Ferritin, metabolic syndrome and its components

Elsevier Editorial System(tm) for Atherosclerosis

Manuscript Draft

Manuscript Number: ATH-D-18-00284R1

Title: FERRITIN, METABOLIC SYNDROMEAND ITS COMPONENTS: A SYSTEMATIC REVIEW AND META-ANALYISIS Running title: Ferritin, metabolic syndrome and its components

Article Type: Research paper

Section/Category: Clinical & Population Research

Keywords: iron, metabolic syndrome, insulin resistance

Corresponding Author: Dr. Jose Manuel Fernandez-Real, PhD, MD

Corresponding Author's Institution: Hospital Dr.Josep Trueta

First Author: Milton Fabian Suárez-Ortegón

Order of Authors: Milton Fabian Suárez-Ortegón; Eduardo Ensaldo-Carrasco; Ting Shi; Stela McLachlan; Jose Manuel Fernandez-Real; Sarah H Wild

Abstract: BACKGROUND AND AIMS: Mechanisms for the association between iron stores and risk factors for diabetes and cardiovascular disease, such as metabolic syndrome (MetS) and its components, are still not clear. We evaluated the associations between ferritin levels, MetS and its individual components, and potential role of confounding, in a meta-analysis.

METHODS: We searched articles in MEDLINE and EMBASE until February 14th/2018. There were two approaches: Meta-analysis 1) of cross-sectional and longitudinal studies and 2) of only cross-sectional studies. Meta-regressions were conducted to identify sources of heterogeneity in the associations of ferritin with MetS and its individual components.

RESULTS: Information from 27studies (5 prospective) was systematically reviewed and 22 studies were meta-analysed. The pooled OR for MetS by increased ferritin was 1.78(95%CI: 1.60-1.97) in the meta-analysis 1, and 1.70(95%CI: 1.49-1.95 in the meta-analysis 2. The pooled association was weaker in studies that adjusted for hepatic injury markers (meta-regression coefficient (95% CI): -0.34(-0.60,-0.09) P= 0.008) and body mass index (BMI)(meta-regression coefficient(95% CI): -0.27(-0.53,-0.01) P = 0.039). Among MetS components, the pooled association with increased ferritin was strongest with high triglycerides [OR (95%CI): 1.96(1.65-2.32)] and high glucose levels [OR 95%CI: 1.60(1.40-1.82)]. Higher cut-off points used to define high ferritin concentrations were more strongly associated with high triglycerides [meta-regression coefficient (95% CI): 0.22 (0.03, 0.041), P = 0.023].

CONCLUSIONS: High triglycerides and glucose are the components more strongly associated with ferritin. Hepatic injury and BMI appear to influence the ferritin-MetS association, and a threshold effect of high

ferritin concentration on the ferritin-high triglyceridesassociation was observed.

Highlights

- The associations between ferritin and metabolic syndrome (MetS) were meta-analysed.
- Associations of ferritin with each MetS component were meta-analysed.
- Ferritin was positively associated with MetS.
- High triglycerides and glucose are the components more strongly linked to ferritin.
- Hepatic injury and BMI appear to influence the ferritin-MetS association.

Highlights

Ferritin, metabolic syndrome and its components: A systematic review and meta-analysis

Milton Fabian Suárez-Ortegón[1,2*], Eduardo Ensaldo-Carrasco[1], Ting Shi[1], Stela McLachlan[1], José Manuel Fernández-Real[3*], Sarah H. Wild [1]

1. Centre for Population Health Sciences, University of Edinburgh, Edinburgh-Scotland
2. Nutrition Group, Universidad del Valle, Cali-Colombia
3. Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona, CIBEROBN (CB06/03/010) and Instituto de Salud Carlos III (ISCIII), Girona-Spain

*Corresponding authors:

MF Suarez-Ortegon

Centre for Population Health Sciences-University of Edinburgh. Teviot Place. EH8 9AG. Edinburgh-United Kingdom.

Email: Milton.Suarez@ed.ac.uk.

JM Fernández-Real

Department of Diabetes, Endocrinology and Nutrition, Hospital "Dr JosepTrueta", Carretera de França s/n, 17007, Girona-Spain.

Email:jmfreal@idibgi.org

Abstract

Background and aims: Mechanisms for the association between iron stores and risk factors for diabetes and cardiovascular disease, such as metabolic syndrome (MetS) and its components, are still not clear. We evaluated the associations between ferritin levels, MetS and its individual components, and potential role of confounding, in a meta-analysis.

Methods: We searched articles in MEDLINE and EMBASE until February 14th, 2018. There were two approaches: meta-analysis of 1) cross-sectional and longitudinal studies and 2) only cross-sectional studies. Meta-regressions were conducted to identify sources of heterogeneity in the associations of ferritin with MetS and its individual components.

Results: Information from 27 studies (5 prospective) was systematically reviewed and 22 studies were meta-analysed. The pooled OR for MetS by increased ferritin was 1.78 (95%CI: 1.60-1.97) in the meta-analysis 1, and 1.70 (95%CI: 1.49-1.95) in the meta-analysis 2. The pooled association was weaker in studies adjusted for hepatic injury markers (meta-regression coefficient (95% CI): -0.34 (-0.60,-0.09) p= 0.008) and body mass index (BMI) (meta-regression coefficient (95% CI): -0.27 (-0.53,-0.01) p= 0.039). Among MetS components, the pooled association with increased ferritin was strongest with high triglycerides [OR (95%CI): 1.96 (1.65-2.32)] and high glucose levels [OR 95%CI: 1.60 (1.40-1.82)]. Higher cut-off points used to define high ferritin concentrations were more strongly associated with high triglycerides [meta-regression coefficient (95% CI): 0.22 (0.03, 0.041), p= 0.023].

Conclusions: High triglycerides and glucose are the components more strongly associated with ferritin. Hepatic injury and BMI appear to influence the ferritin-MetS association, and a threshold effect of high ferritin concentration on the ferritin-high triglycerides association was observed.

Key words: iron, metabolic syndrome, insulin resistance

Abbreviations

MetS, metabolic syndrome; GGT, gamma-glutamyltranspeptidase/transferase; HDL-C, HDL cholesterol; HOMA-IR, homeostatic model assessment- insulin resistance; BMI, body mass index.

Introduction

Several systematic reviews and meta-analyses have described positive associations between increased iron stores, estimated as ferritin levels, and risk of type 2 diabetes mellitus (T2D) (1-3). Metabolic syndrome, a cluster of clinical and biochemical cardiovascular risk markers, known as MetS components, has been described as a risk factor for T2D and cardiovascular disease (4). Although the relationship between serum ferritin and MetS has been evaluated in several studies, there is limited reviewed evidence for the association between ferritin and MetS. One meta-analysis reported an overall positive association but did not investigate associations of ferritin with individual components of the MetS (5). To date, it is not known whether serum ferritin is equally associated with each MetS abnormality or if there are components that would

explain most of the ferritin-MetS association. Moreover, the role of important confounders such as BMI and hepatic function markers or threshold effects of ferritin levels in the overall association across published studies has not been evaluated previously. Several recent studies on the topic have been published between 2014 and 2018, which have not been included in the previous meta-analysis, justifying an updated review to address the gaps mentioned above with more statistical power. Therefore, we conducted a systematic review and meta-analysis of ferritin, MetS, and its individual components, and explored sources of heterogeneity in the association.

Materials and methods

Search strategy

Two authors (MFSO and EEC) searched and selected articles from PubMed and EMBASE databases up to February 14, 2018. The following search terms were used: metabolic syndrome.mp. or metabolic syndrome X; ferritin or ferritin blood level or iron or body iron stores.mp. No restrictions regarding study design or article type were applied in the search, but unpublished reports were not considered. There were no disagreements about which studies to include, so advice from a third researcher was not needed. Only full texts and abstracts in English language were considered.

Prevalence of MetS components were an additional outcome in this systematic review/meta-analysis. However, specific search terms of MetS components (e.g. glucose, glycaemia, blood sugar levels, blood pressure) were not used since in a preliminary exploration, studies on iron markers and individual MetS components or MetS-related variables that did not include MetS as outcome were heterogeneous in terms of effect estimates and adjustments (many of them unadjusted), which would

have made a quantitative analysis difficult. Studies on MetS as outcome were used to describe associations with MetS components as well, so the individual association between ferritin and each MetS component was evaluated in those studies providing this additional information.

Study selection

Eligibility criteria were studies that included participants from the general adult population with descriptions of associations, stratified by gender and age groups or adjusting for these covariates at a minimum. Study populations exclusively composed of children, pregnant women, obese individuals, or people with a specific diagnosis were not considered. Studies of animals or genetic polymorphisms and reports of *in vitro* experiments were also excluded. If two or more studies were based on the same population and same study design, the study with larger sample size was included. If the sample sizes were similar between studies of similar populations, the study with more robust adjustment was selected. If there were two studies with the same population but with different designs, both studies were selected, but they were analysed separately (see more detail below in data analysis).

Data extraction and risk of bias

The data extracted from the selected articles were name of the study, publication year, country, time of survey or baseline survey, age (range or estimates), study design, sample size, percentage of male individuals, duration of follow-up, prevalence/incidence of MetS, MetS definition, ferritin levels, cut-off values for high ferritin, and covariates for adjustments. Risk of bias was evaluated according to modified criteria of the Newcastle-Ottawa scale modified by Orban and Huth (3) in

terms of representativeness, adjustment for confounders, description of exposure and outcome, and duration of follow-up (if prospective design) (Supplemental file Newcastle-Ottawa scale). The maximum and minimum scores were 7 and 0, respectively, and the higher the score the lower the likelihood of bias. Although representativeness is very complex to evaluate because a study can be representative of a specific location or group of people, we defined a study as representative if it was based on a national/regional health/nutrition survey, an epidemiological population-based study, or if, for instance, random selection was reported in the recruitment of participants.

Data synthesis and analysis

Odds ratios (ORs), hazard ratios (HRs) or relative risks (RRs), and their confidence intervals were extracted from the results described in the studies. Five studies describing mean ferritin levels reported by categories of MetS (yes/no) were retained for the systematic review but excluded from the meta-analyses. For these studies we did not use any method to derive the ORs for the association between mean ferritin levels and MetS, since these methods assume normal distribution of the variable, and distribution of ferritin is frequently skewed across diverse general populations (6, 7). In addition, a meta-analysis of mean differences for the four studies was not feasible owing to different effect estimates reported, in terms of normal mean, standardised mean, and mean of change in ferritin levels.

We decided to conduct the meta-analysis on ferritin and MetS by using two approaches: meta-analysis of cross-sectional studies and prospective studies [meta-analysis 1] and meta-analysis of cross-sectional studies only [meta-analysis 2]. The main rationale

behind that decision was that there were few prospective studies, and it was necessary to ensure higher statistical power for meta-regression and sub-group analyses. The other reason was that some populations reported both cross-sectional and longitudinal associations, so it was relevant to determine the effect of both kinds of estimates on the pooled estimates and subgroup analyses. The meta-analysis on ferritin and MetS components did not require a similar approach since all of the studies describing associations between ferritin and MetS components were cross-sectional in design, with the only exception being Vari et al., who reported both cross-sectional and prospective associations (8). We used cross-sectional findings from the study by Vari et al. for this meta-analysis of the association between ferritin and MetS components.

We pooled estimates from the studies by using an inverse-variance weighted random-effects model. The I^2 statistic was used to estimate heterogeneity in terms of the proportion of total variation in the estimates of meta-analysis explained by heterogeneity. For the meta-analysis 1 of cross-sectional and prospective studies, because most of the studies provided OR as effect estimate, hazard ratios, ORs and relative risks were assumed to approximate the same effect estimate of OR. Meta-regression analyses were conducted to evaluate the potential factors accounting for heterogeneity in the associations between ferritin-MetS and between ferritin-Mets components throughout the selected studies. The factors were: study design (cross-sectional or prospective), type of effect estimate (OR, HR, relative risk), geographic region (Asia, Europe, America), adjustment for BMI (yes/no), adjustment for CRP (yes/no), adjustment for any inflammatory marker (yes/no), adjustment for hepatic function markers (yes/no), sample size (<500 or ≥500), sample size (<1000 or ≥1000), ferritin assay (chemiluminescence QLA, radiometry, RIA; inmunoturbidimetry, TIA;

others), average ferritin levels reported, and cut-off points reported for the highest category of ferritin levels. For these latter two factors, we calculated quartiles specific of sex and menopausal status or whole population as reported in each study selected. In meta-regression analysis, if the meta-regression coefficient is negative, it indicates an inverse association between the potential factor of heterogeneity and the association evaluated. For instance, if the characteristic of adjusting for inflammation markers (yes v. no) across the studies shows a negative meta-regression coefficient in relation to the pooled ferritin-MetS association, this indicates that adjusting for inflammation markers attenuates the pooled association. Sub-group analyses in terms of stratified ferritin-MetS or ferritin-MetS components associations by factors of heterogeneity were performed for those factors that were found significantly associated in the meta-regression analyses. Publication bias was evaluated by using Begg's and Egger's test as well and visualisation of funnel plots. A p value < 0.05 was considered statistically significant All analyses were processed using STATA 14.0 software (Statistics/Data Analysis, Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA, 800-STATA-PC).

Results

Studies with the same population and decisions made

Information on these cases (9-23)] is provided in the Supplemental file.

Studies selected

Figure 1 summarises the process of identifying and selecting the studies. We identified 27 studies that described the association between ferritin and MetS of which 18 were

included in the meta-analyses and systematic review and five only contributed to the systematic review (Table 1). Among the studies selected, there were 9 studies for the meta-analysis on the association between ferritin and the five MetS components. Three studies were prospective only, and two reported cross-sectional and prospective evaluations. The rest of the studies (81%) were cross-sectional analyses.

MetS definitions, geographic location, and types of source

Information on MetS definitions used (24-27) and populations' characteristics is described in the Supplemental file and shown in Table 1.

Adjustments

Two studies exclusively involved post-menopausal women (28), another, only women (both pre and post-menopausal) (10), and four, only men (15, 16, 29, 30). Information on adjustment variables used in the studies is shown in Table 1. Since basic adjustments for age and sex were the inclusion criteria for this systematic review, all of the studies showed either adjustments or stratified results for age and sex.Eleven studies reported adjustment for BMI (9, 10, 18-20, 29, 31-35), 11 for CRP levels as marker of sub-clinical/clinical inflammation, (9, 14, 16-20, 23, 31, 33, 34, 36), and 6 reported adjustments for both covariates (18-20, 31, 33, 34). Four from those with no covariate of CRP, adjusted for white blood cell count (15, 37, 38) or other inflammatory markers such as fibrinogen levels (35). Thirteen studies reported adjustments for hepatic function in terms of transaminase levels (9, 10, 16, 19, 20, 23, 28, 30, 32, 33, 37, 38)or non-alcoholic fatty liver disease (39), two, for family history of chronic diseases (32, 34), and five,for the surrogate of insulin resistance HOMA-IR (10, 15, 16, 23, 37), of which four did not adjust for BMI (15, 16, 23, 37). With the exception of eight studies

(8, 17, 36, 37, 39-42), all others adjusted for alcohol intake. Two articles included education level as covariate (9, 40), out of which one additionally adjusted for variables such as urban or rural residence and income (40). However, this latter study did not adjust for other factors.

Average ferritin concentrations and cut-off values defining high ferritin

Median/mean values of ferritin levels and cut-offs of ferritin defining high concentration reported in the studies selected are shown in Supplemental Tables 1 and 2, respectively. The values are grouped by sex/menopausal status/sex-specific tertiles and quartiles. All of the studies described cut-offs for high ferritin lower than suggested reference values (>200 µg/L in women, >300 µg/L in men) (5), with the exception of Kilani et al. (326 µg/L in men) (19, 20) and Tang et al. (459.9 (cross-sectional study) and 426.6 µg/L (prospective study) in men) (29).

Risk of bias

Supplemental tables 3 and 4 describe our evaluation of risk of bias in cross-sectional and prospective studies, respectively. The median score for risk of bias, which is inversely related to opportunity of bias, was 4. Two cross-sectional studies, i.e. Sun et al. (34) and Jehn et al. (31), reached the maximum possible score of 7 for lower risk of bias (Supplemental Table 3). Of note, many studies with very robust adjustments did not obtain high scores, presumably because one of the assessment criteria was the simultaneous adjustment for BMI and inflammatory markers. Failure to report coefficients of variation in ferritin measurements was another common reason for not obtaining higher scores (Supplemental tables 3and 4).

Ferritin and metabolic syndrome: Results of the meta-analysis and meta-regression

Information from 78,851 individuals was obtained when cross-sectional and prospective studies were analysed together (meta-analysis 1; 19 studies). The pooled OR for MetS by high levels of ferritin (*vs.* lowest levels) was 1.78 (95% CI: 1.60–1.97) [heterogeneity $p< 0.001$; $I^2$57.2%] (Fig. 2A). When prospective effect estimates were replaced by cross-sectional effect estimates in the case of articles or populations providing both associations (meta-analysis 2; 16 studies; 82,332 participants), the pooled OR for MetS for the highest levels of ferritin (*vs.* lowest levels) was 1.70 (95% CI: 1.49–1.95) [heterogeneity $p< 0.001$; I^2 79.2%] (Fig. 2B). The overall ORs for MetS components by high levels of ferritin (*vs.* lowest levels) (meta-analysis 3) are shown in Fig. 3. High triglycerides and glucose were the components more strongly associated with high levels of serum ferritin [high fasting glucose 1.60 (1.40–1.82) heterogeneity $p< 0.001$; I^2 77.8%]; high triglycerides 1.96 (1.65–2.32) heterogeneity $p< 0.001$; I^2 82.8%]; low HDL-C 1.47 (1.30–1.66) [heterogeneity $p< 0.001$; I^2 60.7%]; and high blood pressure 1.13 (1.04–1.23) [heterogeneity $p= 0.074$; I^2 34.7%]. Supplemental Fig. 1-5 show detailed forest plots for the association between serum ferritin and MetS components.

The meta-regression analysis with study characteristics as independent variables is shown in Table 2. In meta-analysis 1, the pooled estimates for association between ferritin and MetS was stronger when RIA (reference category) was the laboratory method for ferritin measurement than with other methods (Table 2) [meta-regression coefficient (95% CI): -0.09 (-0.018,-0.002), $p= 0.045$]. Pooled ORs for MetS by sub-groups of laboratory method are shown in Supplemental Fig. 7. No others potential

factors of heterogeneity were identified for the meta-analysis 1. However, in the meta-analysis of only cross-sectional studies, adjusting for BMI and adjusting for hepatic markers (yes *vs.* no) attenuated the association between ferritin and MetS[BMI meta-regression coefficient (95% CI): -0.27 (-0.53,-0.01) p= 0.039; hepatic markers meta-regression coefficient (95% CI): -0.34 (-0.60,-0.09) p= 0.008] (Table 2). Supplemental Fig. 8 and 9 provide stratified odds ratios by groups of studies adjusting and not adjusting for BMI and hepatic markers, respectively. As in meta-analysis 1, ferritin assay was also found as source of heterogeneity for ferritin –MetS association although with marginal statistical significance (p=0.077) (Table 2).

The meta-regression analysis also showed that adjusting for CRP strengthened the association of ferritin with high triglycerides and high glucose (Supplemental Table 5) (Supplemental Fig. 10 and 11). On the other, hand the ferritin-high blood pressure association was attenuated in studies adjusting for BMI (Supplemental Table 5) (Supplemental Fig. 12). In studies with lower risk of bias (risk of bias score > median score), high ferritin was less strongly associated with high triglycerides, WC, and blood pressure (Meta-regression p< 0.038) (Supplemental Table 5) (Supplemental figures 13, 14 and 15). In addition, higher cut-off points used to define high ferritin concentrations were more strongly associated with high triglycerides [meta-regression coefficient (95% CI): 0.22 (0.03, 0.041), p= 0.023] (Table 3) (Supplemental Fig. 16).

Findings from studies for the systematic review but not included in the meta-analysis

All these articles described significantly higher levels of ferritin in cases with MetS. More details on these associations are provided in the Supplemental file.

Sensitivity analyses

Information on sensitivity analyses is provided in the Supplemental file.

Publication bias

The funnel plot for the ferritin-MetS association was asymmetrical with most of the studies located on the top left of the diagram (Supplemental Fig. 17). However, according to Begg's and Egger's tests, there was no evidence for publication bias (p=0.713 and p=0.299, respectively).

Discussion

The meta-analysis suggested a positive overall association between ferritin and MetS. The meta-regression for the ferritin-MetS association identified weaker associations when the studies adjusted for BMI and hepatic function. With regard to the overall association between ferritin and MetS components, stronger positive associations were observed with triglycerides and fasting glucose in comparison with other components. Moreover, subgroup and meta-regression analyses also showed that in studies with higher cut-off points defining upper categories of ferritin levels, the association with high triglycerides was stronger.

Ferritin and MetS: comparison with previous systematic review/meta-analyses

In the present meta-analysis, we describe a similar pooled overall positive OR for ferritin and MetS to that recently reported by Abril-Ulloa et al. (5), [(1.76 (95% CI:

1.57–1.97) *vs.* 1.73 (1.54–1.95), respectively]. However, the present meta-analysis had several differences from the previous one. First, the inclusion criteria of the present systematic review/meta-analysis required adjustment for at least age and sex. Second, there were four additional prospective studies (18, 20, 23, 29) and six additional cross-sectional studies (19, 28-30, 36, 41). Third, we explored adjustment for BMI and hepatic function markers and threshold effects of ferritin values across study populations as sources of influence on the overall ferritin-MetS association. Lastly, associations between ferritin and individual MetS components were also described to identify whether there were any differences.

Factors influencing the ferritin-MetS association

Neither Abril-Ulloa et al. (5) nor we found that study design, kind of effect estimate, geographic area, and study size influenced the ferritin-MetS association. The trend identified but not discussed by Abril-Ulloa et al. of a stronger association in studies which used immunoradiometric assays for ferritin measurement than in those which used other assays ($p = 0.091$) (5), was statistically significant ($p = 0.045$) in the present meta-analysis. In contrast to the study of Abril-Ulloa et al. (5), in this updated meta-analysis, adjustment for CRP levels was not identified as a source of heterogeneity for the ferritin-MetS association. A possible explanation is that Abril-Ulloa et al. (5) included some articles reporting unadjusted associations. We found that adjusting for CRP strengthened the pooled association with high triglycerides and glucose, similar to the effect observed by Abril-Ulloa et al. for the ferritin-MetS association which was unexpected. CRP levels are considered a confounder since inflammation increases ferritin levels because ferritin is also a phase-acute reactant (43), and cardiometabolic

risk has been widely associated with inflammatory response (44). One would expect effect estimates for ferritin-MetS or ferritin-triglycerides association to be attenuated in CRP-adjusted models rather than the pattern observed.

There were no differences in average ferritin levels or cut-off values for high ferritin by category of laboratory assay (data not shown). Therefore, the influence of the assay in the heterogeneity of ferritin-MetS association cannot be attributed to the threshold effect of the values of ferritin measurement. Since the meta-analysis by Abril-Ulloa et al. also described a similar finding (5), possible explanations should be considered. However, there are no major differences in the accuracy of the current methods for measuring serum ferritin to explain the heterogeneity observed. The heterogeneity of the ferritin-MetS association by ferritin assay could also be a chance finding.

Adjusting for BMI and hepatic function markers (mostly transaminases) attenuated the pooled ferritin-MetS association across the studies evaluated. BMI is a well-known anthropometric predictor of cardiometabolic diseases (CMD) (45) and is positively correlated with iron stores (46). Obesity, estimated as high BMI, is also associated with both iron deficiency and increased ferritin. It appears that adipocytokines stimulate synthesis and secretion of the hormone hepcidin which inhibits intestinal iron absorption and release by tissues, causing iron deficiency (47)]. Similarly, low-grade inflammation in obesity can lead to increasing ferritin levels even in the context of iron deficiency (47)]. Iron excess in obesity could be explained by mechanisms of IR affecting iron homeostasis (48)]. Thus, adjusting for BMI allows investigation of whether any ferritin-MetS association exists independently of obesity. More than half of the studies included did not adjust for BMI, and their authors did not give a rationale for

not using BMI as covariate. Meanwhile, because ferritin is mostly produced in the liver, damage to hepatic cells positively influences circulating ferritin levels because it gets released into the bloodstream (49)]. Similarly, hepatic function markers have been associated with cardiovascular risk factors (50)]. In future research, the role of adjustment for BMI, hepatic function markers for evaluating confounding, effect modification, and potential underlying mechanisms should be considered.

Ferritin and MetS: pooled association *vs.* inconsistencies

Although the meta-analysis identified a pooled positive significant association between ferritin and MetS, there were several studies describing non-significant association. For instance, Zelberg et al. did not find a significant association in an Israeli population (39), and Kilani et al., in men or women (20). Interestingly, along with the latter study, the studies by Jehn et al. (31), Kim et al. (33), Lee et al. (9), and Shi et al. (40) failed to find an independent association in men, a demographic subgroup with higher iron status. There were no consistent associations by sex or menopausal status, with some studies reporting associations in women but not in men and others reporting the reverse.

Ferritin and MetS components

Stronger associations were observed between ferritin and high triglycerides or high fasting glucose than with other components of the MetS. There is growing experimental evidence that metabolism of glucose and of iron are interrelated and in a bidirectional way (43, 51). For instance, in murine models, starvation-induced gluconeogenesis promoted iron hepatic deposition, and high hepatic stores of iron caused hyperinsulinemia by decreasing insulin extraction or affecting insulin signalling (43). This latter effect of iron could promote dyslipidaemia owing to high triglycerides. The

association between ferritin and triglycerides could also be two-way based on findings in animals, where high-fat diets stimulated intrahepatic deposition of iron (43). In light of the above, high levels of glucose and triglycerides appear to be the components that make the largest contribution to a positive association between ferritin and MetS. The finding that the association between ferritin and MetS remained significant after adjustment for IR (HOMA-IR) in the four studies that included this adjustment is interesting. In two of these studies that showed unadjusted and adjusted associations, a marked attenuation of the association was observed only in one (OR (95%CI) 3.45 (3.03–3.92) to 1.99 (1.70–2.33)) (15). The above points imply that association between ferritin and MetS is not entirely explained by the associations with hyperinsulinemia and that there are alternative and still unknown, underlying mechanisms.

The subgroup analysis of the association of ferritin and MetS components suggested the presence of heterogeneity between the studies. For instance, the high blood pressure-ferritin association was weaker when the studies adjusted for BMI as was found for the ferritin-MetSassociation. On the other hand, there were other sources of influence specific to individual associations between ferritin and other MetS components. The association between ferritin and high triglyceride was significantly influenced by the cut-off value for high ferritin reported in the studies. Meanwhile, studies with greater risk of bias can overestimate specific associations between ferritin and increased WC, triglycerides, and blood pressure on the basis of low representativeness and/or non-adjustment for BMI. It is unclear why these factors were not similarly found as sources of influence in the ferritin-MetS association. The above discrepancy suggests that each component of MetS may have specific patterns of association with ferritin regardless of the pattern with the risk cluster.

Ferritin, MetS and its components: role of non-alcoholic hepatic steatosis (NASH) and fatty liver?

Our findings on stronger association of ferritin with high glucose and triglycerides, components highly related to insulin resistance, plus the influence of BMI on the ferritin-MetS association may involve liver alterations. In fact, insulin resistance and ferritin have been described as major determinants of non-alcoholic fatty liver disease in apparently healthy obese patients (52). Serum ferritin concentrations were also significantly higher in NASH patients than in the patients with simple steatosis (53). In this latter study, the serum ferritin level was associated with insulin resistance, with an area under the ROC curve of 0.732 for distinguishing NASH from simple steatosis (p= 0.005; 95% CI, 0.596-0.856). Thus, high ferritin levels, in addition to be a marker of MetS, could constitute a marker of fatty liver in obese people that usually have high triglyceride and glucose levels. In this context, and to close the circle, serum ferritin levels have been described to be associated with vascular damage in patients with non-alcoholic fatty liver disease (54).

Strengths and limitations

To the best of our knowledge, this study appears to be the first meta-analysis on ferritin, MetS, and its individual components. In addition, the investigation of the influence of adjustments for body mass and hepatic function and of threshold effects of ferritin on the ferritin-MetS association across the studies represents another novel contribution. On the other hand, some findings from the subgroup and meta-regression analysis were not consistent throughout the sensitivity analysis. This implies limitations in statistical power or chance findings arising from multiple testing. Given the different assumptions

in the calculation of effect estimates from prospective and cross-sectional studies, analysing them together might not be appropriate, although no heterogeneity by effect estimate or study design was detected in the subgroup meta-regression analysis. However, this potential limitation was balanced by conducting an additional meta-analysis specific to cross-sectional studies with all the studies reporting associations as ORs.

In conclusion, the meta-analysis suggests a significant overall positive association between ferritin and MetS. Hepatic injury, BMI, and type of ferritin assay appear to influence the ferritin-MetS association. It also appears to exist a threshold effect of high ferritin concentration on the associations with high triglycerides. High triglycerides and glucose are the MetS components most strongly associated with ferritin levels and could explain most of the association with the risk cluster known as MetS.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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LEGENDS TO FIGURES

Figure 1. Flow chart for the selection of eligible studies included in the systematic review/meta-analysis of the association between ferritin and metabolic syndrome.

Figure 2. Forest plots describing the association (odds ratio 95 % confidence interval) between ferritin and metabolic syndrome in: (A) cross-sectional and longitudinal studies [Meta-analysis 1] and (**B)** only cross-sectional studies [Meta-

analysis 2]. Studies are stratified by sex, menopausal status or presented both sexes depending on the way the association was reported in each article. Diamonds are pooled estimates from inverse variance weighted effects random models.

Figure 3. Overall pooled odds ratios (95 % confidence interval) for association between high levels of ferritin (*vs.* lowest levels) and each MetS component. Detailed forest plots for these associations are shown in supplemental material.

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
Jehn et al., 2004 (31)	Cross-sectional	U.S/ U.S population	NHANES III /1988-1994	≥20	20.1	6044	17.5% (Men), 10.2% (Premenopausal women), and 27.8% (postmenopausal women)	NCEP ATP-III	yes	yes	Alcohol intake and smoking
Vari et al.,2007	Cross-sectional/Pr	France/Users insured by	DESIR/NP	30-65	49.7	944	21% (Men), 8%	IDF NCEP ATP-III	No	No	None

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
(8)	ospective (6 years follow-up)	French Social Security					(Premenopausal women), and 24% (postmenopausal women)	NCEP revised			
Zelber-Sagi et al., 2007 (39)	Cross-sectional	Israel	First Israeli National Health and Nutrition Survey/2003-2004	24-70	52.7	349	NP as a total	NCEP ATP-III	No	No	Non-alcoholic fatty liver disease

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STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS										
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**	
									BMI	**CRP**	**Other adjustments**
Shi et al., 2008 (40)	Cross-sectional	China/China population	National Nutrition Survey /2002	>20	45.9	1294	9.4% (men) and 18% (women)	IDF	No	No	Residence (urban/rural), education level, and income
Sun et al., 2008 (34)	Cross-sectional	China/ China	NHAPC/ 2005	50-70	43	3289	42.3%	NCEP ATP-III	Yes	Yes	Alcohol intake, smoking, family history of chronic diseases, dietary factors, IL-6, TNF-R2,

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
Cho et al., 2011 (10)	Cross-sectional	Korea/ Korea population	KNHANES /2007	36.9 ± 8.2 (Premenopausal) and 64.8 ± 9.5 (Postm	0 (1691 and 1391 pre and postmenopausal women, respectively)	3082	10.6 (Premenopausal) and 41.9 (Postmenopausal)	NCEP ATP-III and the Korean Society for Study of Obesity (WC cut-off points)	Yes	No	HOMA-IR, alcohol intake, smoking history, exercise, intake of energy, iron, hemoglobin, ASAT, ALAT, and hormone
											adiponectin, PAI-1, and RBP4)

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
				enopausal)							therapy use (postmenopausal women)
Kim et al., 2011 (33)	Cross-sectional	Korea/ Users of a Health Promotion Centre in Seoul	NP/2008	20-89	52.7	12090	NP	NCEP ATP-III	Yes	Yes	Smoking, alcohol use, and menopause status (women).
Lee et	Cross-	Korea/	KNHANES	>20	42.5	6311	16.3% (Men),	NCEP ATP-III	Yes	No	Alcohol intake,

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS

Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
al.,2011 (9)	sectional	Korea population	IV /2008				9.5% (Premenopausal women), and 31.5% (postmenopausal women)	and the Korean Society for Study of Obesity (WC cut-off points)			smoking, educational level, AST and ALT.
Ryoo et al., 2011 (15)**	Cross-sectional	Korea/ Employees from companies	NP/2008	40.5 ± 6.5	100	1858 1	13.8	NCEP ATP-III	No	No	Alcohol intake, recent smoking status, total protein, GGT, log

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
		in an Annual health check-up									(hsCRP), WBC, ALT, ApoB, TIBC, serum creatinine and HOMA-IR
Yoon et al., 2012 (23)	Prospective (5 years follow-up)	Korea/Korean Rural Population	Korean Genomic Rural Cohort/NP	>40	49.8	861	13.3	Harmonized definition	No	Yes	HOMA-IR, adiponectin, leptin, ALT, exercise, alcohol intake and

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
											smoking status
Park et al., 2012 (16)	Prospective (5 years follow-up)	Korea/ Employees from companies in an Annual health checkup	NP/2005-2010	30-59	100	1902 2	16.3	Harmonized definition	No*	Yes	WBC, GGT, HOMA-IR, serum creatinine, TIBC, smoking status, regular exercise, alcohol intake, hypertension, diabetes
Chang et	Cross-	Taiwan/	NAHSIT	≥19	47.4	2654	43.1% (Men),	NCEP ATP-III	Yes	No	GOT, GTP, ALK,

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS

Authors, year (Ref)	Study	Location/ Universe	Study-Survey /Year of survey	Age range (years)*	Male (%)	*n*	Prevalence of metabolic syndrome	MetS definition	Adjustments		
									BMI	CRP	Other adjustments
al.,2013 (32)	sectional	Taiwan population	/2005-2008				26.5% (women)	for Asia Pacific			Amylase, BUN, UA, creatinine, homocysteine, past smoker, alcohol intake, betel nut intake, haemoglobin, iron deficiency anemia, and family history of

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
											chronic diseases
Li et al. , 2013 (14)	Cross-sectional	China/China population	CHNS /2009	≥18	46.6	8441	19.9% (Men), 25.4% (women)	NCEP ATP-III for Asia Americans	No	Yes	Nationality, alcohol intake and smoking
Kilani et al., 2014 (19)	Cross-sectional	Switzerland/ Population from Lausanne	The CohorteLausannoise/ 2003-2006	35-75	47.2	5498	29.4% (Men) 8.3% (premenopausal women) and 25.5% (postmenopausal	NCEP ATP-III	Yes	Yes	Alcohol intake, smoking, iron supplement and altered hepatic markers

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
							women)				
Ledesma et al., 2015 (30)	Cross-sectional	Spain/ workers from a factory in Zaragoza	The Aragon Workers' Health Study/2009-2019	19-65	100	3386	27.1	Harmonized definition	No	No	History of blood donations, alcohol intake and transaminases
Seo et al., 2015 (28)	Cross-sectional	Korea/ Users of a health promotion	NP/2008-2010	>40	0	280	25~%	NCEP ATP-III (BMI used instead of waist	No	No	Alcohol intake, haemoglobin, transaminases and hormone status

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
		center in Seoul						circumference)			(E2, total testosterone, FSH, and TSH)
Tang et al., 2015 (29)	Cross-sectional/Prospective (4 years follow-up)	China/ Men from Guangxi	Fangchenggang Area Males Health and Examination Survey/2009-2013	17-88	100	2417	Prevalence :12.7 % Incidence: 9.42%	NCEP ATP-III for Asia Americans	Yes	No	Physical activity, family history of chronic diseases, alcohol intake and smoking status
Kilani et	Prospective	Switzerland/	The	35-75	42.8	3271	22.6% (Men), and	NCEP ATP-III	Yes	Yes	Alcohol intake,

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
al., 2015 (20)	(5.5 years follow-up	Population from Lausanne	CohorteLausannoise/ 2003-2006				16.5 % (women)				smoking, iron supplement and altered hepatic markers
Suarez-Ortegon et al., 2016 (35)	Cross-sectional	Croatia/ population from the villages Vis and Komiza	Dalmatians Research Programme/ 2003-2004	18-93	39.1	725	50.7% (Men) 33.8% (premenopausal women) and 88.6% (postmenopausal	Harmonized definition	Yes	No	Fibrinogen levels, smoking,and alcohol consumption

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS											
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**		
									BMI	**CRP**	**Other adjustments**
							women)				
Cho et al., 2017 (38)	Cross-sectional	Korea/ Korea population	KNHANES /2010-2012	58.7±0.4	0	2734	Not provided for the whole population. MetS prevalence was 40.3%-64.8% from the lowest till highest quartile of ferritin	NCEP ATP-III	No	No	Smoking, alcohol consumption, regular exercise, and leukocyte count
Chen et	Cross-	China/	Population-	25-75	47.5	2786	42% (Men), and	IDF	No	No	Serum creatinine,

Table 1. Characteristics of the studies included in the systematic review and meta-analysis and in the systematic review only of the association between ferritin and metabolic syndrome (seven pages table)

STUDIES INCLUDED IN THE SYSTEMATIC REVIEW AND META-ANALYSIS										
Authors, year (Ref)	**Study**	**Location/ Universe**	**Study-Survey /Year of survey**	**Age range (years)***	**Male (%)**	***n***	**Prevalence of metabolic syndrome**	**MetS definition**	**Adjustments**	
									BMI	**CRP**	**Other adjustments**
al., 2017 (37)	sectional	Population from Pinggu district, Beijing	based study / 2012-2013				45% (women)				ALT, Neutrophils/Lymphocytes ratio, frequency of pork consumption and HOMA-IR

STUDIES INCLUDED ONLY IN THE SYSTEMATIC REVIEW

Authors	Study	Location/ Universe	Study-Survey /Year of survey	Age range (years)	Male (%)	*n*	Prevalence of metabolic syndrome	MetS definition	Adjustments		
									BMI	CRP	Others adjustments
Martinelli et al.,2012 (36)	Cross-sectional	Italy/ Val Borbera population	Val Borbera/NP	>18	44.3	1391	21.9%	Harmonized definition	No	Yes	C282Y HFE mutation, haemoglobin, uric acid, and creatinine

Hamalainen et al.,2012 (17)	Cross-sectional	Finland/Middle –aged subjects from Pieksamaki who were born in 1942,1947,1952,1957 or 1962	NP/2003-2004	52.1 ± 6.2 years (men) and 52.1 ± 6.2 years (women)	44.5	766	53% (men), 40% (women)	NCEP ATP-III	No	Yes	Smoking, alcohol intake and physical activity

Hamalainen et al.,2014 (18)	Prospective (6.5 years follow-up)	Finland/Middle –aged subjects from Pieksamaki who were born in 1942,1947,1952,1957 or 1962	NP/1998-2004	45.3 ± 6.2 years (men) and 45.1 ± 6.5 years (women)	41.8	691	Incidence :18%	Harmonized definition	Yes	Yes	Smoking, alcohol intake and physical activity
Iwanaga et al., 2011 (41)	Cross-sectional	Japan/in individuals from a worksite lifestyle intervention study	NP/2007	41.2 ± 10.4 years	42.7	685	13.6 (men), 1.7 (women)	Japanese criteria	No	No	None

Padwal et al., 2015 (42)	Cross-sectional	India/ Patients from Outpatient department University Medical College, Pune	2013	≥30	50%	90	Not apply. Age-sex matched case-control study (50 cases with MetS)	NCEP ATP-III	No	No	None

***** * Or mean (SD) of age if age range not provided. ** This study used BMI instead of waist circumference as surrogate for central obesity. Ref, reference. NP, Not provided.

Table 2. Effect of diverse studies' characteristics on the pooled ferritin-MetS association

	Prospective and cross-sectional studies (Meta-analysis 1)			Cross-sectional studies only (Meta-analysis 2)		
	Number of studies*	**Meta-regression coefficient (95%CI)**	**P value**	**Number of studies***	**Meta-regression coefficient (95%CI)**	**P value**
Study design (Cross-sectional v. Prospective)	19 (36)	-0.11 (-0.35,0.11)	0.320	18 (33)	**	**
Measure of association (Odds ratio/ Hazard ratio/ Relative risk)	19 (36)	0.10 (-0.19,0.39)	0.497	18 (33)	**	**
Region (Asia/ Europe/America)	19 (36)	-0.02 (-0.19,0.14)	0.782	18 (33)	-0.07 (-0.29,0.14)	0.484
Adjusted for BMI (Yesv. No)	19 (36)	-0.14 (-0.35,0.05)	0.154	18 (33)	**-0.27 (-0.53,-0.01)**	**0.039**
Adjusted for CRP (Yesv. No)	19 (36)	0.14 (-0.06,0.34)	0.178	18 (33)	-0.08 (-0.37,0.21)	0.578
Adjusted for at least one inflammatory marker (Yesv. No)	19 (36)	0.08 (-0.12,0.29)	0.394	18 (33)	-0.06 (-0.34,0.21)	0.631
Adjusted for at least one hepatic function marker (Yesv. No)	19 (36)	-0.16 (-0.36,0.04)	0.121	18 (33)	**-0.34 (-0.60,-0.09)**	**0.008**
Ferritin assay (RIA/ QLA/ TIA/Other)	19 (36)	**-0.09 (-0.18,-0.002)**	**0.045**	18 (33)	-0.11 (-0.23,0.01)	0.077
Sample size≥1000	19 (36)	0.07 (-0.05,0.29)	0.528	18 (33)	-0.05 (-0.38,0.28)	0.748

Risk of bias (score ≥ median score) (Yesv. No)	19 (36)	-0.09 (-0.30,0.11)	0.375	18 (33)	0.06 (-0.21,0.33)	0.653
Sex/menopausal-specific quartiles (1-2-3-4) of mean/median ferritin levels	19 (36)	-0.01 (-0.11,0.08)	0.742	18 (33)	-0.09 (-0.21,0.03)	0.144
Sex/menopausal-specific quartiles (1-2-3-4) of cut-off points reported for highest category of ferritin levels	18 (33)	0.07 (-0.03,0.17)	0.187	17 (30)	0.004 (-0.14,0.15)	0.955

* The first number describes number of studies, and second number (in parenthesis) means sex/menopausal status groups from each study.

**These characteristics do not apply since all studies in the meta-analysis 2 were cross-sectional and reported the same kind of effect estimate: Odds ratio (95% confidence interval).

Dr. Arnold von Eckardstein
Editor-in-Chief
Geesje M. Dallinga-Thie
Co-Editor
Atherosclerosis

May 1st , 2018

Ref.: Ms. No. ATH-D-18-00284

FERRITIN, METABOLIC SYNDROME AND ITS COMPONENTS: A SYSTEMATIC REVIEW AND META-ANALYISIS

Dear Drs von Eckardstein and Dallinga-Thie,

Thank you for your e-mail of April 8th. We appreciate all your comments and suggestions as well as those from the reviewers of our manuscript.

A point-by-point response to the associate editor's and reviewers' comments is enclosed. All suggestions have been addressed. Modifications throughout the manuscript are in red font. We would be pleased to provide additional information or to further modify the text.

The current version of article has 4408 words (introduction, methods, results and discussion) due to the addition of a new and pertinent paragraph in discussion section suggested by the reviewer #1. There are 200 words more in the legends of tables and figures. We believe that additional reduction of words count would imply to loose clarity in methods and discussion. We hope this little excess of words can be accepted taking into account that the article is not only a meta-analysis but also a systematic review.

We look forward to hearing from you. Thank you very much for your attention to our manuscript.

Sincerely yours,
José Manuel Fernández-Real and Milton Fabian Suárez-Ortegón

The authors are grateful for the reviewer' comments which have contributed to clarify the message of our paper and to improve the quality of our submission.

The specific comments are addressed below:

Reviewers' comments

Reviewer #1

Milton Fabian Suárez-Ortegón et al. have performed a meta-analysis and systematic review on the association between ferritin and metabolic syndrome and its components. The main conclusion is that there is a clear association between ferritin and MS particularly with triglycerides and glucose components.

The role of ferritin in the context of metabolic diseases remains uncertain and clinicians could be mislead by high ferritin concentrations in these patients. Therefore, this study is welcome because contributes to establish the association of ferritin levels and metabolic diseases.

The study has been very well conducted. The quality controls applied to studies included in the analyses are robust. The number of studies included is large enough. Therefore the results are highly reliable.

R/ Many thanks for this opinion.

At the end the authors show a strong association between TG, G and BMI that probably determine high ferritin levels independently of metabolic syndrome definition. It is known that these three factors are associated to fatty liver that is associated to high ferritin levels, even in the absence of liver damage (high transaminases). I think that in the discussion such association should be better addressed and the role of fatty liver and NASH as high ferritin levels determinants, must be taken into account at least in the discussion. Are high ferritin levels a marker of MS or a marker of fatty liver in obese people that usually have high TG and glucose levels?

R/ We fully agree with this comment. We greatly acknowledge this idea. We have added a paragraph at the end of discussion section, as follows:

"**Ferritin, MetS and its components: role of non-alcoholic hepatic steatosis (NASH) and fatty liver?**

Our findings on stronger association of ferritin levels with high glucose and triglycerides,

components highly related to insulin resistance, plus the influence of BMI on the ferritin-MetS association may involve liver alterations. In fact, insulin resistance and ferritin have been described as major determinants of non-alcoholic fatty liver disease in apparently healthy obese patients (52). Serum ferritin concentrations were also significantly higher in NASH patients than in the patients with simple steatosis (53). In this latter study, the serum ferritin level was associated with insulin resistance, with an area under the ROC curve of 0.732 for distinguishing NASH from simple steatosis (P = 0.005; 95% CI, 0.596-0.856). Thus, high ferritin levels, in addition to be a marker of MetS, could constitute a marker of fatty liver in obese people that usually have high triglyceride and glucose levels. In this context, and to close the circle, serum ferritin levels have been described to be associated with vascular damage in patients with non-alcoholic fatty liver disease (54)."

From my point of view the data in supplementary material are more clinically relevant that the tables in the paper. I suggest including at least a figure showing the data from the Forest plots between ferritin and the MS components, probably showing only the overall results for each variable, while tables 2 and 3 could be send to supplementary material.

R/ We agree with this comment of the reviewer. We have created a new figure, Figure 3, which show overall pooled estimates for associations between high ferritin (v. low ferritin) and each MetS component. Supplemental figures 1-5, show the detailed forest plots for the above associations. We sent the Table 3 to the supplemental material, and this is now the new Supplemental Table 5. We kept Table 2 in the main manuscript since the Journal enables until 5 tables/figures. The current manuscript has two tables and three figures. Here we present the modifications in results section:

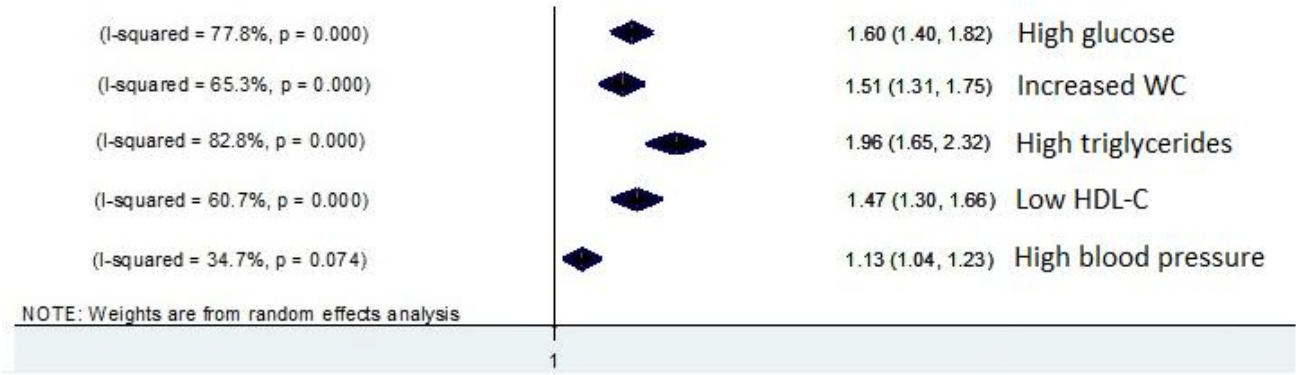

Figure 3. Overall pooled odds ratios (95 % confidence interval) for association between high levels of ferritin (vs. lowest levels) and each MetS component. Detailed forest plots for these associations are shown in supplemental material.

"The overall ORs for MetS components by high levels of ferritin (vs. lowest levels) (meta-analysis 3) are shown in Figure 3. High triglycerides and glucose were the components more strongly associated with high levels of serum ferritin [high fasting glucose 1.60 (1.40–1.82)

heterogeneity P < 0.001; I^2 77.8%]; high triglycerides 1.96 (1.65–2.32) heterogeneity P < 0.001; I^2 82.8%]; low HDL-C 1.47 (1.30–1.66) [heterogeneity P < 0.001; $I^2$60.7%]; and high blood pressure 1.13 (1.04–1.23) [heterogeneity P = 0.074; $I^2$34.7%]. Supplemental figures 1-5 show detailed forest plots for the association between serum ferritin and MetS components."

"The meta-regression analysis also showed that adjusting for CRP strengthened the association of ferritin with high triglycerides and high glucose (Supplemental Table 5) (Supplemental figures 10 and 11). On the other, hand the ferritin-high blood pressure association was attenuated in studies adjusting for BMI (Supplemental Table 5)……"

Reviewer #2

The paper by Suarez-Ortegon et al reports a systematic review and meta-analysis on the potential association of circulating ferritin levels with the MetS. The authors meta-analyzed this relationship by 2 approaches: meta-analysis of cross-sectional/longitudinal studies and only cross-sectional studies. Moreover, a subgroup analysis considering the association of ferritin with MetS components was also conducted.

The paper deals with a relevant issue of significant clinical relevance.
The paper appears consequential in its sections and is clearly readable. The Authors conclude that high TG and glucose are the components of the MetS more associated with ferritin levels. Moreover, liver disease and BMI strongly influenced the ferritin-MetS association.

The statistical approach appears correct and solid in reaching sound conclusions. Overall, more than 78,000 individuals were included in the analysis. The Discussion section is of appropriate length and properly discusses the data obtained within the literature context.
R/ Many thanks for this opinion.
Specific and minor comments:

Highlights
The first 2 sentences may be re-written using 1) an impersonal wording (not "we...") and 2) making the second statement independent from the first one.
R/ The two first highlights have been corrected as the reviewer suggested:

"- The associations between ferritin and metabolic syndrome (MetS) were meta-analysed.
- Associations of ferritin with each MetS component were meta-analysed."

Introduction, line 6:
at least here at the beginning add the word "mellitus" after...type 2 diabetes ....

R/ Added as the reviewers suggested:
"Several systematic reviews and meta-analyses have described positive associations between increased iron stores, estimated as ferritin levels, and risk of type 2 diabetes mellitus (T2D)."

Methods, Data synthesis and analysis, second page of this section, line 16:
"quimioluminiscencia" appears to be the correct word in Spanish. Use "chemiluminescence" here, in English.

R/ Corrected as the reviewers suggested:

"ferritin assay (chemiluminescence QLA, radiometry, RIA; inmunoturbidimetry, TIA; others),....."

Reviewer #3

The associations of ferritin levels, metabolic syndrome and the individual components of metabolic syndrome have been investigated in a meta-analysis of 22 studies. It has been concluded that high triglycerides and glucose are the metabolic syndrome components that are more strongly associated with ferritin. It was also found that hepatic dysfunction and BMI influence the ferritin-metabolic syndrome association. A threshold effect of high ferritin concentration on the ferritin-high triglycerides association was also found. This is an interesting report that extends a number of previous reports of an association of ferritin levels with metabolic syndrome.
Overall, this is a valuable addition to previous studies of the relationship between ferritin and the metabolic syndrome.

R/ Many thanks for these opinions.
Some of the grammar could be improved.

R/ Grammar and style have been revised by a professional academic proof-reading service.
Otherwise, there are no issues requiring attention.

Editorial Office comments

-Atherosclerosis applies formatting guidelines to all accepted papers, with the aim of

improving their readability.
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- Make sure to apply the formatting requirements to all figures and tables where necessary (e.g. style of p values, gene and protein nomenclature).

R/ Checked as requested.

- Make sure to use uniform lettering and sizing of your original artwork, including letters to indicate panels, throughout all figures.

R/ Checked as requested.

- Make sure to submit high resolution versions of each figure.

R/ High resolution figures have been uploaded.

Statement of Originality

The manuscript has been submitted only to *Atherosclerosis*, and it will not be submitted elsewhere while under consideration. This article has not been published elsewhere, and, if accepted, it will not be published elsewhere—either in similar form or verbatim—without permission of the editors.

All authors are responsible for reported research, and have participated in the concept and design; analysis and interpretation of data; drafting or revising of the manuscript. All authors have approved the manuscript as submitted.

*Conflict of interest form

AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Signed by all authors as follows:

March 5th, 2018

Milton Fabian Suárez-Ortegón, Eduardo Ensaldo-Carrasco, Ting Shi, Stela McLachlan, José Manuel Fernández-Real, Sarah H. Wild

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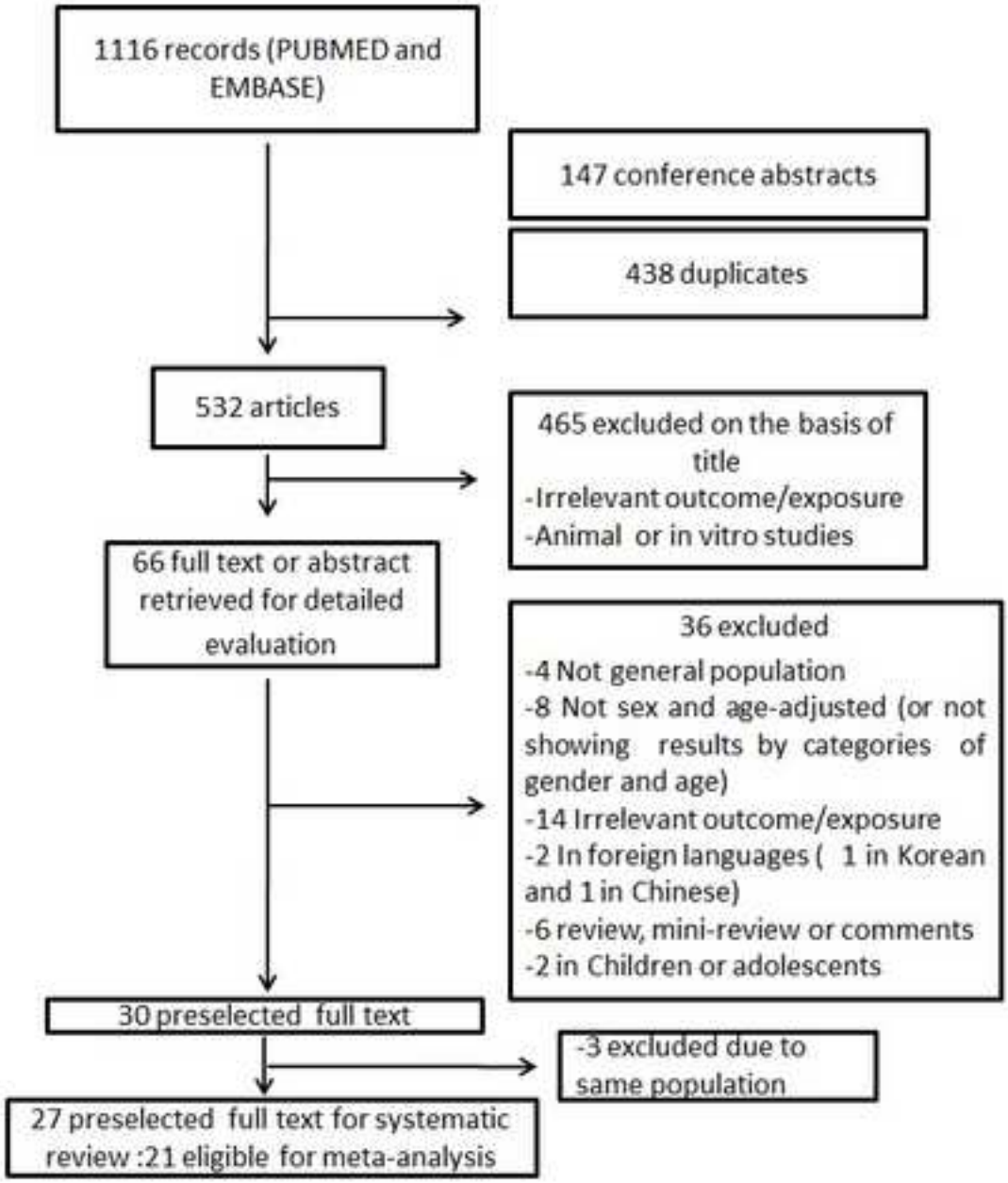

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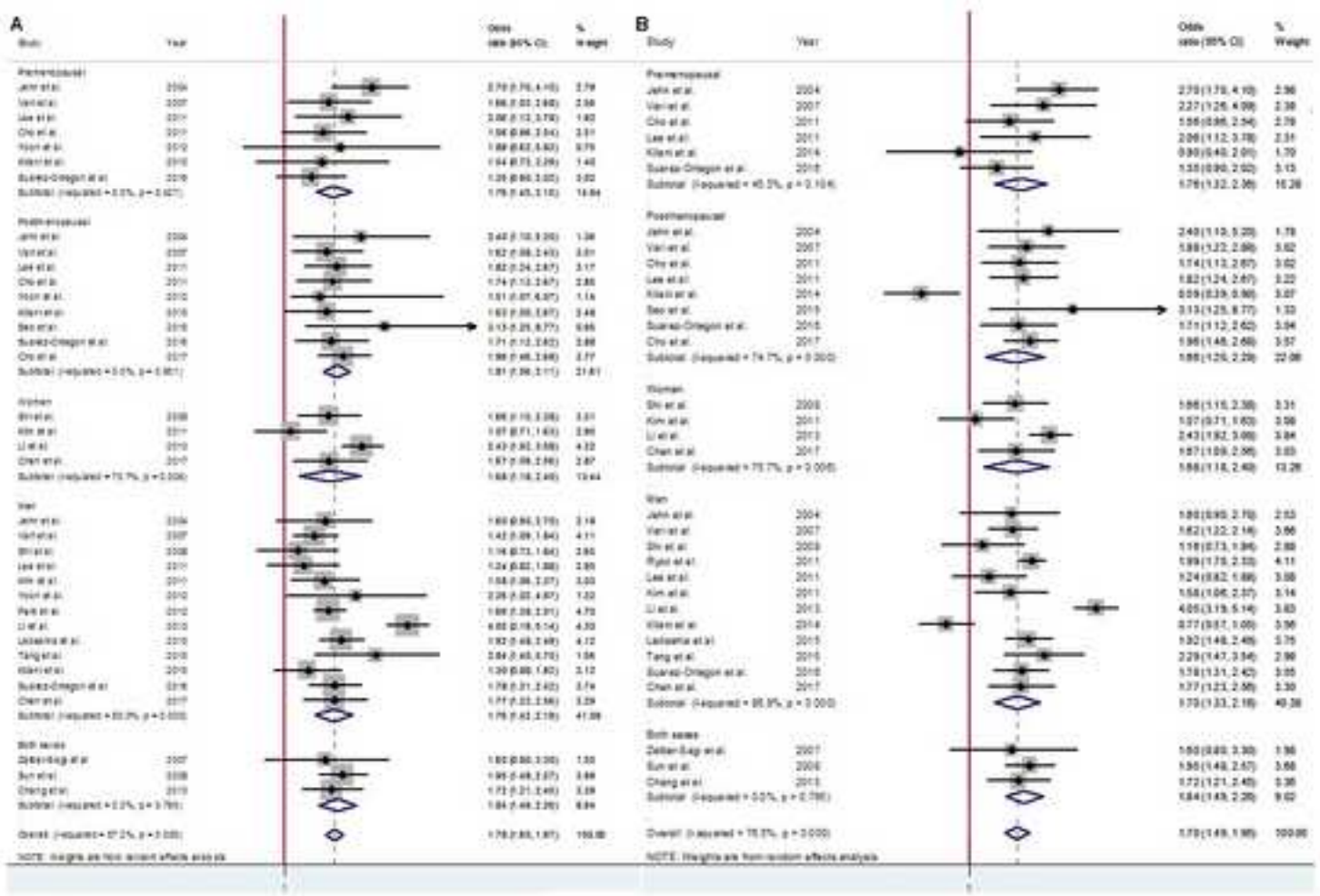

Figure 2

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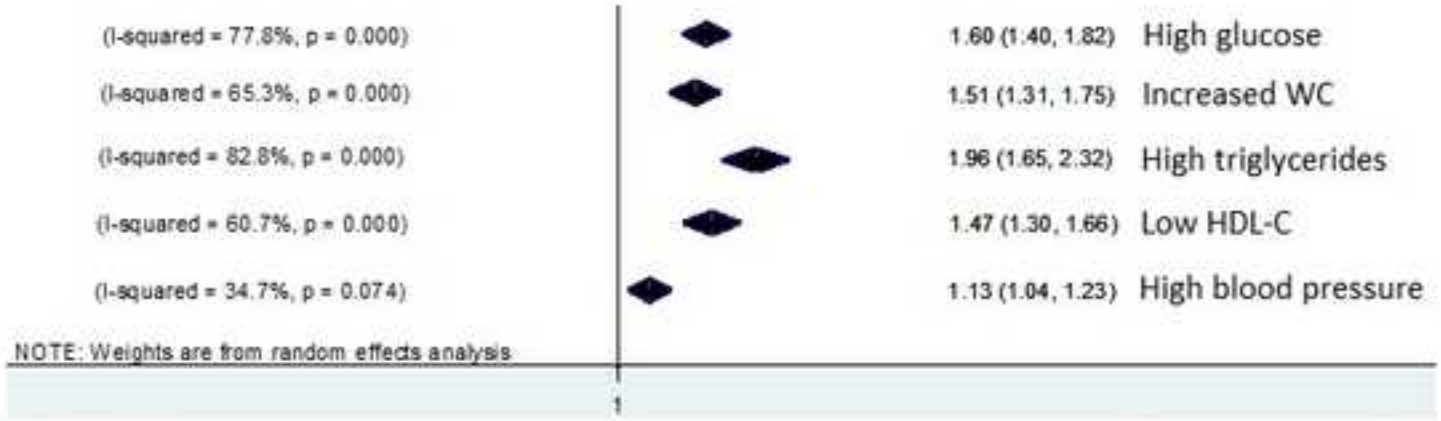

Figure 3.

Supplementary Material for online publication only

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Atherosclerosis style guide checklist

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