Original Article

Dietary iron and zinc intakes and nonalcoholic fatty liver disease: A meta-analysis

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Background and Objectives: The differences of dietary iron and zinc intakes between patients with nonalcoholic fatty liver disease (NAFLD) and controls remain controversial. The meta-analysis aimed to explore the differences of dietary iron and zinc intakes between NAFLD patients and healthy subjects. **Methods and Study Design:** A systematic literature search was performed up to July 2021 in databases of PubMed, Embase, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), and Wanfang. Using a random-effects model, the differences of dietary iron and zinc intakes between cases and controls were calculated as standardized mean differences (SMDs) with 95% confidence intervals (CIs). A total of 21 studies from 19 articles with 6639 cases were included. **Results:** The pooled estimate showed no difference in dietary iron consumption in the NAFLD groups compared with control groups. The difference became significant in Asia (SMD=0.16; 95% CI: 0.04, 0.28; I^2=89.1%; $p_{heterogeneity}$<0.001) as well as in cross-sectional studies (SMD=0.12; 95% CI: 0.07, 0.17; I^2=4.7%; $p_{heterogeneity}$=0.350). The difference in dietary zinc intake between cases and controls was not significant. We noticed a statistically significant increase of dietary zinc intake in NAFLD compared to controls in studies using food-frequency questionnaire (FFQ) to evaluate dietary intake (SMD=0.15; 95% CI: 0.10, 0.20; I^2=12.2%; $p_{heterogeneity}$=0.332). **Conclusions:** Our findings indicated that dietary iron intake in patients with NAFLD was higher than healthy subjects in Asia.

Key Words: iron, zinc, dietary intake, nonalcoholic fatty liver disease, meta-analysis

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases, it is defined as liver excessive fat accumulation, without any type of virus-related liver disease, or an intake of alcohol less than 20g per day.[1-3] NAFLD encompasses a series of liver abnormalities, including simple steatosis, steatohepatitis and fibrosis, which may further progress to cirrhosis and hepatocarcinoma.[1,2] The prevalence of NAFLD is increasing, with a prevalence of 25.24% globally.[4,5] The data from a large administrative claims database of the U.S. showed that the total annual cost of care per NAFLD patient with private insurance was $7804 for a new diagnosis and $3789 for long-term management,[6] it has exerted increasing burden on the health care system.

Many studies have suggested that NAFLD is a multifactorial disease. Obesity, insulin resistance, metabolic syndrome, type 2 diabetes mellitus and dyslipidemia contribute to the development of NAFLD, and oxidative stress is also a mediator of hepatocellular injury in NAFLD.[7-10] The drug treatment of NAFLD has limited effects, existing clinical treatments of NAFLD mainly include diet changes, increasing physical activity and lifestyle modifications.[11,12] Given the side-effects of some drugs and the poor compliance of patients on lifestyle modification, increasing attention is being paid to nutritional interventions. Some dietary antioxidant factors play a pivotal role in NAFLD, such as vitamin E, flavonoids, and carotenoids.[13-15] As essential trace elements for humans, iron and zinc play an important role in a variety of metabolic processes.[16] Zinc is also identified as an antioxidant in liver.[17] Studies *in vivo* and *in vitro* have shown that zinc may have beneficial effects on insulin resistance, glucose and lipid metabolism.[18-20] However, excessive iron accumulation could exert toxic effects on liver to initiate and catalyze oxygen radicals.[21]

Previous evidence has indicated that NAFLD subjects might suffer from iron overload and zinc deficiency.[22,23] However, the conclusions regarding the differences of dietary iron and zinc intakes between patients with NAFLD and controls have been inconsistent. For example, dietary iron intake of NAFLD groups was higher than that control groups in some studies,[24-26] whereas no significant difference was found in other studies.[27,28] For dietary

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zinc intake, a lower intake in cases compared with controls was reported in some studies,[28,29] while several studies found that the consumption of dietary zinc in cases was significantly higher than that in controls,[24,30] the significant difference was not found in other studies.[26,31] Therefore, we performed this meta-analysis to compare dietary iron and zinc intakes between NAFLD patients and healthy subjects.

METHODS

Search strategy

We searched the electronic databases of PubMed, Embase, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), and Wanfang up to July 2021 without language restrictions. Iron, zinc, dietary, nutrient, and nonalcoholic fatty liver disease, NAFLD, nonalcoholic fatty liver, nonalcoholic steatohepatitis, NASH were used as search terms. Moreover, we manually searched the references of original included articles to identify potential studies that might have been missed.

Study selection

The titles and abstracts of all identified articles were independently screened by two authors, and any divergence of opinion was resolved through group discussion. The inclusion criteria were as follows: 1) observational studies (cohort, case-control, or cross-sectional studies); 2) the exposure of interest was dietary iron or zinc intake; 3) the outcome of interest was NAFLD, NASH or abnormally elevated level of alanine aminotransferase (ALT) in the absence of other liver diseases, such as autoimmune liver diseases, viral hepatitis and drug-induced liver disease; 4) studies that provide sufficient data. The following studies were excluded: randomized controlled trials (RCTs), animal studies, *in vitro* studies, meta-analyses, reviews, and commentaries.

Data extraction

Two authors performed the data extraction independently. If the two authors disagreed about the extraction, it was resolved by discussion with a third author. The following data were extracted: name of first author, year of publication, country, region, study design, sample size, age, gender, methods of cases diagnosis and dietary survey, exposure (intake of dietary iron and/or zinc), matched variables, adjusted variables, cases definition, and the means with standard deviations (SDs). If the SDs were not reported directly, we would convert 95% confidence interval (CI) or standard error of mean (SEM) to SD according to the equations listed in the Cochrane handbook.[32]

Statistical analysis

The random-effects model was used to calculate the summary estimates as standardized mean differences (SMDs) with 95% CIs. The I^2 statistic was used to evaluate heterogeneity among studies. The I^2 values of 25%, 50% and 75% as cut-off points represent low, moderate, and high degrees of heterogeneity respectively.[33] Meta-regression with a single covariate analysis was carried out to explore potential sources of heterogeneity. Moreover, the adjusted R^2 was calculated to examine the degree of the between-study heterogeneity accounted for the suspected covariates.[34] Subgroup analyses by the region, study design, sample size, gender, cases diagnosis method, dietary survey method, matched variables, and adjusted variables were conducted. Influence analyses were conducted to investigate the effect of one study on the pooled effects by removing one study in every turn. Publication bias was assessed by visual inspection of the funnel plot and Egger's test ($p<0.1$).[35] All p values were two-sided and $p<0.05$ was considered significant. Data were analyzed with Stata 11.0 (Stata CORP, College Station, TX).

RESULTS

Literature search and study characteristics

Figure 1 shows the flow chart of literature search. A total of 15710 articles were identified from PubMed, Embase, Web of Science, Cochrane Library, CNKI, Wanfang, and manual search after removing duplicated articles. Of these, there were 186 articles left after reading titles and abstracts. After reading full text for the second selection step, 167 articles were excluded for various reasons (five did not provide valid data, four did not provide iron or zinc intake in healthy subjects, and 158 only reported serum iron or zinc level). Finally, 19 articles were eligible for inclusion in the meta-analysis.[24-31,36-46] Two of the articles were divided into male and female for analysis, respectively.[29,31] In addition, we combined the data from three age groups (20-39, 40-59, and ≥60 y) in the article by Toshimitsu et al,[30] and combined the data from lean and obese subjects in the article by Li et al.[26] Overall, a total of 21 studies from 19 articles were included for data analysis. Among them, 20 studies from 18 articles reported dietary iron intake[24-31,36-38,40-46] and 13 studies from 11 articles reported dietary zinc intake.[24,26-31,36,39,44,45]

The characteristics of each included study are displayed in Table 1. Of all the included studies, outcomes of 13 studies were NAFLD, 5 studies were NASH, 2 studies were NAFLD and NASH and 1 study was suspected pediatric NAFLD defined by elevated ALT level. Eighteen studies were case-control studies,[24-31,37-43,45] and the remaining three were cross-sectional studies.[36,44,46] There were 15 studies from Asia (Iran,[27,28,37,39,45] China,[24-26,38,40] Korea,[31,36,46] and Japan[30]), two from America (Canada,[44] and America[42]), and four from Europe (Italy,[29,41] and Portugal[43]), with a total of 6639 cases and 24488 controls. Twenty studies were conducted among adults,[24-31,36-45] and one study was conducted among children and adolescents.[46]

Overall difference in dietary iron intake between NAFLD cases and controls

Among the 20 studies on the association between dietary iron intake and NAFLD, 4 studies showed that dietary intake of iron in cases was higher than controls,[24-26,36] two studies found a lower consumption of iron in patients with NAFLD,[41,43] while the other 14 studies indicated no significant difference.[27-31,37,38,40,42,44-46] The combined effect demonstrated that the difference in dietary iron intake was not significant between NAFLD patients and healthy controls (SMD=-0.07; 95% CI: -0.28, 0.14; I^2=96.7%; $p_{heterogeneity}$ <0.001) (Figure 2).

Table 1. Characteristics of the included studies

Author, Year	Country	Region	Study design	Sample size		Mean age or age range	Gender (F/M)	Cases diagnosis method	Dietary survey method	Exposure	Matched variables	Adjusted variables	Cases definition
				Case	Control								
Zolfaghari, 2016[28]	Iran	Asia	Case-control	159	158	20-60	F+M	US	24-h dietary recall	Iron and zinc	Age, gender	Energy intake, age, gender	Recently diagnosed NAFLD
Han, 2014 [31]	Korea	Asia	Case-control	66	116	Case: 51.9 Control: 38.6	F	US	24-h dietary recall, 4-d dietary record	Iron and zinc	-	Age group	NAFLD
Han, 2014 [31]	Korea	Asia	Case-control	103	63	Case: 39.9 Control: 39.2	M	US	24-h dietary recall, 4-d dietary record	Iron and zinc	-	Age group	NAFLD
Wu, 2012 [24]	China	Asia	Case-control	144	144	18-82	F+M	US	24-h dietary recall	Iron and zinc	Age, gender	Physical exercise, alcohol drinking history	NAFLD
Federico, 2017 [56]	Italy	Europe	Case-control	50	1326	Case: 37-78 Control: 18-89	F	Liver biopsy	7-d dietary record	Iron and zinc	-	-	NASH
Federico, 2017 [56]	Italy	Europe	Case-control	74	1000	Case: 39-75 Control: 18-89	M	Liver biopsy	7-d dietary record	Iron and zinc	-	-	NASH
Zheng, 2015 [25]	China	Asia	Case-control	215	215	21-80	F+M	US	FFQ	Iron (heme and non-heme iron)	-	-	NAFLD
Chang, 2014 [36]	Korea	Asia	Cross-sectional	3087	7024	Case: 55.1 Control: 52.9	F+M	US	FFQ	Iron and zinc	-	-	NAFLD
Kani, 2013 [27]	Iran	Asia	Case-control	100	100	37.9	F+M	US	4-d dietary record	Iron and zinc	-	-	NAFLD (new cases)
Toshimitsu, 2007 [57]	Japan	Asia	Case-control	46	8964	≥20	F+M	Liver biopsy	3-d dietary record	Iron and zinc	-	-	Simple steatosis, NASH
Farhangi, 2016 [37]	Iran	Asia	Case-Control	75	76	20-50	F+M	US	SFFQ	Iron	Age, gender	-	NAFLD
Lotfi, 2019 [39]	Iran	Asia	Case-control	200	400	20-60	F+M	US	SFFQ	Zinc	Age, gender	Age,BMI, MET, energy	NAFLD

F: female; M: male; US: ultrasonography; h: hour; d: day; wk: week; NASH: Nonalcoholic Steatohepatitis; FFQ: food-frequency questionnaire; SFFQ: semi-quantitative food-frequency questionnaire; BMI: body mass index; MET: metabolic equivalent task; ALT: alanine aminotransferase.

Table 1. Characteristics of the included studies (cont.)

Author, Year	Country	Region	Study design	Sample size		Mean age or age range	Gender (F/M)	Cases diagnosis method	Dietary survey method	Exposure	Matched variables	Adjusted variables	Cases definition
				Case	Control								
Liu, 2017 [38]	China	Asia	Case-control	280	280	19-70	F+M	US	SFFQ	Iron (heme and non-heme iron)	Age, gender	-	NAFLD (new cases)
Vahid, 2019 [45]	Iran	Asia	Case-Control	295	704	20-80	F+M	US	FFQ	Iron and zinc	Age, gender	-	NAFLD
Li, 2018 [26]	China	Asia	Case-Control	169	182	Case: 44.85 Control:43.75	F+M	US	FFQ	Iron and zinc	Age, gender, BMI	-	NAFLD
Musso, 2003[41]	Italy	Europe	Case-Control	25	25	37	F+M	US	7-ddietary record	Iron	Age, gender, BMI	-	NASH
Cortez-Pinto, 2006[43]	Portugal	Europe	Case-control	45	856	27-68	F+M	Liver biopsy	SFFQ	Iron	Age,gender,alcohol consumption	Age, gender, BMI	NASH
Silva, 2014 [59]	Canada	America	Cross-sectional	74	27	Case: 45.66 Control: 38.0	F+M	Liver biopsy	7-d dietary record	Iron and zinc	-	-	Simple steatosis, NASH
Chalasani, 2004[60]	America	America	Case-Control	21	19	Case: 41 Control: 43	F+M	Liver biopsy	3-wk dietary record	Iron	Age, gender, BMI	-	NASH
Kim, 2020 [46]	Korea	Asia	Cross-sectional	138	1536	10-18	F+M	ALT	24-h dietary recall	Iron	-	-	Suspected pediatric NAFLD

F: female; M: male; US: ultrasonography; h: hour; d: day; wk: week; NASH: Nonalcoholic Steatohepatitis; FFQ: food-frequency questionnaire; SFFQ: semi-quantitative food-frequency questionnaire; BMI: body mass index; MET: metabolic equivalent task; ALT: alanine aminotransferase.

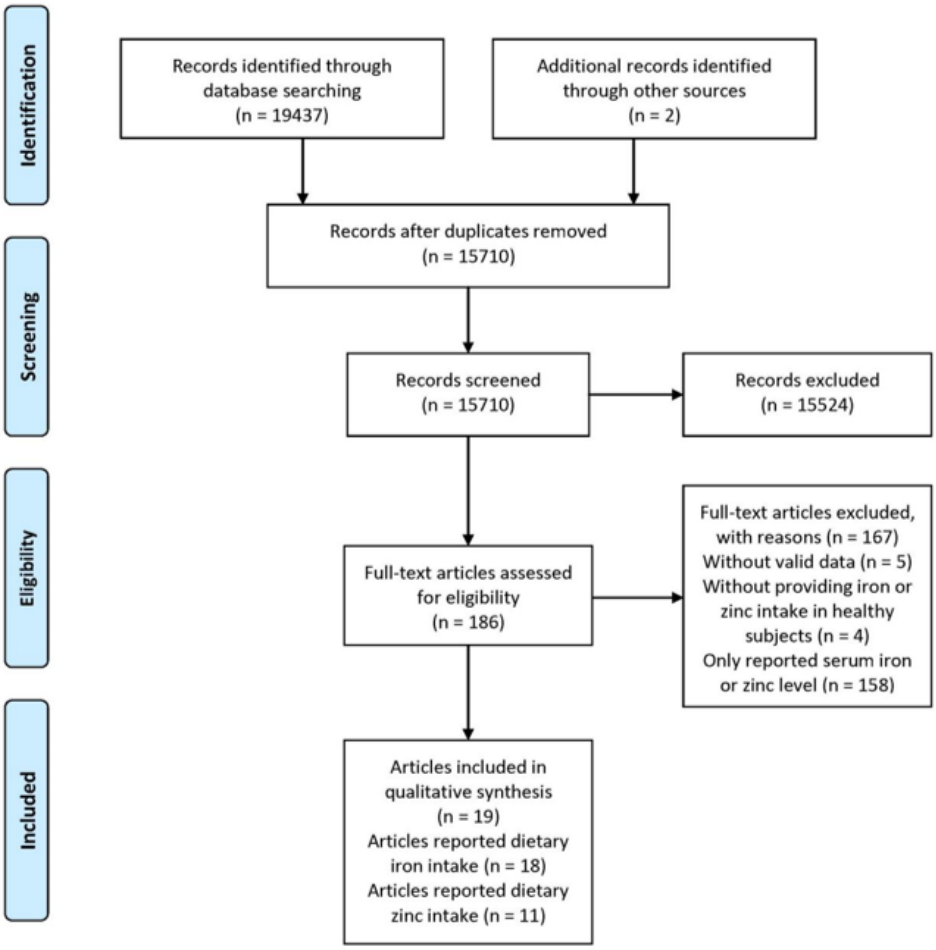

Figure 1. Flow chart of articles included in the meta-analysis.

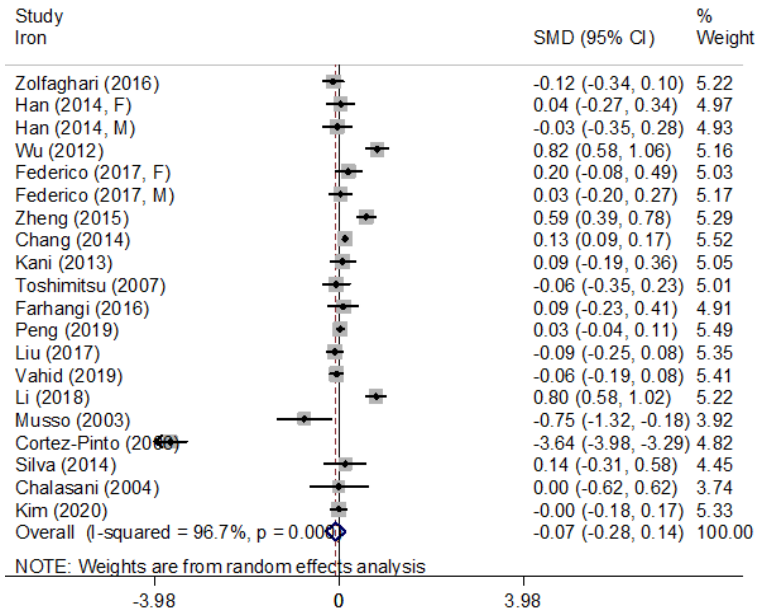

Figure 2. Forest plot for the difference in dietary iron intake between cases and controls. The pooled effect was calculated using a random-effects model. The diamonds denote summary risk estimate, and horizontal lines represent 95% CI. SMD, standardized mean difference; CI, confidence interval.

Overall difference in dietary zinc intake between NAFLD cases and controls

Among the 13 studies on the association between dietary zinc intake and NAFLD, 5 studies revealed that dietary zinc intake in cases was significantly higher than that controls.[24,29,30,36,44] On the contrary, the other 2 studies found a lower intake of dietary zinc in cases compared with controls.[28,29] The remaining 6 studies showed no significant difference.[26,27,31,39,45] The overall difference with respect to dietary zinc intake between cases and controls was not significant (SMD=0.18; 95% CI: -0.03, 0.38; I^2=93.9%; $p_{heterogeneity}$<0.001) (Figure 3).

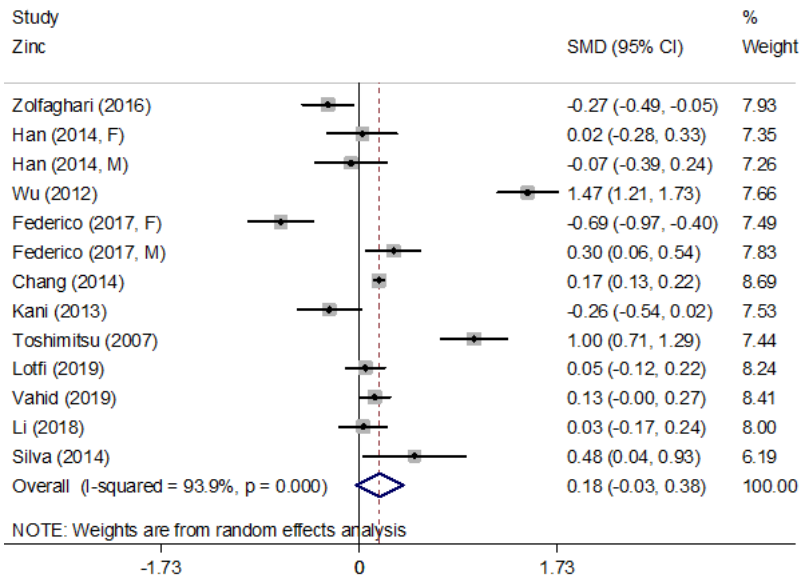

Figure 3. Forest plot for the difference in dietary zinc intake between cases and controls. The pooled effect was calculated using a random-effects model. The diamonds denote summary risk estimate, and horizontal lines represent 95% CI. SMD, standardized mean difference; CI, confidence interval.

Subgroup analysis and meta-regression

As shown in Table 2, subgroup analysis by region demonstrated a significant increased intake of dietary iron in cases compared with controls when conducted in Asia (SMD=0.16; 95% CI: 0.04, 0.28). When stratified by study design, the pooled SMD was statistically significant among cross-sectional studies (SMD=0.12; 95% CI: 0.07, 0.17). With stratified analysis based on sample size, the pooled SMD (95% CI) was 0.14 (-0.15, 0.42) in samples < medium (390.5) and -0.26 (-0.56, 0.05) in samples ≥ medium (390.5). In subgroup analysis by gender, the consumption of dietary iron between cases and controls was not significant. Stratifying by dietary survey method, the pooled SMD was -0.24 (95% CI: -0.61, 0.14) for studies using food-frequency questionnaire (FFQ) and 0.06 (95% CI: -0.12, 0.24) for studies using non-FFQ. When stratified by matched variables, the pooled SMD was significant among studies not matched by age and gender (SMD=0.12; 95% CI: 0.01, 0.24). Subgroup analysis by adjusted variables showed a significant increased intake of dietary iron in cases compared with controls among studies not adjusted by any variables (SMD=0.11; 95% CI: 0.00, 0.23). All *p* values from meta-regression were found to be >0.05.

As shown in Table 3, in subgroup analysis by gender, the intake of dietary zinc was significantly higher in NAFLD patients than that in healthy controls among studies conducted in both genders (SMD = 0.30; 95% CI: 0.05, 0.55). Stratified by dietary survey method, the pooled SMD was 0.15 (95% CI: 0.10, 0.20) for studies using FFQ. All *p* values from meta-regression were >0.05.

Influence analysis and publication bias

The results of influence analysis are shown in Figure 4 and Figure 5. With respect to dietary iron intake between cases and controls, significant change occurred after removing Cortez-Pinto's study,[43] which changed the pooled difference from (SMD=-0.07; 95% CI: -0.28, 0.14) to (SMD=0.13; 95% CI: 0.02, 0.24). With respect to dietary zinc intake between cases and controls, significant change occurred after removing Zolfaghari's study,[28] and Federico's study (for female),[29] respectively, which changed the pooled difference from (SMD=0.18; 95% CI: -0.03, 0.38) to (SMD=0.22; 95% CI: 0.00, 0.43), and (SMD=0.25; 95% CI: 0.05, 0.45), respectively.

No publication bias was found regarding dietary iron intake (*p*=0.403) as well as dietary zinc intake (*p*=0.982) by using Egger's test and the funnel plot (Figure 6 and Figure 7).

DISCUSSION

Twenty studies for dietary iron intake and thirteen studies for dietary zinc intake were included in the present meta-analysis. The summary estimates indicated that the differences in dietary iron and zinc intakes were not significant between NAFLD patients and healthy subjects. In the subgroup analyses, cases had a higher intake of dietary iron than controls in Asia as well as in cross-sectional studies. The difference in dietary zinc intake became significant in studies using FFQ to evaluate dietary intake.

The mechanisms underlying the development and progression of NAFLD are complex. Initially, Day et al. proposed the pathogenesis of NAFLD: "two-hit" hypothesis.[47] The first hit is hepatic accumulation of lipids that results from insulin resistance. The second hit is hepatic injury that caused by oxidative stress and inflammatory reaction on the basis of the first hit. In 2016, "multiple-hit" hypothesis was proposed,[48] dietary factors, environmental factors, insulin resistance, gut microbiota, hormones secreted from the adipose tissue, inflammatory cytokines, oxidative stress, and genetic and epigenetic factors act together. Previous study has demonstrated that iron overload could generate reactive oxygen species through Fenton reaction, resulting in oxidative stress,[49] which may contribute to the development of NAFLD.[50] Therefore, combined with our results, we speculated that iron overload may partly be the result of increased dietary iron intake among subjects, and further lead to NAFLD.

Table 2. Subgroup analysis and meta-regression for the difference in dietary iron intake between cases and controls

	Studies (n)	SMD (95% CI)	Heterogeneity		Meta-regression		
			I^2 (%)	*p* value	I^2 residual (%)	Adjusted R^2 (%)	*p* value†
Region					96.34%	15.22%	
Asia	14	0.16 (0.04, 0.28)	89.1%	<0.001			0.056
Non-Asia	6	-0.67 (-1.96, 0.62)	98.7%	<0.001			
Study design					96.87%	-5.14%	
Case-control	17	-0.11 (-0.42, 0.20)	97.2%	<0.001			0.730
Cross-sectional	3	0.12 (0.07, 0.17)	4.7%	0.350			
Sample size					96.84%	-0.24%	
< medium (390.5)	10	0.14 (-0.15, 0.42)	88.3%	<0.001			0.342
≥ medium (390.5)	10	-0.26 (-0.56, 0.05)	98.2%	<0.001			
Gender					97.08%	-11.49%	
Both genders	16	-0.11 (-0.35, 0.14)	97.4%	<0.001			0.867
Female	2	0.13 (-0.08, 0.33)	0.0%	0.425			0.901
Male	2	0.01 (-0.18, 0.20)	0.0%	0.739			1.000
Dietary survey method					96.91%	-2.97%	
Non-FFQ	12	0.06 (-0.12, 0.24)	78.5%	<0.001			0.491
FFQ	8	-0.24 (-0.61, 0.14)	98.7%	<0.001			
Matched by age and gender					96.81%	-0.42%	
Yes	10	-0.28 (-0.76, 0.20)	98.3%	<0.001			0.340
No	10	0.12 (0.01, 0.24)	67.6%	0.001			
Adjusted variables					96.70%	5.85%	
Yes	5	-0.58 (-1.90, 0.74)	99.1%	<0.001			0.161
No	15	0.11 (0.00, 0.23)	84.5%	<0.001			

n: the number of studies; SMD: standardized mean difference; CI: confidence interval; FFQ: food-frequency questionnaire.
†Gender (male as reference) was included as dummy variable in meta-regression.

Table 3. Subgroup analysis and meta-regression for the difference in dietary zinc intake between cases and controls

	Studies (n)	SMD (95% CI)	Heterogeneity		Meta-regression		
			I^2 (%)	*p* value	I^2 residual (%)	Adjusted R^2 (%)	*p* value†
Region					94.28%	-6.35%	
Asia	10	0.22 (-0.00, 0.45)	94.3%	<0.001			0.601
Non-Asia	3	0.02 (-0.70, 0.75)	94.0%	<0.001			
Study design					94.40%	-8.51%	
Case-control	11	0.16 (-0.15, 0.46)	94.9%	<0.001			0.728
Cross-sectional	2	0.25 (-0.01, 0.51)	46.1%	0.173			
Sample size					94.41%	-9.09%	
< medium (351)	6	0.23 (-0.36, 0.82)	96.0%	<0.001			0.803
≥ medium (351)	7	0.14 (-0.06, 0.35)	91.6%	<0.001			
Gender					94.20%	1.43%	
Both genders	9	0.30 (0.05, 0.55)	94.9%	<0.001			0.671
Female	2	-0.33 (-1.03, 0.36)	91.2%	0.001			0.436
Male	2	0.13 (-0.24, 0.49)	71.1%	0.063			1.000
Cases diagnosis method					94.40%	-8.34%	
Ultrasonography	9	0.14 (-0.08, 0.36)	93.7%	<0.001			0.722
Liver biopsy	4	0.27 (-0.45, 0.99)	95.7%	<0.001			
Dietary survey method					94.40%	-8.72%	
Non-FFQ	9	0.22 (-0.24, 0.68)	95.9%	<0.001			0.733
FFQ	4	0.15 (0.10, 0.20)	12.2%	0.332			
Matched by age and gender					94.42%	-7.38%	
Yes	5	0.28 (-0.18, 0.74)	96.5%	<0.001			0.631
No	8	0.12 (-0.15, 0.39)	91.5%	<0.001			
Adjusted variables					94.42%	-8.83%	
Yes	5	0.24 (-0.34, 0.83)	96.6%	<0.001			0.775
No	8	0.14 (-0.08, 0.35)	91.3%	<0.001			

n: the number of studies; SMD: standardized mean difference; CI: confidence interval; FFQ: food-frequency questionnaire.
†Gender (male as reference) was included as dummy variable in meta-regression.

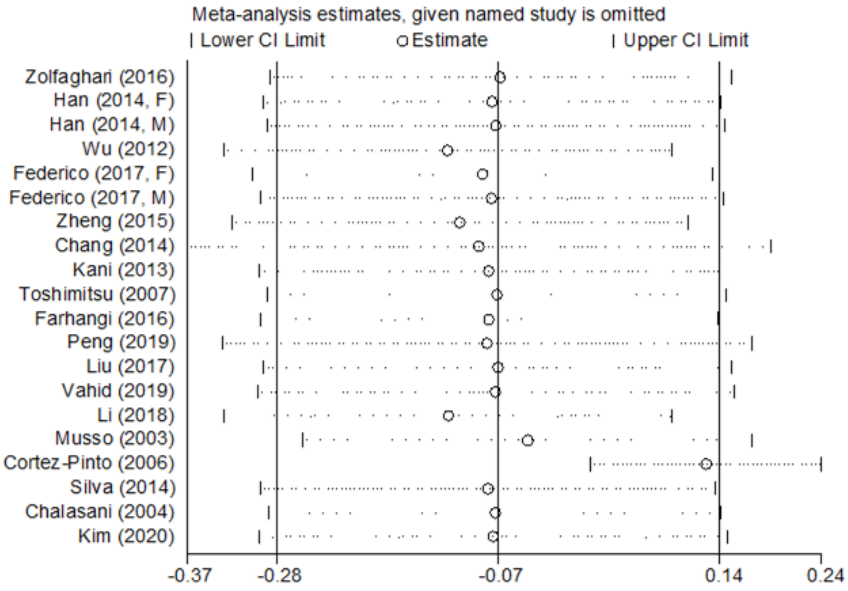

Figure 4. Influence analysis for the difference in dietary iron intake between cases and controls.

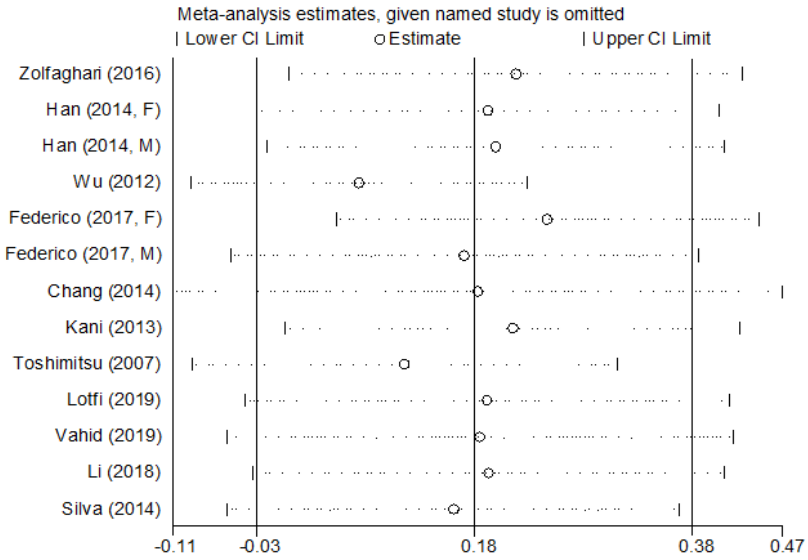

Figure 5. Influence analysis for the difference in dietary zinc intake between cases and controls.

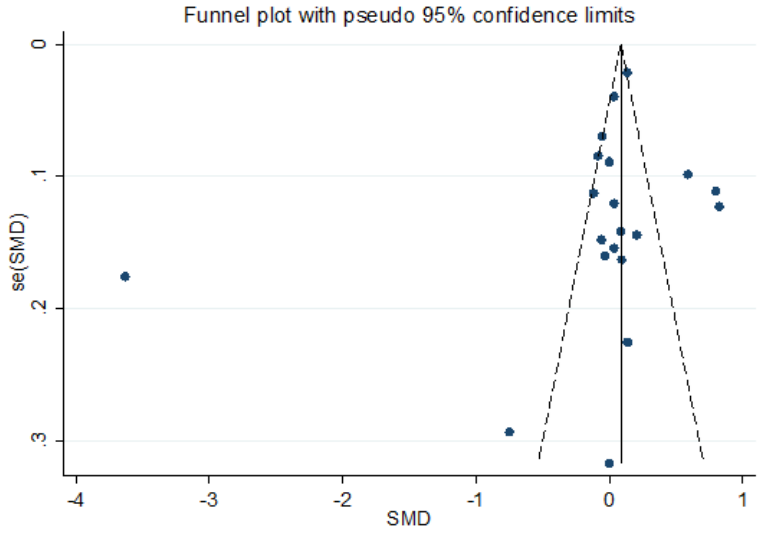

Figure 6. Funnel plot for the difference in dietary iron intake between cases and controls.

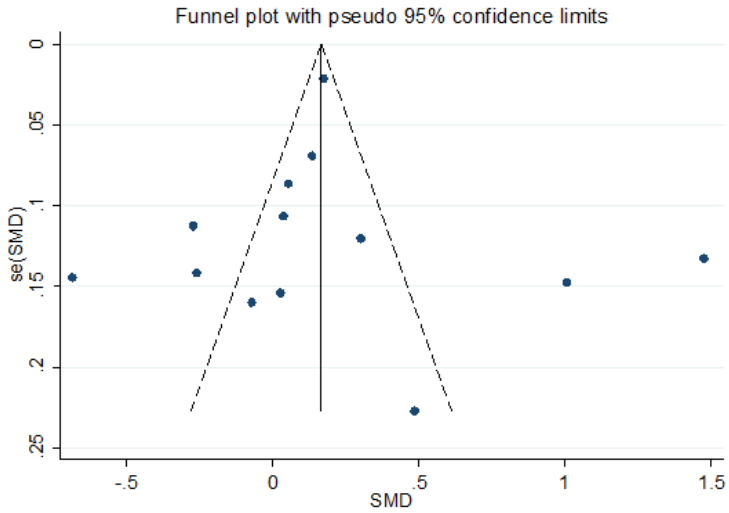

Figure 7. Funnel plot for the difference in dietary zinc intake between cases and controls.

With respect to dietary zinc intake, based on published research, higher consumption of grains, which are rich in zinc, could lead to an increased intake of zinc.[51] Moreover, a case-control study found that NAFLD patients had a higher level of intake of grains than healthy individuals.[52] Therefore, an increased consumption of dietary zinc may on this basis be thought to increase the risk of NAFLD in our study.

In the present analysis, the high heterogeneity existed between studies, which could still not be eliminated by subgroup analysis and meta-regression. The heterogeneity of the included studies might probably explain by: 1) the differences of the inclusion/exclusion criteria between the included studies; 2) the diversity in basic characteristics of participants (age, body mass index (BMI), physical activity, energy intake, dietary habits, and NAFLD-related diseases); 3) the evaluation of dietary intake was self-reported, which had the risk of under-reporting;[53] 4) the differences in the severity and stage of NAFLD. For instance, some studies selected new patients diagnosed with NAFLD,[27,28,38] while patients from other studies may have already changed their dietary habits due to the advice of their family physician.[54] Kim et al. conducted study among suspected pediatric NAFLD subjects diagnosed by alanine aminotransferase (ALT).[46] Still some studies differentiated simple NAFLD from NASH,[29,30,41-44] but others did not; 5) only eight studies adjusted for or matched for potentially confounders other than age and gender;[24,26,28,39-43] 6) different food sources of iron may differ in their effects on NAFLD. Further influence analyses revealed that the study by Cortez-Pinto et al[43] might be the source of heterogeneity of the meta-analysis with respect to dietary iron intake, and the studies by Zolfaghari et al,[28] and Federico et al[29] might be the sources of heterogeneity of the meta-analysis with respect to dietary zinc intake. The results indicated that the combined test performance of this meta-analysis might be influenced by a few of the studies. Therefore, conclusions should be carefully drawn and need further study.

The present study has several advantages. First, it is probably the first to investigate the differences of dietary iron and zinc intakes between NAFLD patients and healthy subjects using meta-analysis. Second, we had a total of 31127 individuals, which enhanced the statistical power and the reliability of our findings. Third, there was no significant publication bias, indicating the stability of the pooled estimates.

However, some limitations should also be mentioned. First, all of the included studies were observational studies, the causality cannot be easily determined due to the lack of cohort studies. Further prospective studies with a large sample-size are needed to validate the associations between these mineral intakes and NAFLD risk. Second, as a method of NAFLD diagnosis, ultrasonography has a much lower accuracy than liver biopsy. However, the invasive nature of liver biopsy limits the sample size. Third, all dietary survey methods have the risk of an underestimation of the dietary intake, or even an over estimation, especially in overweight and obese subjects.[53,55] Fourth,the background information in studies included, such as food culture or pattern, alcohol intake, other personal behaviours, body fatness and its distribution, comorbidities like hepatitis, diabetes, pre-diabetes or insulin resistance, is not sufficient to explore their individual or conjoint effects on the associations between dietary iron and zinc intakes and NAFLD. Moreover, only three studies analyzed the different sources of dietary iron.[25,38,40] In view of the different absorption rates between non-heme iron and heme iron, the intake of dietary iron from different sources must be known to reach any firm conclusions.

Conclusion

The present study indicates that the intake of dietary iron is significantly increased in NAFLD patients compared to healthy controls in Asia. This might have implications for the prevention and treatment of NAFLD.

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AUTHOR DISCLOSURES

The authors would like to declare no conflict of interest.

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