	69.6
Sex						
Male	5	1.12 (0.96–1.31)	64.7	3	0.98 (0.76–1.26)	76.4
Female	5	1.22 (0.98–1.51)	59.8	4	1.02 (0.87–1.19)	1.6
Location						
America	4	1.31 (1.11–1.56)	0	4	0.97 (0.84–1.13)	53.4
Non-America	10	1.16 (0.99–1.37)	62.2	6	1.01 (0.84–1.21)	38.3
No of participants						
≥10,000	8	1.11 (1.00–1.24)	45.1	4	0.98 (0.77–1.24)	64.3
<10,000	6	1.32 (0.92–1.63)	65.2	6	0.99 (0.88–1.12)	24.2
Duration of follow-up						
≥10	7	1.10 (0.96–1.25)	26.4	5	1.01 (0.91–1.11)	24.1
<10	7	1.26 (0.94–1.68)	68.3	5	0.92 (0.69–1.24)	58
Study quality						
Score ≤ 7	6	1.18 (0.99–1.40)	64.3	4	0.95 (0.87–1.04)	0
Score > 7	8	1.21 (1.01–1.45)	57.0	6	1.05 (0.87–1.28)	49.4

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2014.09.002.

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