Original Article

Dietary iron intake and risk of death due to cardiovascular diseases: A systematic review and dose–response meta-analysis of prospective cohort studies

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Background and Objectives: Many studies have investigated the association between dietary iron intake and death due to cardiovascular disease (CVD), but the results were inconsistent. We performed a dose–response meta-analysis to quantitatively assess the risk of CVD mortality with dietary intake of iron (total iron, heme iron, and non-heme iron). **Methods and Study Design:** PubMed and Embase databases were searched for articles published up to February 21, 2019. Prospective cohort studies were included if reporting relative risks (RRs) and 95% confidence intervals (CIs) for risk of CVD mortality associated with dietary iron intake. Restricted cubic splines were used to model the dose–response association. **Results:** We included eight articles (19 studies including 720,427 participants [46,045 deaths due to CVD]) in the meta-analysis. When comparing the highest versus lowest level of dietary heme iron intake, the pooled RR for CVD mortality was 1.19 (95% CI, 1.01–1.39). With a 1-mg/day increase in dietary heme iron intake, the pooled RR for death due to CVD, stroke, coronary heart disease, and myocardial infarction were 1.25 (95% CI, 1.17–1.33), 1.17 (1.04–1.32), 1.25 (0.70–2.22), and 1.17 (0.55–2.50) respectively. The association between dietary iron intake and CVD mortality was linear ($p_{nonlinearity}>0.05$). **Conclusions:** Higher dietary intake of heme iron was associated with a greater risk of CVD mortality. Reducing consumption of heme iron may help to prevent premature death due to CVD.

Key Words: cardiovascular disease, mortality, dietary iron intake, dose–response meta-analysis, prospective cohort studies

INTRODUCTION

Cardiovascular disease (CVD) mortality, the leading cause of death, accounted for 31.8% deaths worldwide in 2017 and has increased by 21.1% in the past 20 years.[1] More than 25% of the CVD mortality must be reduced to lower non-communicable disease-related premature mortality by 25% by 2025 according to the 25×25 Global Action Plan launched by the World Health Organization in 2013.[2,3] The global action plan emphasizes the importance of diet, and relevant preventive approaches are essential to control this serious situation[4] and achieve the target.[2]

Iron, an essential nutrient for humans, has important biological functions, including oxygen transportation, cellular respiration, and vitamin A generation.[5] Iron supplementation is widely used to prevent anemia especially in developing countries.[6] Nevertheless, recent published

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studies have shown increased dietary iron intake, especially heme iron, positively associated with diabetes mellitus,[7] metabolic syndrome,[8] and CVD.[9] A previous meta-analysis did not find dietary intake of iron (total iron, heme iron, and non-heme iron) associated with coronary heart disease (CHD) mortality.[10] However, the meta-analysis missed one study[11] and additional studies reporting a positive association have been published recently.[12, 13] Moreover, the meta-analysis did not quantify the association between dietary iron intake and CHD mortality and included only four relevant articles.[10]

We conducted a systematic review and dose–response meta-analysis to estimate the association between dietary intake of iron (total iron, heme iron, and non-heme iron) and risk of CVD mortality.

METHODS

Search strategy and selection criteria

The PubMed and Embase databases were searched for all articles published up to February 21, 2019 by using the literature search strategy (Supplementary table 1) and with restriction on English language. Reference lists of identified articles were manually searched for relevant articles.

Studies were included if they: (1) they were prospective cohort studies of participants aged ≥18 years; (2) dietary iron intake was assessed and at least divided into three levels at baseline; (3) the study assessed risk of death due to CVD (total CVD, CHD, stroke, and myocardial infarction [MI]); and (4) the article reported the multivariate-adjusted relative risks (RRs) or hazard risks (HRs) and 95% confidence intervals (CIs) for the outcomes associated with dietary iron intake.

Studies were excluded if they were: (1) conference summaries or clinical trial reports; and (2) derived from the same cohort, secondary analyses, or combined analysis of other cohort studies. If studies reported total CVD and types of CVD, the information for types of CVD was used in the subgroup analyses. We followed the PRISMA criteria for reporting of Meta-analyses of Observational Studies in Epidemiology.[14]

Data extraction and quality assessment

Two independent researchers (M.H. and R.Q.) initially screened all titles and/or abstracts, and M.H. screened the 19 potentially relevant articles identified from the initial screening (Figure 1). All discrepancies were resolved by discussion with another investigator (D.Z.). The following information was extracted from articles: first author name, publication year, country, study name, sample size, number of deaths, type of CVD, follow-up duration, sex, baseline age, dietary iron intake assessment, CVD mortality assessment, amount of dietary iron intake, RRs/HRs and 95% CIs for dietary iron intake category, CVD deaths per dietary iron category, total participants or person years per dietary iron category, and variables adjusted for in the included studies. If the required information could not be obtained from the original articles, we contacted the authors for additional information.

The quality of each study was assessed by the Newcas-

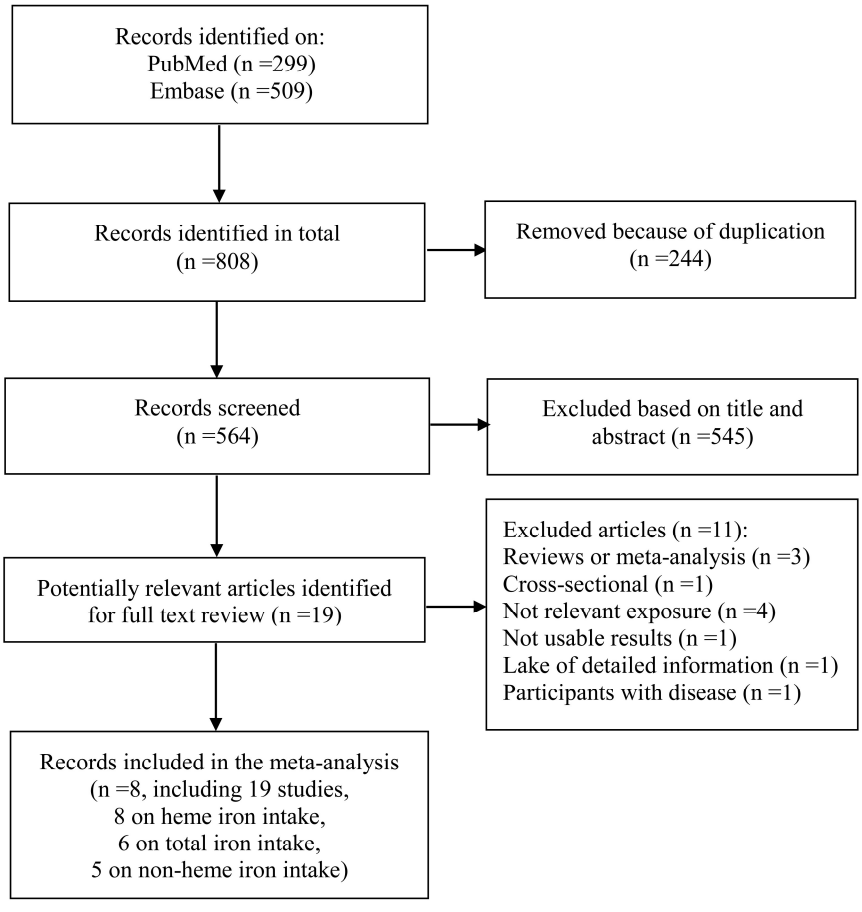


Figure 1. Flowchart of study selection.

tle-Ottawa Scale (NOS).[15] Scores ranged from 0 to 9 points, with higher scores indicating higher study quality. Scores of 0-3, 4-6, and 7-9 were considered as poor, fair, and good quality, respectively.

Data synthesis and analysis

The multivariate-adjusted RRs (with 95% CIs) were used as the effective risk estimates for all included studies, and HRs were considered as RRs.[16] Articles that stratified the data by sex or dietary iron types were considered independent studies. Data reporting the results separately by alcohol consumption were pooled in the fixed-effects model before inclusion in the meta-analysis.[17] A random-effects model was used to pool RRs and 95% CIs for CVD mortality for the highest versus lowest iron intake level and per 1-mg/day increase in heme iron intake and per 5-mg/day increase in total iron and non-heme iron intake if heterogeneity I^2 ≥50%; otherwise, a fixed-effects model was used.

Generalized least squares regression was used to estimate a study-specific dose–response association. The DerSimonian and Laird random-effects model[18] was used to pool the study-specific dose–response RR estimates. First, a linear association was assumed; study-specific RR estimates were calculated per 1-mg/day increase in heme iron intake and per 5-mg/day increase in total iron and non-heme iron intake and then pooled, respectively. In addition, we examined a possible nonlinear association by using restricted cubic splines with three knots at the 25th, 50th, and 75th percentiles of the distribution. The p value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to zero.

Heterogeneity was tested by Cochran Q and I^2 statistics.[19] p<0.10 was considered statistically significant for the Q statistic, and I^2 ≈25%, 50%, 75% were considered low, moderate, and high heterogeneity, respectively.[20] Subgroup analyses were conducted by type of CVD mortality, sex, region, follow-up duration, and sample size. A sensitivity analysis was performed by omitting one study at a time to assess the stability of the results and potential sources of heterogeneity. Publication bias was evaluated by Egger's and Begg's test with p<0.10 indicating potential publication bias. All analyses involved using Stata 12.1 (Stata Corp, College Station, TX). Two-tailed p<0.05 was considered statistically significant if not specified.

RESULTS

We identified 808 potentially relevant articles from PubMed and Embase databases; eight articles (19 prospective cohort studies) with a total of 720,427 study participants and 46,045 deaths due to CVD mortality cases were finally included in the meta-analysis (Figure 1). Eight studies provided information on the association between CVD mortality and dietary heme iron intake,[11-13,17,21-23] six studies on dietary total iron intake,[11,21,23,24] and five studies on non-heme iron intake.[11,13,17,21] Figure 1 details the selection and exclusion process.

The characteristics of the prospective cohort studies are in Table 1. Among the eight articles, two were from Asia,[21,24] three from the United States,[13,17,23] and three from Europe.[11,12,22] Sample size ranged from 90611 to 536,96513 and follow-up duration from 4.0 years[23] to 15.6 years.[13] Four articles included both men and women.[13,21,22,24] All studies were graded as good quality and most studies adjusted adequately for several potential confounders (Supplementary table 2).

Dietary heme iron intake and CVD mortality

Eight studies from seven articles reported an association between dietary heme iron intake and risk of CVD mortality. When comparing the highest versus lowest level of dietary heme iron intake, the pooled RR for CVD mortality was 1.19 (95% CI, 1.01–1.39; I^2 =67.5%; $p_{\text{heterogeneity}}$ =0.003; Supplementary figure 1). One study was not eligible for the dose–response analysis because of lacking of information on dietary heme iron intake.[11] In the dose–response analysis, the pooled RR for CVD mortality was 1.25 (95% CI, 1.17–1.33; I^2 =27.9%; $p_{\text{heterogeneity}}$ =0.216; Figure 2) per 1-mg/day increase in dietary of heme iron. Furthermore, we found no evidence of a non-linear

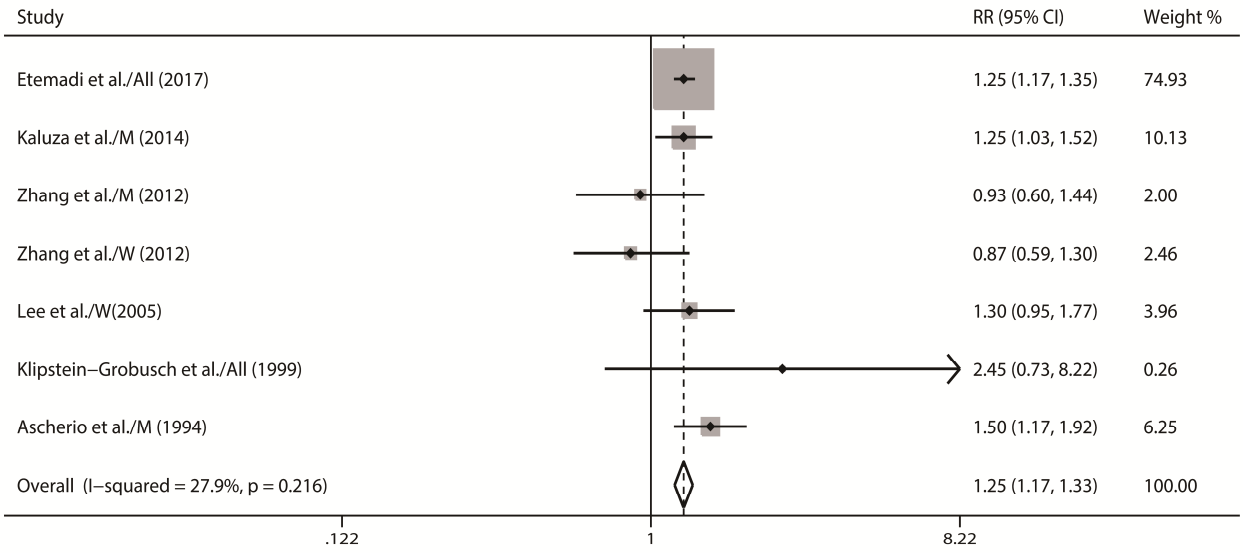


Figure 2. Forest plot for intake of dietary heme iron intake (per 1-mg/day) and risk of cardiovascular disease mortality.

Table 1. Characteristics of included prospective cohort studies.

First author, publication year	Country	Sample size (% men)	Mean or range age (years)	Follow-up (years)	Exposure assessment	Outcome assessment	Endpoint (no of cases)
Shi, (2017) [24]	China	2,832 (45.90)	46.64	9.8	3-day weighed food diary	Household visit and death registry	CVD mortality (70)
Etemadi, (2017) [13]	United States	536,965 (58.94)	62.16	15.6	124-item DHQ	Death master file and National Death Index	CVD mortality (40,580)
Kaluza, (2014) [12]	Sweden	36,882 (100)	45-79	11.7	96-item FFQ	Death registry	MI mortality (678)
Zhang, (2012) [21]	Japan	58,615 (39.38)	40-79	14.7	33-item FFQ	Death certificate	CVD mortality (2,690)
Casiglia, (2011) [11]	Italy	906 (0)	61.1	10.0	138-item FFQ	Death registry and physician's file	CVD mortality (83)
Lee, (2005) [17]	United States	34,492 (0)	55-69	15.0	127-item FFQ	Death registry and National Death Index	CVD mortality (1,767)
Klipstein-Grobusch, (1999) [22]	Netherlands	4,802 (NA)	≥55	6.0	170-item SFFQ	Death registry	MI mortality (30)
Ascherio, (1994) [23]	United States	44,933 (100)	40-75	4.0	133-item FFQ	Medical records	CHD mortality (147)

CVD: cardiovascular disease; CHD: coronary heart disease; DHQ: diet history questionnaire; FFQ: food frequency questionnaire; MI: myocardial infarction; SFFQ: semi-quantitative food frequency questionnaire; NA: not available.

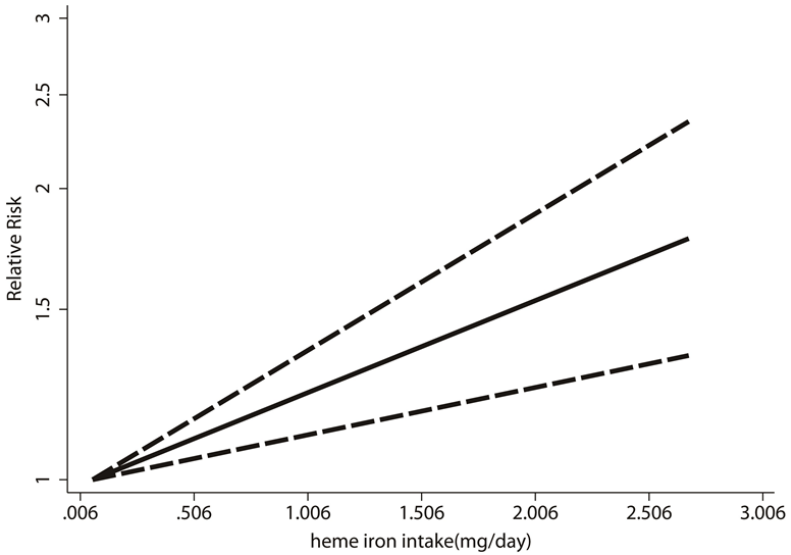


Figure 3. Linear dose–response association between dietary heme iron intake and cardiovascular diseases mortality.

association between dietary heme iron intake and CVD mortality ($p_{nonlinearity}$ =0.394; Figure 3).

Dietary total iron intake and CVD mortality

Six studies from four articles were included in the meta-analysis of the association between dietary total iron intake and risk of CVD mortality. The pooled RR of CVD mortality for the highest versus lowest level of dietary total iron intake was 1.04 (95% CI, 0.91–1.20; I^2 =41.2%; $p_{heterogeneity}$ =0.130; Supplementary figure 2). One study was not eligible for the dose–response analysis because the RR could not be calculated for the increase.[24] Five studies were included. We found no association between risk of CVD mortality and dietary total iron intake per 5-mg/day increase (RR 0.97, 95% CI 0.91–1.05; I^2 =46.6%; $p_{heterogeneity}$ =0.112; Supplementary figure 3). The association between dietary total iron intake and risk of CVD mortality was linear ($p_{nonlinearity}$ =0.635; Supplementary figure 4).

Dietary non-heme iron intake and CVD mortality

Five studies from four articles were included in the analysis of dietary non-heme iron intake and risk of CVD mortality. The pooled RR of CVD mortality for the highest versus lowest level of dietary total iron intake was 0.93 (95% CI, 0.76–1.14; I^2 =66.8%; $p_{heterogeneity}$ =0.017; Supplementary figure 5). One study was not eligible for the dose–response analysis due to lack of information on dietary heme iron intake.[11] Four studies were included and the pooled RR for CVD mortality was 1.02 (95% CI, 0.97–1.07; I^2 =0.0%; $p_{heterogeneity}$ =0.731; Supplementary figure 6) per 5-mg/day increase in dietary non-heme iron intake. We found a linear association between dietary non-heme iron intake and risk of CVD mortality ($p_{nonlinearity}$ =0.209; Supplementary figure 7).

Subgroups analyses, sensitivity analyses, and publication bias

Subgroups analyses were conducted by types of CVD mortality, sex, region, follow-up duration, and sample size (Table 2). A 1-mg/day increase in dietary heme iron intake was associated with a 17% increase in stroke mortality (RR 1.17, 95% CI 1.04–1.32) but not CHD mortality (RR 1.25, 95% CI 0.70–2.22) and MI mortality (RR 1.17, 95% CI 0.55–2.50). As well, a 1-mg/day increase in dietary heme iron intake predicted increased CVD mortality among men, Americans, and both follow-up duration and sample size groups (p<0.05).

On sensitivity analysis of dietary heme iron intake and risk of CVD mortality by removing 1 study at a time, none of the individual studies changed the pooled risk substantially. Similar findings were observed in sensitivity analyses of dietary total iron and non-heme iron intake. We found no publication bias (Supplementary figure 8-S10) by Begg's test for dietary intake of heme iron (p=0.621), total iron (p=0.624), and non-heme iron (p=0.174) and by Egger's for dietary intake of heme iron (p=0.807), total iron (p=0.984), and non-heme iron (p=0.274).

DISCUSSION

Our meta-analysis found a positive association between risk of CVD mortality and dietary intake of heme iron but not total iron or non-heme iron. The relative risk of CVD mortality was increased 19% with the highest versus lowest dietary heme iron intake level and 25% for each 1-mg/day increase in dietary heme iron intake. On subgroup analyses, dietary heme iron intake was associated with risk of stroke mortality but not CHD and MI mortality. The association between dietary heme iron intake and risk of CVD mortality was robust for men and Americans.

The results were consistent with another meta-analysis[10] (including four cohort articles) reporting no association of dietary iron intake (even if heme iron intake) and risk of CHD mortality. Specifically, we performed dose–response analyses and found a linear association between dietary heme iron intake and risk of CVD mortality. Furthermore, we found dietary heme iron intake associated with CVD mortality in men and Americans. One study found higher accumulation of stored iron in men and postmenopausal women than premenopausal women because the latter have lower iron deposits due to

Table 2. Dose–response subgroup analysis of risk of CVD mortality with heme iron intake.

Subgroups	Dose–response analysis (per 1-mg/day)			
	N	RR (95% CI)	I^2 (%)	$p_{heterogeneity}$
All studies	7	1.25 (1.17–1.33)	27.9	0.216
Diseases type				
CHD	3	1.25 (0.70–2.22)	55.7	0.104
Stroke	3	1.17 (1.04–1.32)	22.8	0.274
MI	4	1.17 (0.55–2.50)	71.8	0.014
Sex				
Men	3	1.29 (1.11–1.49)	45.5	0.160
Women	2	1.08 (0.73–1.60)	59.2	0.117
Region				
America	3	1.27 (1.19–1.36)	0.0	0.378
Non-America	4	1.14 (0.97–1.34)	40.3	0.170
Follow-up year				
<10	2	1.53 (1.20–1.95)	0.0	0.436
≥10	5	1.23 (1.16–1.31)	17.7	0.302
Sample size				
<10,000	1	2.45 (0.73–2.96)	-	-
≥10,000	6	1.25 (1.17–1.33)	29.8	0.211

CVD: cardiovascular disease; RR: relative risk; CI: confidence interval; CHD: coronary heart disease; MI: myocardial infarction.

menstruation.[25] As well, increased stored iron was previously found associated with risk of CVD mortality.[26,27] Americans consume more red meat[28] and dietary heme iron[17,21] than do non-Americans, which might explain the inconsistent association between Americans and non-Americans. Our results found a positive association of heme iron intake with stroke mortality, while not with CHD or MI mortality. The potential mechanisms are unclear, but one possible reason may be that the population of CHD and MI mortality subgroups in our analysis were mainly non-Americans who consume less red meat and more non-heme iron, which results in the non-significant association. Further research is needed to verify our findings.

Dietary iron includes heme and non-heme iron, and the two forms have different dietary sources, absorption mechanisms, and metabolic pathways.[29] The regulation of intestinal iron absorption is important because of no physiological pathway for excreting iron.[30] Iron ions circulate bound to plasma transferrin and accumulate within cells in the form of ferritin, and assessing the concentration of serum ferritin is a clinically useful measure of iron storage.[30,31] More than two thirds of the body's iron content are incorporated into hemoglobin in developing erythroid precursors and mature red cells.[30] Iron balance is tenuous; both iron deficiency and iron overload are deleterious. Iron (blood) losses and/or insufficient iron intake/absorption from dietary sources can cause iron deficiency, and excess dietary iron intake may result in iron overload. Heme iron is absorbed at a much greater rate and is less influenced by iron status and other components in diet than is non-heme iron,[32,33] which causes excess iron deposition and may explain the differential risk of CVD mortality with dietary heme iron and non-heme iron intake.

Several potential mechanisms likely account for the association between dietary heme iron intake and risk of CVD mortality. Increased dietary heme iron intake can induce oxidative stress biomarkers and lipid peroxidation[34,35] and has been found be associated with makers of inflammation.[36] All of these pathways can contribute to atherosclerosis development.[37-40] Moreover, epidemiological studies have demonstrated increased dietary heme iron intake associated with metabolic syndrome[8] and diabetes mellitus,[7] known risk factors for CVD mortality.

The primary strength in our analysis is that we conducted a dose–response analysis to quantify the associations and evaluate the direction of these associations. However, the study has some limitations. First, the groups were classified by dietary iron intake at baseline, and the possible changes in dietary iron intake during follow-up were not considered. Second, dietary iron intake was assessed by food frequency questionnaires, so the inevitable information bias for diet might suggest potential misclassification of exposure. Finally, residual confounding was not completely avoided even in the fully adjusted models.

Conclusion

We found a significantly positive association of risk of CVD mortality and dietary intake of heme iron but not total iron or non-heme iron. Our findings may have great public health significance in guiding people to reduce their consumption of heme iron-rich foods to prevent premature death due to CVD. Non-heme iron often exists in plant foods and increasing plant food intake will benefit health.

AUTHOR DISCLOSURES

The authors declare no conflict of interest. This study was supported by the National Natural Science Foundation of China (grant nos. 81373074, 81402752 and 81673260); the Natural Science Foundation of Guangdong Province (grant no. 2017A030313452).

REFERENCES

1. GBD2017 Causes of Dealth Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1736-88. doi: 10.016/S0140-6736(18)32203-7.
2. World Health Organization. Who Global NCD Action Plan 2013–2020. Geneva, Switzerland: WHO; 2013.
3. Bonita R, Magnusson R, Bovet P, Zhao D, Malta D, Geneau R et al. Country actions to meet UN commitments on non-communicable diseases: a stepwise approach. Lancet. 2013; 381:575-84. doi: 10.1016/S0140-6736(12)61993-X.
4. Ren Y, Zhang M, Luo X, Zhao J, Yin L, Pang C et al. Secular trend of the leading causes of death in China from 2003 to 2013. Afr Health Sci. 2017;17:532-7. doi: 10.4314/ahs.v17i2.29.
5. Sakata S, Iwai K. (Various functions and toxicity of iron). Tanpakushitsu Kakusan Koso. 2007;52:982-7. (In Japanese)
6. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet. 2016;387(10021):907-16. doi: 10. 1016/S0140-6736(15)60865-0. .
7. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. BMC Med. 2012;10:119. doi: 10.1186/741-7015-10-119.
8. Dos Santos Vieira DA, Hermes Sales C, Galvao Cesar CL, Marchioni DM, Fisberg RM. Influence of haem, non-haem, and total iron intake on metabolic syndrome and its components: A population-based study. Nutrients. 2018;10: 314. doi: 10.3390/nu10030314.
9. Fang X, An P, Wang H, Wang X, Shen X, Li X et al. Dietary intake of heme iron and risk of cardiovascular disease: a dose-response meta-analysis of prospective cohort studies. Nutr Metab Cardiovasc Dis. 2015;25:24-35 doi: 10.1016/j.numecd.2014.09.002.
10. Hunnicutt J, He K, Xun P. Dietary iron intake and body iron stores are associated with risk of coronary heart disease in a meta-analysis of prospective cohort studies. J Nutr. 2014; 144:359-66. doi: 10.3945/jn.113.185124. .
11. Casiglia E, Tikhonoff V, Bascelli A, Giordano N, Caffi S, Andreatta E et al. Dietary iron intake and cardiovascular outcome in Italian women: 10-year follow-up. J Womens Health. 2011; 20:1565-71. doi: 10.089/jwh.2011.780.
12. Kaluza J, Larsson SC, Hakansson N, Wolk A. Heme iron intake and acute myocardial infarction: a prospective study of men. Int J Cardiol. 2014;172:155-60. doi: 10.1016/j.ijcard.2013.12.176.
13. Etemadi A, Sinha R, Ward MH, Graubard BI, Inoue-Choi M, Dawsey SM et al. Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. BMJ. 2017; 357:j1957. doi: 10.136/bmj. j.
14. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D et al. Meta-analysis of observational studies in

epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283:2008-12. doi: 10.1001/jama.283.15.2008.
15. Wells GA, Shea BJ, O'Connell D, Peterson J, Welch V, Losos M et al. The Newcastle–Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. Ottawa (Canada): Ottawa Health Research Institute; 2019.
16. Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. Am J Epidemiol 2012;175:66-73. doi: 10.1093/aje/kwr265.
17. Lee DH, Folsom AR, Jacobs DR, Jr. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: the Iowa Women's Health Study. Am J Clin Nutr. 2005;81:787-91. doi: 10.1093/ajcn/81.4.787.
18. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88. doi: 10.016/0197-2456(86)90046-2.
19. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557-60. doi: 10.1136/bmj.327.7414.557.
20. Liu X, Zhang D, Liu Y, Sun X, Han C, Wang B et al. Dose-response association between physical activity and incident hypertension: A systematic review and meta-analysis of cohort studies. Hypertension. 2017;69:813-20. doi: 10.1161/HYPERTENSIONAHA.116.08994. .
21. Zhang W, Iso H, Ohira T, Date OC, Tanabe N, Kikuchi S et al. Associations of dietary iron intake with mortality from cardiovascular disease: the JACC study. J Epidemiol. 2012;22:484-93. doi: 10.2188/jea.je20120006.
22. Klipstein-Grobusch K, Grobbee DE, den Breeijen JH, Boeing H, Hofman A, Witteman JC. Dietary iron and risk of myocardial infarction in the Rotterdam Study. Am J Epidemiol. 1999;149:421-8. doi: 10.1093/oxfordjournals.aje.a009829.
23. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation. 1994;89:969-74. doi: 10.1161/01.cir.89.3.969.
24. Shi Z, Zhen S, Zhou Y, Taylor AW. Hb level, iron intake and mortality in Chinese adults: a 10-year follow-up study. Br J Nutr. 2017;117:572-81 doi: 10.1017/S000711451700040X.
25. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet. 1981;1(8233):1293-4. doi: 10.016/s0140-6736(81)92463-6.
26. Kadoglou NPE, Biddulph JP, Rafnsson SB, Trivella M, Nihoyannopoulos P, Demakakos P. The association of ferritin with cardiovascular and all-cause mortality in community-dwellers: The English longitudinal study of ageing. PLoS One. 2017;12:e0178994. doi: 10.1371/journal.pone.
27. Stack AG, Mutwali AI, Nguyen HT, Cronin CJ, Casserly LF, Ferguson J. Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population. QJM. 2014;107:623-33. doi: 10.1093/qjmed/hcu045. .
28. Bezerra IN, Goldman J, Rhodes DG, Hoy MK, Moura Souza A, Chester DN et al. Difference in adult food group intake by sex and age groups comparing Brazil and United States nationwide surveys. Nutr J. 2014;13:74 doi: 10.1186/475-2891-13-74.
29. Hallberg L. Advantages and disadvantages of an iron-rich diet. Eur J Clin Nutr. 2002;56(Suppl 1):S12-8. doi: 10.1038/sj.ejcn.1601348.
30. Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999;341:1986-95. doi: 10.056/NEJM199912233412607.
31. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. Cell. 2010;142:24-38. doi: 10.1016/j.cell.2010.06.028.
32. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91:1461S-7S. doi: 10.3945/ajcn.2010.28674F.
33. Miret S, Simpson RJ, McKie AT. Physiology and molecular biology of dietary iron absorption. Annu Rev Nutr. 2003;23:283-301. doi: 10.1146/annurev.nutr.23.011702.73139. .
34. Gueraud F, Tache S, Steghens JP, Milkovic L, Borovic-Sunjic S, Zarkovic N et al. Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. Free Radic Biol Med. 2015;83:192-200. doi: 10.1016/j.freeradbiomed.2015.02.023. .
35. Romeu M, Aranda N, Giralt M, Ribot B, Nogues MR, Arija V. Diet, iron biomarkers and oxidative stress in a representative sample of Mediterranean population. Nutr J. 2013;12:102. doi: 10.1186/475-2891-12-102.
36. de Oliveira Otto MC, Alonso A, Lee DH, Delclos GL, Jenny NS, Jiang R, Lima JA, Symanski E, Jacobs DR Jr, Nettleton JA. Dietary micronutrient intakes are associated with markers of inflammation but not with markers of subclinical atherosclerosis. J Nutr. 2011;141: 1508-15. doi: 10.3945/jn.111.138115.
37. Forstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res. 2017;120:713-35. doi: 10.1161/CIRCRESAHA.116.309326.
38. Shao B, Heinecke JW. HDL, lipid peroxidation, and atherosclerosis. J Lipid Res. 2009;50:599-601. doi: 10.1194/jlr.E900001-JLR200. .
39. Hu J, Xi D, Zhao J, Luo T, Liu J, Lu H et al. High-density Lipoprotein and Inflammation and Its Significance to Atherosclerosis. Am J Med Sci. 2016;352:408-15. doi: 10.1016/j.amjms.2016.06.014. .
40. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12:204-12. doi: 10.1038/ni.2001.

Supplementary table 1. Systematic literature review search terms and strategy.

Search terms for PubMed
#1 ("Iron, Dietary" [Mesh] OR "dietary iron" [Title/Abstract] OR "non-heme iron" [Title/Abstract] OR "heme iron" [Title/Abstract] OR "haem iron" [Title/Abstract] OR "iron intake" [Title/Abstract] OR "iron consumption" [Title/Abstract])
#2 ("mortality" [Mesh] OR "death" [Mesh] OR "mortality" [Title/Abstract] OR "death" [Title/Abstract] OR "deaths" [Title/Abstract] OR "fatal" [Title/Abstract])
#1 AND #2
Search terms for Embase
#1 iron intake/ OR dietary iron.mp. OR non-heme iron.mp. OR heme iron .mp. OR haem iron.mp. OR iron intake.mp. OR iron consumption.mp.
#2 mortality/ OR death/ OR mortality.mp. OR death.mp. OR deaths.mp. OR fatal.mp.
#1 AND #2

CVD: cardiovascular disease; RR: relative risk; CI: confidence interval; CHD: coronary heart disease; MI: myocardial infarction.

Supplementary table 2. Association between dietary iron intake and cardiovascular disease mortality in the included studies.

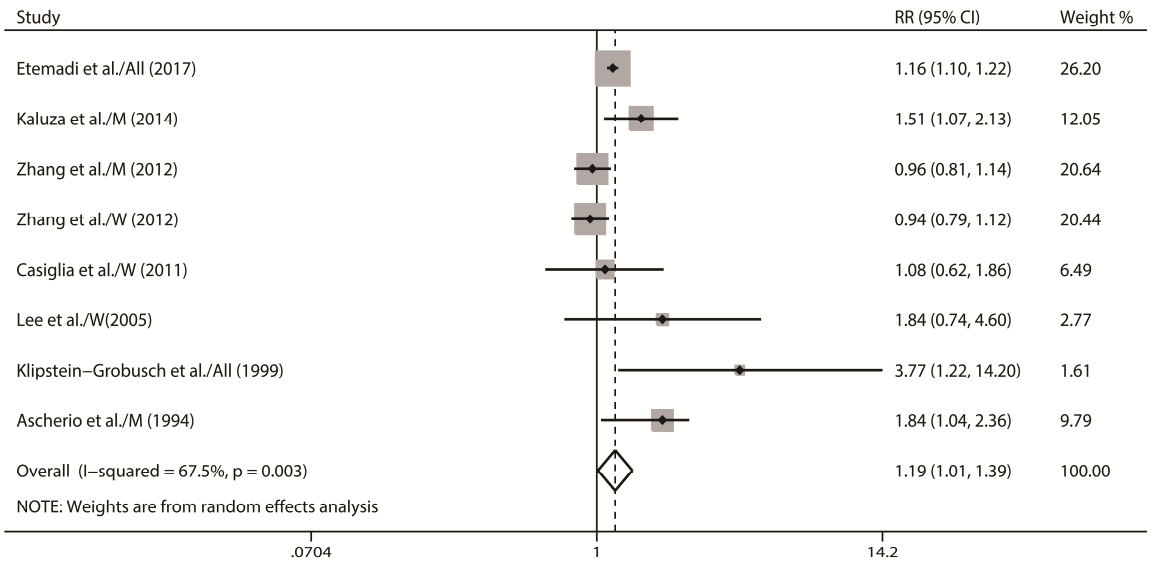
First author, publication year	Comparison	RRs or HRs (95% CI)	Adjusted variables
Shi, (2017) [24]	Dietary total iron intake (men): highest (median 41 mg/day) vs lowest (median 17.7 mg/day) quintile	1.09 (0.32–3.72)	Age, smoking, alcohol drinking, leisure time physical activity, education, occupation, region, BMI, diabetes, hypertension, intake of energy, fat and fibre.
	Dietary total iron intake (women): highest (median 33.8 mg/day) vs lowest (median 14.8 mg/day) quintile	2.88 (0.74–11.24)	
Etemadi, (2017) [13]	Dietary heme iron intake: highest (median 0.74 mg/day) vs lowest (median 0.12 mg/day) quintile	1.16 (1.10–1.22)	Sex, age at entry to study, marital status, ethnicity, education, fifths of composite deprivation index, perceived health at baseline, history of heart disease, stroke, diabetes, and cancer at baseline, smoking history, BMI, vigorous physical activity, usual activity throughout day, alcohol consumption, fruit and vegetable intakes, total energy intake.
Kaluza, (2014) [12]	Dietary heme iron intake: highest (median 2.68 mg/day) vs lowest (median 1.04 mg/day) quintile	1.51 (1.07–2.13)	Age, education, smoking status and pack–years of smoking, BMI, 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, quintiles of energy-adjusted intakes of protein, saturated fat, PUFA, cholesterol, fiber, vitamin E, β-carotene, vitamin C, potassium, sodium, calcium, magnesium.
	Dietary non-heme iron intake: highest (median 16.8 mg/day) vs lowest (median 9.4 mg/day) quintile	0.93 (0.67–1.30)	
Zhang, (2012) [21]	Dietary total iron intake (men): highest (median 10.58 mg/day) vs lowest (median 5.12 mg/day) quintile	1.27 (1.01–1.58)	BMI, smoking status, ethanol intake, history of hypertension, history of diabetes mellitus, sports time, walking time, educational status, perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy.
	Dietary total iron intake (women): highest (median 9.81 mg/day) vs lowest (median 5.14 mg/day) quintile	0.94 (0.77–1.15)	
	Dietary heme iron intake (men): highest (median 0.44 mg/day) vs lowest (median 0.07 mg/day quintile	0.96 (0.81–1.14)	
	Dietary heme iron intake (women): highest (median 0.48 mg/day) vs lowest (median 0.06 mg/day) quintile	0.94 (0.79–1.12)	
	Dietary non-heme iron intake (men): highest (median 10.19 mg/day) vs lowest (median 3.84 mg/day) quintile	1.04 (0.86–1.29)	
	Dietary non-heme iron intake (women): highest (median 9.46 mg/day) vs lowest (median 3.81 mg/day) quintile	0.99 (0.83–1.19)	
Casiglia, (2011) [11]	Dietary total iron intake: highest (mean 8.7 mg/day) vs lowest (mean 3.9 mg/day) quintile	0.77 (0.41–1.22)	Serum iron, prevalence of anemia, intake of fibers and caffeine, and total daily energy intake.

RRs: relative risks; HRs: hazard ratios; CIs: confidence intervals; BMI: body mass index; PUFA: polyunsaturated fatty acid.

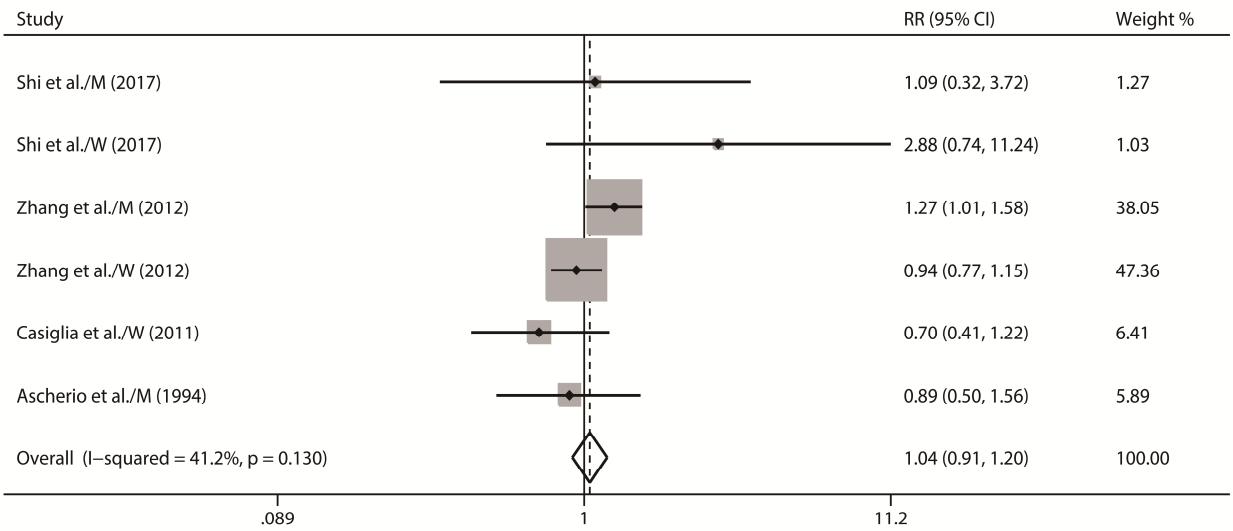
Supplementary table 2. Association between dietary iron intake and cardiovascular disease mortality in the included studies (cont.).

First author, publication year	Comparison	RRs or HRs (95% CI)	Adjusted variables
Lee, (2005) [17]	Dietary heme iron intake: highest (median 2.43 mg/day) vs lowest (median 0.57 mg/day) quintile	1.84(0.74–4.60)	heme iron, non-heme iron, zinc, age, energy intake, BMI, waist-hip ratio, physical activity, cigarette smoking, alcohol consumption, hormone replacement therapy, high blood pressure, saturated fat, trans fat, polyunsaturated fat, folate, β-carotene, vitamin E, and vitamin C.
	Dietary non-heme iron intake: highest (median 2.43 mg/day) vs lowest (median 0.57 mg/day) quintile	1.09 (0.86–1.38)	
K-G, (1999) [22]	Dietary heme iron intake: highest (median 1.36 mg/day) vs lowest (median 0.48 mg/day) quintile	3.77 (1.22–14.2)	age; sex; BMI; pack-years of smoking; equivalent household income, education, alcohol intake, categories of energy-adjusted p-carotene, vitamin C, vitamin E, fat, saturated fat, and cholesterol, use of anti-oxidative vitamin supplements.
Ascherio, (1994) [23]	Dietary total iron intake: highest (mean 37 mg/day) vs lowest (mean 11 mg/day) quintile	0.89 (0.50–1.56)	Age.
	Dietary heme iron intake: highest (median 1.36 mg/day) vs lowest (median 0.48 mg/day) quintile	1.84 (1.04–2.36)	Age, BMI, smoking habits, alcohol consumption, history of hypertension, diabetes, hypercholesterolemia; family history of myocardial infarction; profession; and quintiles of intake of total energy, vitamin E, total iron, heme iron.

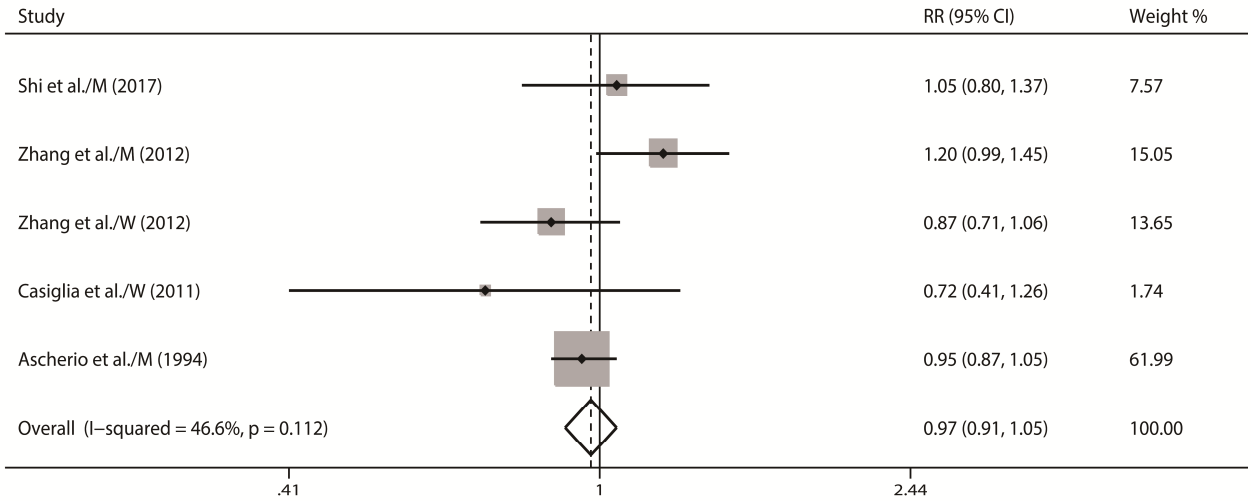
RRs: relative risks; HRs: hazard ratios; CIs: confidence intervals; BMI: body mass index; PUFA: polyunsaturated fatty acid



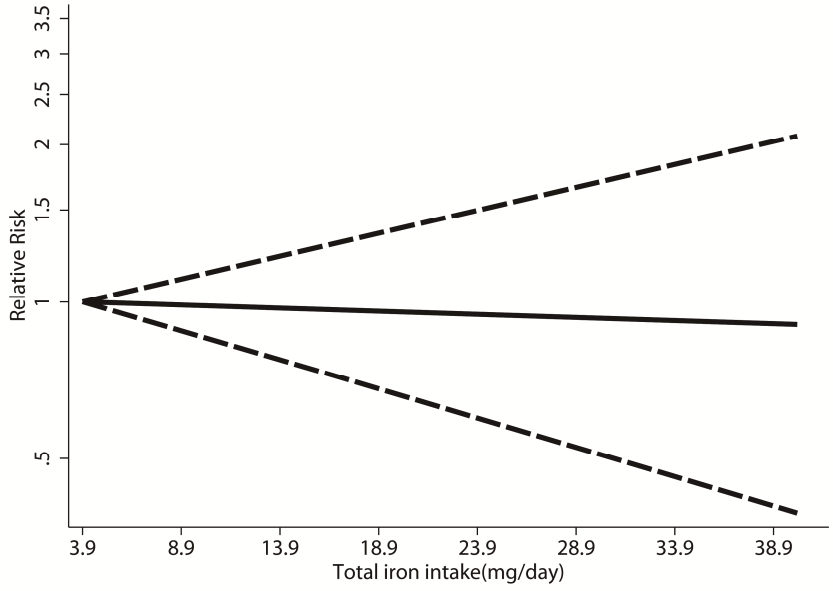
Supplementary figure 1. Forest plot of cardiovascular disease mortality for the highest versus lowest category of dietary heme iron intake.



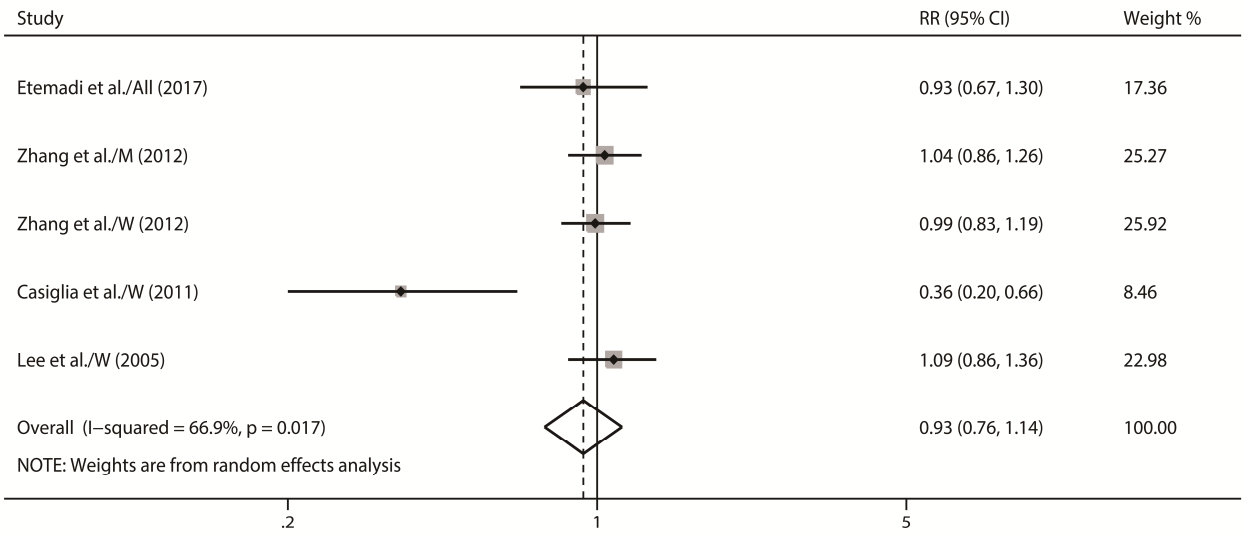
Supplementary figure 2. Forest plot of cardiovascular disease mortality for the highest versus lowest category of dietary total iron intake.



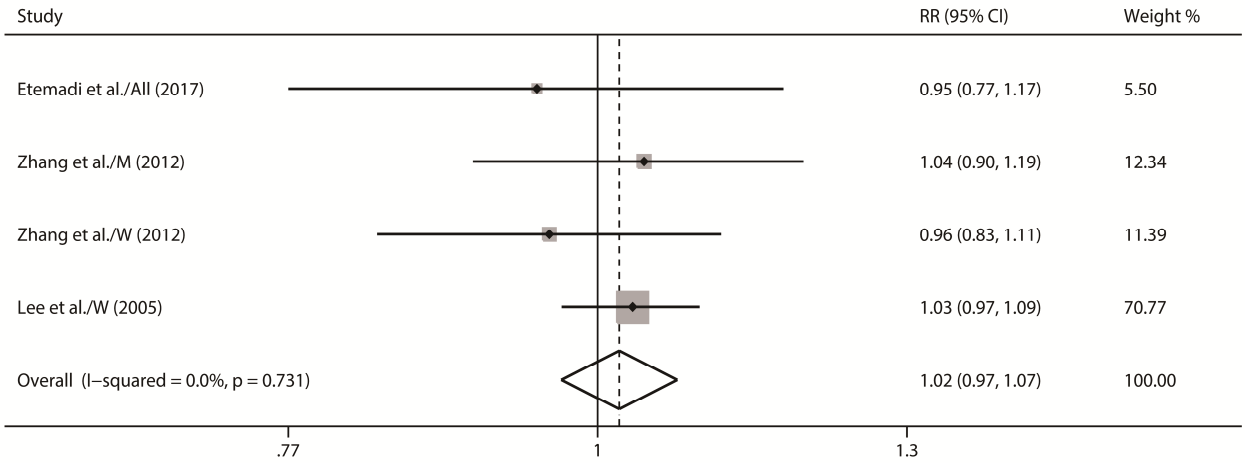
Supplementary figure 3. Forest plot for intake of dietary total iron intake (per 5-mg/day) and risk of cardiovascular disease mortality.



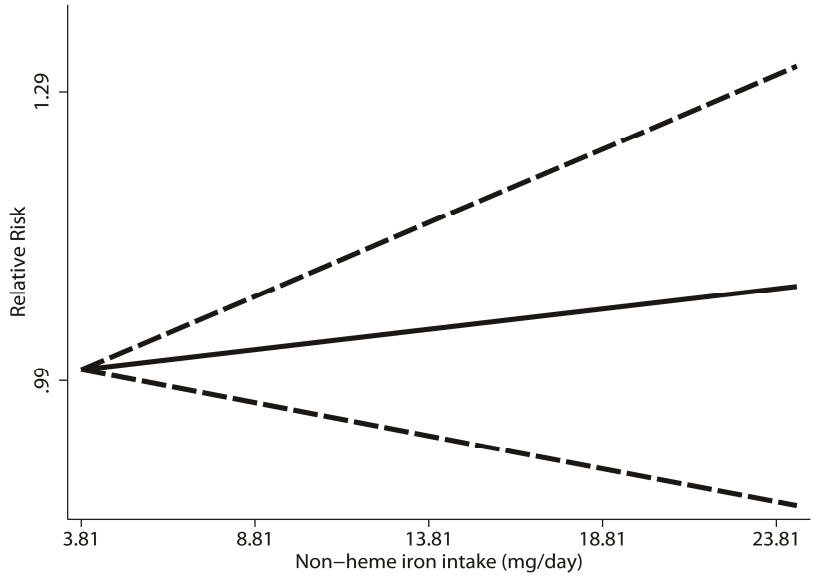
Supplementary figure 4. Linear dose–response association between dietary total iron intake and cardiovascular disease mortality.



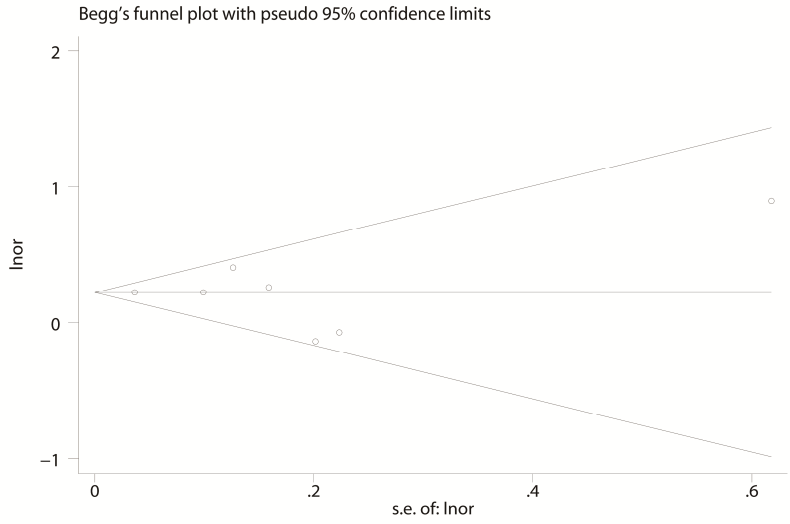
Supplementary figure 5. Forest plot of cardiovascular disease mortality for the highest versus lowest category of dietary non-heme iron intake.



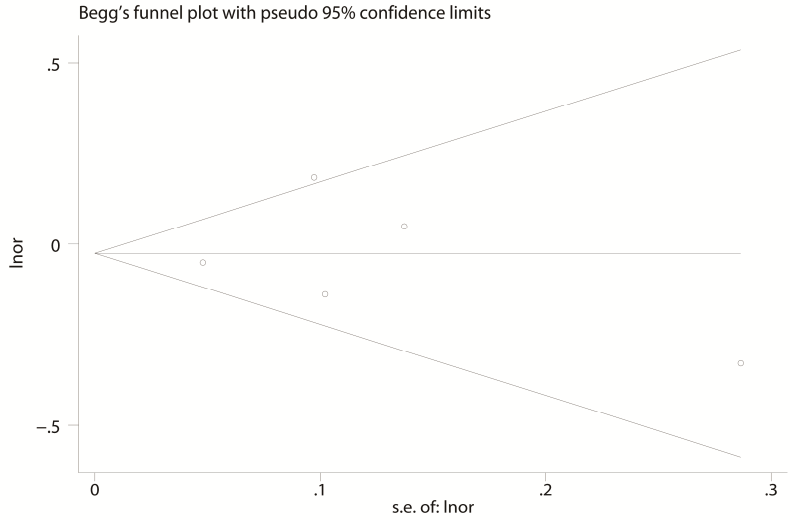
Supplementary figure 6. Forest plot for intake of dietary non-heme iron intake (per 5-mg/day) and risk of cardiovascular disease mortality.



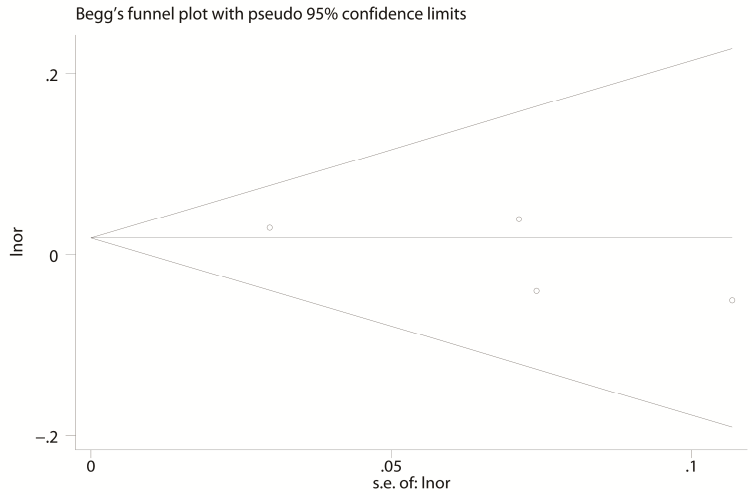
Supplementary figure 7. Linear dose–response association between dietary non-heme iron intake and cardiovascular disease mortality.



Supplementary figure 8. Funnel plot of publication bias for studies reporting the association between dietary heme iron intake and cardiovascular disease mortality.



Supplementary figure 9. Funnel plot of publication bias for studies reporting the association between dietary total iron intake and cardiovascular disease mortality.



Supplementary figure 10. Funnel plot of publication bias for studies reporting the association between dietary non-heme iron intake and cardiovascular disease mortality.