Effect of iron intake on iron status: a systematic review and meta-analysis of randomized controlled trials[1–4]

Amelie Casgrain, Rachel Collings, Linda J Harvey, Lee Hooper, and Susan J Fairweather-Tait

ABSTRACT

Background: The response of status biomarkers to an increase in iron supply depends on several physiologic and environmental factors, which make it difficult to predict the outcome of an intervention.

Objective: We assessed effects of baseline iron status, sex, menopausal status, duration of intervention, iron form, and daily dose on the change in iron status in response to iron supplementation.

Design: A systematic review of randomized controlled trials (RCTs) of iron-supplementation and -fortification trials that assessed effects on hemoglobin, serum ferritin (SF), soluble transferrin receptor, or body iron was conducted. Subgrouping and straight-line and curved metaregression were used to describe the magnitude and dose-responsiveness of effect modifiers with respect to changes in status.

Results: Forty-one RCTs were included; none of the RCTs were judged at low risk of bias. Random-effects meta-analyses showed that iron supplementation significantly improved iron status but with high levels of heterogeneity. Metaregression explained approximately one-quarter of between-study variance in effect size. There were clear effects on SF with study duration (increase in SF concentration/wk: 0.51 μg/L; 95% CI: 0.02, 1.00 μg/L; $P = 0.04$) and dose (increase in SF concentration/g Fe: 0.10 μg/L; 95% CI: 0.01, 0.20 μg/L; $P = 0.036$) and on hemoglobin concentrations with baseline iron status [−0.08 g/dL (95% CI: 0.15, 0.00 g/dL) per 10-μg/L increase in baseline SF concentration; $P = 0.02$]. Insufficient data were available to assess effects on body iron, sex, or menopausal status.

Conclusion: Quantitative relations between baseline iron status, study duration, and iron dose on changes in iron-status biomarkers, which were generated from the meta-analyses, can be used to predict effects of trials of iron supplementation and fortification and to design iron-intervention programs. *Am J Clin Nutr* 2012;96:768–80.

INTRODUCTION

Biomarkers of iron status include hemoglobin, plasma or serum ferritin (SF)[5], soluble transferrin receptor (sTfR), and total body iron, calculated from the ratio of SF and sTfR. Hemoglobin, which is the protein that carries oxygen around the circulatory system, is the major iron pool in humans and, therefore, is considered to be a functional marker of iron status. Ferritin is the major iron storage protein, and there is a close relation between SF and liver iron stores (1). However, ferritin is also an acute-phase reactant that responds to inflammation, infection, and other disease states, and under these conditions, it does not accurately reflect iron stores. An increased concentration of sTfR in serum (or plasma) is an early sign of iron deficiency (ID) and an indicator of the severity of the insufficiency. sTfR is less affected than SF by an inflammatory state, but sTfR is affected by the rate of erythropoiesis. Body iron stores can be estimated by calculating the ratio of sTfR to SF (2).

The WHO has published a series of sex- and life stage–specific cutoffs for anemia according to the needs and status of population subgroups (eg, hemoglobin concentration of 12 g/dL for nonpregnant women). However, anemia may be caused by dietary deficiencies of nutrients other than iron (ie, vitamin B-12, folate, and vitamin A) or by inflammation that results from infection (3). Hence, to ascertain that anemia is caused by ID, hemoglobin needs to be measured in conjunction with another biomarker of iron status, such as SF. The relation between iron intake and status is complicated by factors that determine bioavailability (ie, dietary and physiologic factors that affect iron absorption) (4, 5), making it difficult to predict the response to iron supplementation.

The aim of this systematic review was to describe the relation between supplemental iron intake and status (assessed from hemoglobin, SF, sTfR, or body iron measurements) by using data from randomized controlled trials (RCTs) in healthy volunteers in whom iron intakes were changed in a quantitative manner for a period of time that was sufficient to effect a change in iron status. Secondary aims included the assessment of the influence of dose, sex, menopausal status, baseline status, or the form of iron in the supplement on this relation.

[1] From the Norwich Medical School, University of East Anglia, Norwich, United Kingdom.

[2] This article does not necessarily reflect the views of the Commission of the European Communities or its future policy in this area.

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[4] Address correspondence to SJ Fairweather-Tait, Department of Nutrition, Norwich Medical School, Norwich Research Park, University of East Anglia, Norwich NR4 7JT, United Kingdom. E-mail: s.fairweather-tait@uea.ac.uk.

[5] Abbreviations used: EURRECA, EURopean micronutrient RECommendations Aligned; ID, iron deficiency; IDA, iron-deficiency anemia; MD, mean difference; RCT, randomized controlled trial; SF, serum ferritin; sTfR, soluble transferrin receptor.

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METHODS

Methods were based on those described in the Cochrane Handbook (6), and the protocol, which is shown on the EURopean micronutrient RECommendations Aligned (EURRECA) intranet (www.eurreca.org), was developed within the EURRECA Network of Excellence (7).

Included studies had to fulfill all of the following criteria: *1*) enrolled a healthy adult population (mean age ≥18 y, with any baseline iron status; excluding highly trained athletes, regular blood donors, and individuals receiving erythropoietin, with chronic disease, or with gastrointestinal infections); *2*) subjects were randomly assigned to receive an iron supplement, fortified food, or rich natural dietary sources compared with a placebo or no dietary intervention; *3*) iron status was reported at baseline and study end (or the change from baseline) by using hemoglobin, SF, sTfR, and/or body iron (2); *4*) iron dose reported and/or dietary intake assessed; and *5*) written in English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, Portuguese, or Serbian.

MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed), EMBASE (OvidSP; www.ovid.com), and Cochrane Library CENTRAL (www.thecochranelibrary.com) databases were searched from inception to February 2012. The complex search used text and indexing terms, truncation, explosion, and Boolean operators and was fully tested to ensure thorough retrieval of relevant publications. The general structure used was [randomized controlled trial] AND [supplementation OR dietary intake OR nutritional status] AND [iron OR ferr*] AND [human] (*see* Table 1 under "Supplemental data" in the online issue for the full EMBASE search strategy). Reference lists of reviews (identified in a separate specific search) and included studies, plus 2 iron experts, were consulted to identify additional potential RCTs. Titles and abstracts, and then full-text articles, were screened (with 10% duplication to ensure continuity and accuracy, with disagreements adjudicated by the review team).

Data were extracted into a specifically designed and tested Access database (Microsoft Corp) file. Training on the optimal use of the database was undertaken to ensure consistency and was followed by duplicate assessment of a 10% random sample that was discussed before the remainder of studies were data extracted. Data were extracted by study (not by publication) and included bibliographic information, location, aim, intervention and control, status measures, population characteristics, results, and statistical analyses. Authors were contacted for missing data, and where necessary, one of the reviewers extracted data from enlarged graphs by using a ruler.

Data for validity assessment, including sequence generation, allocation concealment, blinding, dropouts, funding, and similarity between intervention and control groups at baseline, were extracted and assessed for each study. Other threats to validity were also noted. All studies were included in the data analysis regardless of the outcome of the validity assessment. Risk of bias was determined by using a scale designed by the EURRECA Network of Excellence [on the basis of Cochrane Collaboration methods (6)]. Briefly, if all criteria were met (adequate sequence generation, allocation concealment, blinding, description of dropouts, and similarity at baseline), the study was judged at low risk of bias; if information was missing for one of the criteria but all other criteria were met, risk of bias was deemed unclear; and if more than one criterion was not met or was unclear, the study was judged at high risk of bias.

Data analysis

Random-effects Mantel-Haenszel weighted meta-analysis of all relevant RCTs was carried out in Review Manager software (RevMan Version 5.1; The Nordic Cochrane Centre, The Cochrane Collaboration) (8) to assess the effects of iron supplementation on each status marker at the longest available duration. Where available, the mean (±SD) biomarker change from baseline in supplemented and control groups were used; the mean (±SD) at the endpoint was used when change data were unavailable. When more than one comparable arm was reported (eg, different chemical forms of supplementation), data were combined for the analyses. When the arms were too different (eg, dietary intervention compared with supplementation with a single control group), control group numbers were split between dietary and supplemental intervention groups. Data reported as geometric means, least-square means, or medians were excluded unless arithmetic means (±SDs) were provided by authors when contacted. For each biomarker, studies with a baseline difference between supplemented and control groups larger than the change in status after supplementation in either group were excluded from the analyses for the specific biomarker. Units were standardized for analyses; specifically, when sTfR was reported in nanomoles per liter, it was converted to milligrams per liter by using the sTfR monomer molecular weight (85 kDa) (9). For dose analysis, the quantity of elemental iron was used when provided or was converted by the reviewers by using the information provided (such as the commercial name of the supplement) in the study publication. Positive values (or negative in the case of sTfR) indicated that the biomarker responded to supplementation.

Investigation of effect modifiers

Effects of sex, menopausal status, baseline status, study duration, supplementation form (tablets, meat or heme, fortified foods), and inorganic iron dose were investigated through subgroup meta-analyses and metaregression. Differences between subgroups were assessed by using random-effects meta-analyses. Baseline status was categorized according to the following WHO cutoffs (10): ID (SF concentration <15 μg/L and hemoglobin concentration >12 g/dL in women or >13 g/dL in men) and iron-deficiency anemia (IDA) (SF concentration <15 μg/L and hemoglobin concentration <12 g/dL in women or <13 g/dL in men). Undefined anemia was used to describe a population with hemoglobin concentrations <12 g/dL in women or <13 g/dL in men when no other biomarkers were reported. If only SF was reported, it was assumed that the hemoglobin concentration was normal, and the iron-status categorization was based on the reported concentration of SF and an assumed normal hemoglobin (ID or normal). For studies carried out in mixed-sex populations, cutoffs for women were applied because the majority of participants were often women.

A random-effects metaregression of mean differences (MDs) was used (with assumption of straight-line relations by using the metareg command in the STATA IC11.2 program; StataCorp LP) and, as a sensitivity analysis, by using β values {by algebraically

deriving an estimate from each study of the regression coefficient (slope, b) and its SE [se(b)]} for studies that did not directly report these and then obtaining an estimate of the response curve, with assumption of a bivariate normal linear model on the logarithmic scale (11) by using logistic regression and assuming curved relations. Metaregression was used to assess the effect of baseline iron status (SF), study duration, dose (all as continuous variables), and supplementation form (categorical) on the response of the selected biomarkers to supplementation. There were insufficient data to assess effects of sex and menopausal status (as dichotomous variables) in the metaregression because only one study in men and one study in postmenopausal women were included in the review.

Dose-response analysis

The presence and shape of a dose-response curve was further assessed by using 2- and 3-dimensional plotting tools (Excel, Microsoft Corp, and DataFit version 9.0, Oakdale Engineering).

RESULTS

The searches retrieved 5122 titles and abstracts, of which 334 potentially relevant full-text articles were collected for additional assessment. Forty-one RCTs (presented in 43 articles) were included in the review (**Figure 1**). Characteristics of included studies are presented in **Table 1**. Hemoglobin was the most frequently reported status biomarker (37 of 41 studies; 49 comparisons), SF was reported in 28 RCTs (29 comparisons), and sTfR was reported in 16 studies (22 comparisons). Most studies reported more than one biomarker, and body iron was reported in only 6 RCTs (Figure 1). Study participants were most frequently premenopausal women, often with ID or anemia. Most RCTs were at high risk of bias (24 studies) and the remainder at unclear risk because of insufficient information for accurate assessment (17 studies). Of the quality criteria, blinding and similarity of groups at baseline were the most frequently described and fulfilled (**Figure 2**).

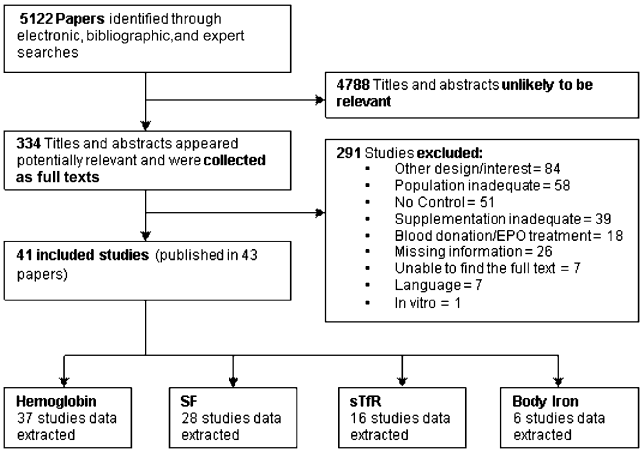

FIGURE 1. Flow diagram of the screening and selection process for the systematic review. Forty-one randomized controlled trials, which were reported in 43 publications, were included in the review, each of which measured different biomarkers of iron status. EPO, erythropoietin; SF, serum ferritin; sTfR, soluble transferrin receptor.

Effect of increased intake from supplemental iron on iron biomarkers

Meta-analyses suggested that iron supplementation, compared with a placebo or control, improved hemoglobin status [MD: +0.51 g/dL; 95% CI: 0.37, 0.65 g/dL; 49 arms (n = 3577); P-effect $<$ 0.00001; I^2 = 83%; P-heterogeneity $<$ 0.00001; **Figure 3**]. The degree of heterogeneity was only slightly reduced for meta-analysis by using curved models (I^2 = 72% for the β model), and thus, straight-line metaregression was used to estimate effect sizes.

SF [MD: 9.19 μg/L; 95% CI: 6.63, 11.75 μg/L; 29 arms (n = 1711); P-effect $<$ 0.00001; I^2 = 73% for both straight-line and β metaregression; P-heterogeneity $<$ 0.00001; data not shown], sTfR [MD: −0.46 mg/L; 95% CI: −0.68, −0.23 mg/L; 22 arms (n = 1472); P-effect $<$ 0.0001; I^2 = 75%; P-heterogeneity $<$ 0.00001], and body iron [MD: 1.89 mg/kg; 95% CI: 1.04, 2.75 mg/kg; 7 arms (n = 682); P-effect $<$ 0.0001; I^2 = 60%; P-heterogeneity = 0.02] were all also improved with iron supplementation (meta-analyses not shown). Although the effect of increasing iron intake on body status was consistently positive, the high levels of heterogeneity between studies for all biomarkers supported the need for an investigation of effect modifiers.

Effect modifiers of supplemental iron intake-status relation

Sex and menopausal status

The assessment of effects of sex and menopausal status was limited by the inclusion of only one study that reported effects in men and one study that reported effects in postmenopausal women, both with only reports on hemoglobin. Subgrouping suggested that effects in men and in postmenopausal women on hemoglobin were very different to those in all women and premenopausal women, respectively (P = 0.004 and P $<$ 0.0001, respectively; **Table 2**), but the shortage of studies meant that only sex could be included in the metaregression for hemoglobin and neither sex nor menopausal status in the metaregression for SF and sTfR. As expected (because the power of this analysis was very low), there was no clear effect of sex on hemoglobin.

Baseline iron status

Subgrouping suggested that subjects with IDA at baseline had significantly greater increases in hemoglobin concentrations than did subjects with ID or normal status (P = 0.006; **Table 2**). Straight-line and β metaregression of effects on final hemoglobin suggested that part of the heterogeneity in effect size could be explained by baseline SF (P = 0.018 for straight-line metaregression and P = 0.052 for β metaregression; **Table 3**). The effect size was an 0.08-g/dL (95% CI: 0.15, 0.00 g/d; P = 0.02) reduction in hemoglobin response per 10-μg/L increase in baseline SF concentration. Although the meta-analyses seemed to indicate an effect of baseline status on the response of SF (P = 0.04; **Table 4**) and sTfR (P = 0.007; **Table 5**), baseline SF did not appear to alter the effects of supplementation on SF or STfR in the metaregression (Table 3).

Duration

Subgrouping by duration suggested a marginally greater effect of supplementation on hemoglobin concentrations when

TABLE 1
Characteristics of included studies[1]

Study: first author, date (reference); location	Population	Intervention	Biomarkers	Remarks
Andersson, 2010 (12); Switzerland	PreMen, ID	14 mg el Fe/d in fortified margarine compared with nonfortified margarine Duration: 32 wk	Hemoglobin, SF[2], sTfR[2], body iron	
Biebinger, 2009 (13); Kuwait	PreMen, ID	20 mg el Fe 5 d/wk in fortified biscuits compared with nonfortified biscuits Duration: 22 wk	Hemoglobin, SF, sTfR, body iron	
Binkoski, 2004 (14); United States	PreMen, normal	100 mg el Fe/d as ferrous sulfate Duration: 3 wk	Hemoglobin, SF	Excluded from analysis (missing data)[3]
Blanco-Rojo, 2011 (15); Spain	PreMen, normal	18 mg el Fe/d in fortified juice compared with nonfortified juice Duration: 16 wk	Hemoglobin, SF, sTfR	
Brutsaert, 2003 (16); Mexico	PreMen, ID	20 mg el Fe/d as ferrous sulfate (plus citrus juice) compared with placebo Duration: 6 wk	Hemoglobin, SF, sTfR	
Callender, 1969 (17); United Kingdom (assumed)	Mixed, status unclear	105 or 100 mg el Fe/d compared with placebo Duration: 2 wk	Hemoglobin	Excluded from analysis (missing data)
Charoenlarp, 1981 (18); Thailand	Women, normal (assumed)	120 or 240 mg Fe/d (unclear whether el Fe or ferrous sulfate) compared with placebo Duration: 14 wk	Hemoglobin	Included volunteers ≥16 y old
Davis, 1992 (19); United States (assumed)	PreMen, normal (hemoglobin assumed)	60 mg el Fe/d as ferrous fumarate compared with placebo Duration: 17 wk	SF	
de Oliveira, 1996 (20); Brazil	Mixed, ID	3.75 mg el Fe/d plus ascorbic acid in fortified water compared with water Duration: 16 wk	Hemoglobin, SF	Assumed an error in SF data
Edgerton, 1979 (21); Sri Lanka	Women, anemia	40 mg el Fe/d as ferrous sulfate compared with placebo Duration: 4 wk	Hemoglobin	Study II excluded as unclear whether different populations
Elwood, 1970 (22); United Kingdom	Women, status unclear	150 mg ferrous carbonate/d compared with placebo Duration: 8 wk	Hemoglobin	Excluded from analysis (missing data)
Elwood 1970 (23); United Kingdom (assumed)	Women, normal (assumed)	Trial I: 10 or 30 mg el Fe/d as ferrous fumarate compared with placebo Duration: 24 wk Trial II: 5 mg el Fe/d as ferrous fumarate compared with placebo Duration: 24 wk	Hemoglobin	
Elwood, 1971 (24); United Kingdom (assumed)	Women, wide status range	2.7 mg/d as fortified bread compared with bread + 2.7 mg Fe/d suppl. compared with bread plus placebo Duration: 52 wk	Hemoglobin	
Elwood, 1966 (25); United Kingdom	Women, wide status range	200 mg el Fe/d as ferrous carbonate compared with placebo Duration: 8 wk	Hemoglobin	Included volunteers ≥15 y old
Ericsson, 1970 (26); Sweden	Mixed (PostMen and men), normal (assumed)	120 mg el Fe/d as ferrous fumarate compared with placebo Duration: 12 wk	Hemoglobin	Results presented for men and postmenopausal women[4]
Flink, 2006 (27) and 2007 (28); Sweden	Mixed, normal (hemoglobin assumed)	20 mg el Fe/d as ferrous sulfate compared with placebo Duration: 12 wk		Included volunteers ≥15 y old
Florencio, 1981 (29); Philippines	Women, anemia	105 mg el Fe/d as ferrous sulfate (500 mg ascorbic acid) compared with placebo Duration: 12 wk		Included volunteers ≥16 y old

(Continued)

TABLE 1 (*Continued*)

Study: first author, date (reference); location	Population	Intervention	Biomarkers	Remarks
Fogelholm, 1994 (30); Finland	PreMen, normal	9 or 27 mg el Fe as heme plus iron fumarate/d compared with placebo Duration: 24 wk	Hemoglobin, SF[2]	
Gershoff, 1977 (31); United States	Mixed (PostMen and men), anemia (men only)	22 mg/d as fortified wheat-based snacks compared with nonfortified snacks (<2.52 mg Fe) Duration: 28 wk	Hemoglobin	Women were postmenopausal
Haas, 2005 (32); Philippines	PreMen, normal	Increase 1.42 mg/d from fortified rice compared with nonfortified rice Duration: 36 wk	Hemoglobin, SF, sTfR, body iron	Excluded from analyses because of missing data
Heath, 2001 (33); New Zealand	PreMen, ID	50 mg el Fe/d as amino acid chelate compared with diet intervention compared with placebo Duration: 16 wk	Hemoglobin, SF[2], sTfR[2]	
Hinton, 2007 (34); United States	Mixed, ID (suppl. group only)	30 mg el Fe/d as ferrous sulfate (plus citrus juice) compared with placebo Duration: 6 wk	Hemoglobin, SF, sTfR	
Brownlie, 2004 (35), and Hinton, 2000 (36); United States	PreMen, ID	20 mg el Fe/d as ferrous sulfate (plus citrus juice) compared with placebo Duration: 6 wk	Hemoglobin, SF, sTfR	Brownlie reported an intake of 16 mg/d
Hodgson, 2007 (37); Australia	Mixed, normal	3.2 mg el Fe/d as increase intake of red meat compared with normal diet Duration: 8 wk	Hemoglobin, SF	
Hotz, 2008 (38); Mexico	PreMen, normal	13 mg el Fe 5 d/wk from fortified rice compared with control rice Duration: 24 wk	Hemoglobin, SF, sTfR, body iron	
Karl, 2010 (39); United States	PreMen, Army recruits	27.9 mg el Fe/d from fortified food bar compared with placebo bar Duration: 9 wk	Hemoglobin, SF, sTfR	Data provided for normal, ID[5], and anemic subgroups
Li, 1993 (40); China	PreMen, IDA	6 or 120 mg el Fe/d as ferrous sulfate compared with placebo Duration: 12 wk	Hemoglobin, SF	Data combined regardless of dose for SF
Lyle, 1992 (41); United States	PreMen, normal	10 or 50 mg el Fe/d as ferrous sulfate compared with diet intervention compared with placebo Duration: 12 wk	Hemoglobin, SF	Intervention involved different diet changes for all groups
Mackintosh, 1988 (42); South Africa	Men, normal	100 mg el Fe as ferric polymaltose ×2/d compared with placebo Duration: 8 wk	Hemoglobin, SF	Excluded from analysis (missing data)[3]
McClung, 2009 (43); United States	PreMen	1 × 15 mg el Fe/d as ferrous sulfate compared with placebo Duration: 8 wk	Hemoglobin, SF, sTfR	Data provided for normal and IDA[5] subgroups
Miller, 1983 (44); United States	PreMen, normal (assumed)	600 mg heme supplement/d compared with placebo Duration: 8 wk	Hemoglobin	Excluded from analysis (missing data)[3]
Murray-Kolb, 2007 (45); United States	PreMen	60 mg el Fe/d as ferrous sulfate compared with placebo Duration: 16 wk	Hemoglobin, SF, sTfR, body iron	Data provided for normal, ID, and IDA subgroups
Rajaram, 1995 (46); United States	PreMen, normal (assumed)	50 mg el Fe/d as ferrous sulfate compared with diet intervention compared with placebo Duration: 24 wk	Hemoglobin	Intervention involved different diet changes for all groups
Roughead, 2000 (47); United States	Mixed, normal	50 mg el Fe/d as ferrous sulfate compared with placebo Duration: 12 wk	Hemoglobin, SF, sTfR	

(Continued)

TABLE 1 (*Continued*)

Study: first author, date (reference); location	Population	Intervention	Biomarkers	Remarks
Taniguchi, 1991 (48); Japan	PreMen, IDA	6 mg el Fe/d and vitamin C compared with placebo, with or without exercise Duration: 9 wk	Hemoglobin, SF	Excluded from analysis (missing data)[3]
Thuy, 2003 (49); Vietnam	PreMen, IDA	10 mg el Fe 6 d/wk as fortified fish sauce compared with unfortified fish sauce Duration: 28 wk	Hemoglobin, SF[2], sTfR[2]	
Van Thuy, 2005 (50); Vietnam	PreMen, normal	7.5 mg el Fe/d as fortified fish sauce compared with unfortified fish sauce Duration: 72 wk	Hemoglobin, SF[2]	SF data excluded (missing data)
Wang, 2009 (51); United States	Mixed, normal (assumed)	130 mg el Fe/d as ferrous sulfate with vitamin C compared with placebo Duration: 12 wk	SF	
Zhu, 1998 (52, 53); United States	PreMen, ID	135 mg el Fe/d as ferrous sulfate (plus citrus juice) compared with placebo Duration: 8 wk	Hemoglobin, SF, sTfR	
Zimmermann, 2005 (54); Thailand	PreMen, ID	12 mg el Fe 6 d/wk as fortified snacks compared with nonfortified snacks Duration: 35 wk	Hemoglobin, SF[2], sTfR[2], body iron	Different supplementation arms combined for analyses

[1] el Fe, elemental iron; ID, iron deficient; IDA, iron-deficiency anemia; Mixed, men and women; PostMen, postmenopausal women; PreMen, premenopausal women; SF, serum ferritin; sTfR, soluble transferrin receptor; suppl., supplemented.

[2] Data were skewed and the issue was addressed in the original report, but arithmetic means (±SDs) were provided for this systematic review.

[3] Study was excluded from the analysis process because of the data format or missing information, and authors could not be contacted or could not provide an alternative format.

[4] Data were excluded from analysis because of excessive baseline differences between intervention and control groups.

[5] These groups were reclassified by our criteria as of normal iron status.

compared with that of controls in shorter-term studies [*P*-differences between subgroups = 0.03; the effect at ≤13 wk was an MD of 0.70 g/dL (95% CI: 0.47, 0.94 g/dL), at >13–26 wk was an MD of 0.39 g/dL (95% CI: 0.15, 0.63 g/dL), and at >26 wk was an MD of 0.20 g/dL (95% CI: −0.12, 0.52 g/dL); Table 2]. Metaregression did not suggest any effect of study duration on the change in hemoglobin concentrations by assuming either straight-line ($P = 0.43$) or curved relations ($P = 0.56$, Table 3).

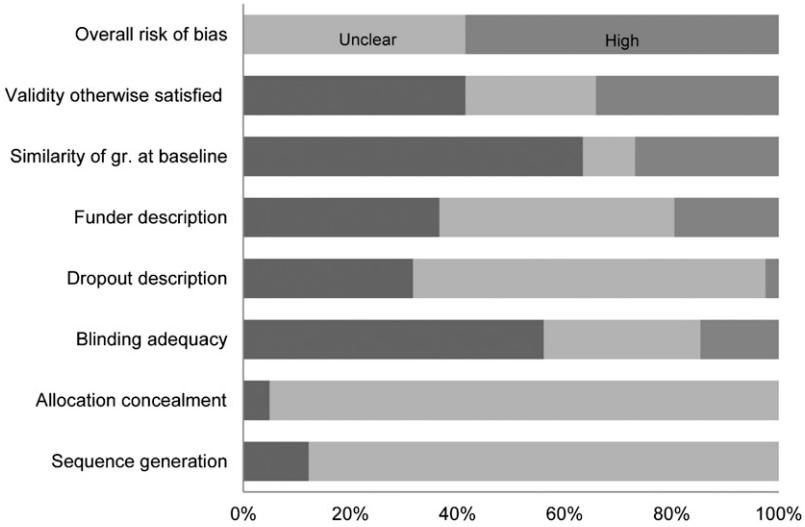

FIGURE 2. Risk of bias assessment. Dark gray (yes) denotes that the criterion was described and fulfilled the validity assessment, medium gray (no) denotes that the criterion was described and did not fulfill the validity assessment, and light gray (unclear) denotes that either the criterion was not described or no decision could be made on the basis of the information provided. Validity was judged as otherwise satisfied if no other source of bias could be identified. gr., group.

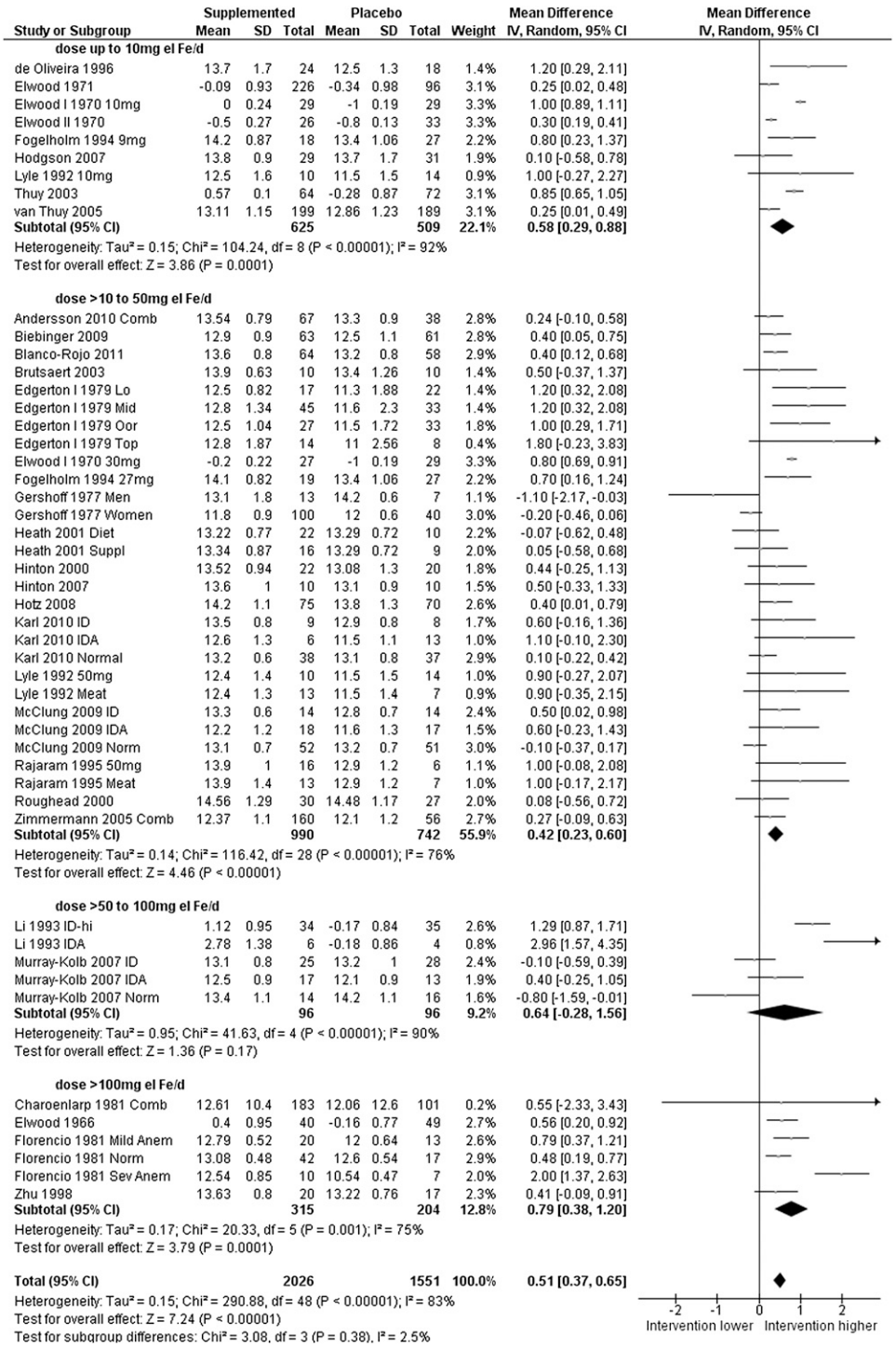

FIGURE 3. Forest plot of the effect of el Fe supplementation on hemoglobin status (mg/dL) subgrouped by supplementation dose. Mean and SDs shown refer to the change in hemoglobin from baseline to study end in the intervention and control arms and the difference between the intervention and control. For each study, the mean difference is represented by a square and the 95% CI by a line; the overall effect (for each subgroup and all studies) is represented by a diamond. Comb, study arms combined; el Fe, elemental iron; ID, iron deficiency; IDA, iron-deficiency anemia; ID-hi, study subgroup with ID or mild IDA (higher hemoglobin value); IV, inverse variance; Lo, "lower-division" study subgroup; Mid, "middle-division" study subgroup; Mild Anem, mildly anemic study subgroup (hemoglobin 11–11.9 mg/dL); Norm, normal baseline status; Oor, "Ooragalla division" study subgroup; Sev Anem, moderately to severely anemic study subgroup (hemoglobin 8.5–10.9 mg/dL); Top, "top-division" study subgroup; I, study trial 1; II, study trial 2.

Similarly, an improvement in sTfR was not dependent on the length of supplementation as shown by both meta-analyses (P-difference between duration subgroups = 0.13; Table 5) and metaregression (P = 0.39 for straight-line metaregression and P = 0.29 for curved-relation metaregression; Table 3). However, although subgrouping was not significant (Table 4), meta-regression analyses showed a clear effect of duration of supplementation on SF compared with control [0.51-μg/L (95% CI: 0.02, 1.00-μg/L; P = 0.04) increase in SF concentration per 1-wk increase in duration; Table 3].

TABLE 2
Effect of sex, menopausal status, duration, supplement form, and baseline iron status on response of hemoglobin to iron supplementation, including all relevant studies in subgrouping[1]

Factor and status	Difference[2]	Studies; participants[3]	I^2	*P*-differences between subgroups
	g/dL	*n*	%	
Overall analysis (all)	0.51 (0.37, 0.65)	49; 3577	83	—
Sex				0.004
Men	−1.10 (−2.17, −0.03)	1; 20	—	
Women (all)	0.51 (0.37, 0.66)	41; 3295	83	
Menopausal status				<0.00001
Premenopausal	0.43 (0.26, 0.60)	28; 2017	71	
Postmenopausal	−0.20 (−0.46, 0.06)	1; 140	—	
Duration				0.03
≤13 wk	0.70 (0.47, 0.94)	24; 1039	74	
>13–26 wk	0.39 (0.15, 0.63)	15; 1128	85	
>26 wk	0.20 (−0.12, 0.52)	7; 1327	88	
Supplement form				0.03
Tablet (nonheme)	0.63 (0.43, 0.83)	28; 1493	84	
Meat or heme	0.63 (0.25, 1.00)	4; 177	10	
Fortified foods	0.27 (0.07, 0.46)	14; 1870	77	
Baseline iron status				0.006
IDA	1.01 (0.59, 1.43)	6; 299	66	
ID	0.27 (0.12, 0.42)	11; 716	0	
Normal	0.38 (0.17, 0.59)	19; 1659	85	
Dose				0.38
≤10 g el Fe/d	0.58 (0.29, 0.88)	9; 1134	92	
>10–50 g el Fe/d	0.42 (0.23, 0.60)	29; 1732	76	
>50–100 g el Fe/d	0.64 (−0.28, 1.56)	5; 192	90	
>100 g el Fe/d	0.79 (0.38, 1.20)	6; 519	75	

[1] All meta-analyses were conducted by using a random-effects methodology. el Fe, elemental iron; ID, iron deficient; IDA, iron-deficiency anemia.
[2] All values are means; 95% CIs in parentheses.
[3] Number of comparisons do not always add up to 49, as listed in the Figure 3 forest plot, because some studies were not classifiable for some criteria or were mixed.

Supplementation form

Subgrouping suggested that fortified foods had less effect on hemoglobin than iron supplements or meat (heme) interventions ($P = 0.03$ for subgroup differences; Table 2), but there were no clear differences in the effect between different iron forms for SF or sTfR (Tables 4 and 5). Metaregression suggested no clear effects of the supplementation type on the change in hemoglobin, SF, or sTfR (Table 3).

Dose

Subgrouping and metaregression did not suggest clear dose-response effects on hemoglobin. For sTfR, an effect for dose was observed in the subgroup analyses (*P*-difference between groups < 0.00001; Table 5). Although only one study was included for each group, the sTfR response was greater in studies that supplemented with the lowest and highest doses, which suggested an absence of a dose effect for sTfR in metaregression analyses. A possible dose effect for SF was observed (with significant effects of dose seen in subgrouping and straight-line metaregression but not in β metaregression) (Table 5). Metaregression suggested an effect size of 0.10 μg/L (95% CI: 0.01, 0.20 μg/L; $P = 0.036$) increase in the SF concentration per 1-g elemental iron increase in dose.

Overall, the following straight-line metaregression equation could predict only 24% of the study variance for the change in hemoglobin on supplementation:

$$\begin{aligned}\text{Change in hemoglobin concentration (g/dL)} = &-0.15 - [0.008 \times \text{baseline SF } (\mu\text{g/L})] \\ &- [0.001 \times \text{dose (mg Fe/d)}] - (0.004 \times \text{weeks duration}) \\ &+ (0.12 \text{ supplement type where nonheme tablets} = 1, \\ &\text{heme supplement or meat diet} = 2, \text{ and food fortification} = 3) \\ &+ (0.52 \times \text{sex where 1} = \text{women}, \\ &3 = \text{men, and 2} = \text{mixed})\end{aligned} \quad (1)$$

Similarly, metaregression explained 26% of between-study variance for SF as follows:

$$\begin{aligned}\text{Change in SF}(\mu\text{g/L}) = &\ 0.012 + [0.101 \times \text{baseline SF } (\mu\text{g/L})] \\ &+ [0.104 \times \text{dose (mg Fe/d)}] \\ &+ (0.508 \times \text{weeks duration}) \\ &- (2.394 \text{ supplement type where nonheme tablets} = 1, \\ &\text{heme supplement or meat diet} = 2 \\ &\text{and food fortification} = 3)\end{aligned} \quad (2)$$

For sTfR, only 4% of between-study variability was explained by straight-line metaregression.

The meta-analysis of the effect of an increasing dose on SF (only in ID premenopausal women to reduce the influence of other factors) is shown in **Figure 4**. Because both dose and duration seemed to be potential modifiers of SF status in the meta-analysis and metaregression, their combined effect was

TABLE 3
Summary of effect modifiers on change in hemoglobin, serum ferritin, and soluble transferrin receptor as assessed by subgrouping, straight-line metaregression, and metaregression that assumed a curved relation[1]

	Subgrouping (P)[2]	Straight-line metaregression (P)	Curved-relation metaregression (P)	Conclusion	Effect size
Baseline status, SF[3]					
Hemoglobin	Yes (0.006)	Yes (0.018)	Marginal (0.052)	Possibly	Mean (95% CI) reduction in serum hemoglobin per 10-μg/L increase in baseline SF: 0.08 g/dL (0.15, 0.00 g/dL); P = 0.02
SF	Yes (0.04)	No (0.28)	No (0.61)	No	—
sTfR	Yes (0.007)	No (0.84)	No (0.21)	No	—
Sex					
Hemoglobin	Yes (0.004)	No (0.11)	—	No	—
SF	—	—	—	Insufficient data	—
sTfR	—	—	—	Insufficient data	—
Menopausal status					
Hemoglobin	Yes (<0.0001)	—	—	Insufficient data	—
SF	—	—	—	Insufficient data	—
sTfR	—	—	—	Insufficient data	—
Duration					
Hemoglobin	Yes (0.03)	No (0.43)	No (0.56)	No	—
SF	No (0.58)	Yes (0.043)	Yes (0.003)	Yes	Mean (95% CI) increase in SF per 1-wk increase in duration: 0.51 μg/L (0.02–1.00 μg/L); P = 0.04
sTfR	No (0.13)	No (0.39)	No (0.29)	No	—
Supplement form					
Hemoglobin	Yes (0.03)	No (0.17)	No (0.17)	No	—
SF	No (0.88)	No (0.28)	Marginal (0.06)	No	—
sTfR	No (0.16)	No (0.36)	Marginal (0.09)	No	—
Dose					
Hemoglobin	No (0.38)	No (0.65)	No (0.21)	No	—
SF	Yes (0.002)	Yes (0.036)	No (0.78)	Possibly	Mean (95% CI) increase in SF per 1-g elemental iron increase in dose: 0.10 μg/L (0.01, 0.20 μg/L); P = 0.036
sTfR	Yes (<0.00001)	No (0.93)	No (0.30)	No	—

[1] SF, serum ferritin; sTfR, soluble transferrin receptor.
[2] Yes implies $P < 0.05$ for chi-square test for differences between subgroups.
[3] Subgrouping was by iron-deficiency anemia, iron-deficient, or normal iron status (as defined by using the WHO criteria); metaregression used SF at baseline as a continuous variable.

explored by using 3-dimensional modeling (**Figure 5**A). Additional analyses of the duration effect only [studies with similar doses (ie, 12–20 mg/d); Figure 5B] or the supplementation effect only [studies of approximately the same duration (ie, 9–22 wk); Figure 5C] were carried out to explore this phenomenon. As shown in Figure 5, with lower doses, the time required to increase SF was longer (Figure 5A). The collective results from several studies suggested that it takes ≥32 wk for SF to increase (Figure 5B) and a dose of ≥50 mg Fe/d is needed to effect a meaningful increase in SF (Figure 5C). These preliminary analyses illustrated the link between the dose and length of supplementation for SF response and should be further explored through modeling analysis.

DISCUSSION

Forty-one RCTs were included in this analysis; 37 RCTs measured hemoglobin, 28 RCTs measured SF, 16 RCTs measured sTfR, and 6 RCTs measured body iron. None of the included studies met all of the quality criteria or described them all fully, and thus, none of the included studies were judged to be at low risk of bias. Random-effects meta-analyses showed that iron supplementation significantly improved the status of all measured biomarkers but with high levels of heterogeneity. When the results of the different types of analysis (subgrouping and metaregression with straight-line and curved relations; Table 3) were compiled, the data suggested that there were clear effects of study duration with an increase in SF response of 0.51 μg/L (95% CI: 0.02, 1.00 μg/L; P = 0.04) per 1-wk increase in duration. Although not as statistically clear, we also observed a tendency for effects of dose on SF response with a 0.10 μg/L (95% CI: 0.01, 0.19 μg/L; P = 0.027) increase in SF per 1-g elemental Fe increase in supplementation dose. A metaregression of the effect of dose on SF indicated that dose was not the only factor that influenced SF response to supplementation (Figure 4); the supplementation duration also affected the SF response (Table 3). Conversely, there was no effect of iron dose on hemoglobin or sTfR response, but there was a significant effect of baseline iron status on the change in hemoglobin concentration, with a 0.08-g/dL (95% CI: 0.15, 0.00 g/dL; P = 0.02) reduction in hemoglobin

TABLE 4
Effect of sex, menopausal status, duration, supplement form, and baseline iron status on response of SF to iron supplementation, including all relevant studies in subgroupings[1]

Factor and status	Difference[2]	Studies; participants[3]	I^2	*P*-difference between subgroups
	g/dL	*n*	%	
Overall analysis, all	9.07 (6.55, 11.58)	30; 1742	72	—
Sex				—
Men	—	0; 0	—	
Women (all)	8.70 (5.91, 11.48)	24; 1510	75	
Menopausal status				—
Premenopausal	8.70 (5.91, 11.48)	24; 1510	75	
Postmenopausal	—	0; 0	—	
Duration				0.58
≤13 wk	8.85 (5.89, 11.80)	17; 695	50	
>13–26 wk	8.54 (3.37, 13.70)	9; 567	82	
>26 wk	12.12 (6.33, 17.91)	3; 449	74	
Supplement form				0.84
Tablet (nonheme)	9.03 (6.26, 11.80)	17; 665	51	
Meat or heme	5.48 (−8.42, 19.39)	2; 80	0	
Fortified foods	9.12 (4.19, 14.05)	10; 966	87	
Baseline iron status				0.04
IDA	13.18 (6.99, 19.37)	5; 300	74	
ID	5.69 (2.28, 9.10)	10; 664	74	
Normal	10.78 (7.31, 14.24)	14; 747	43	
Dose				0.0002
≤10 g el Fe/d	15.45 (10.68, 20.22)	3; 220	0	
>10–50 g el Fe/d	7.20 (4.53, 9.87)	20; 1249	73	
>50–100 g el Fe/d	13.51 (8.43, 18.60)	5; 218	0	
>100 g el Fe/d	19.52 (9.82, 29.22)	2; 55	0	

[1] All meta-analyses were conducted by using random-effects methodology. el Fe, elemental iron; ID, iron deficient; IDA, iron-deficiency anemia; SF, serum ferritin.
[2] All values are means; 95% CIs in parentheses.
[3] Number of comparisons do not always add up to 29 (including study subgroups) because some studies were not classifiable for some criteria or were mixed.

response per 10-μg/L increase in baseline SF, although there was no effect of baseline status on SF or sTfR. Straight-line metaregression by using supplementation dose, supplement type, duration, and baseline SF (plus sex for hemoglobin) accounted for about one-quarter of the between-study variability in hemoglobin and SF responses but only 4% of sTfR response.

Because of the high level of heterogeneity in the overall response to supplementation, other factors besides those associated with diet were investigated. Heterogeneity remained high throughout the process and could not completely be explained despite exhaustive subgroup and sensitivity analysis. For all biomarkers addressed, meta-analysis results showed that the responses of different population subgroups were significantly different, and the improvement observed was only significant for premenopausal women for all biomarkers and in the mixed population for hemoglobin (Table 2). However, data were scarce for the other populations of interest (men and postmenopausal women), which prevented any analyses. From the meta-analysis, it was clear that the presence of IDA at the start of an intervention was a significant determinant of the response to supplementation; subgroup analysis showed an improvement in hemoglobin and SF, but although hemoglobin is widely used to screen for IDA, it has low specificity and sensitivity as a biomarker of iron status (55). The form of iron supplement also had an influence on the response of each biomarker, although the effects were not consistent in the different biomarkers. Body iron is a more recent biomarker of iron status, and not enough data were available to explore how it is influenced by modifiers of iron metabolism. However, as body iron becomes a more common measure of iron status, it will be important to assess its responsiveness to iron intake and the degree to which it is affected by various modifiers.

In this systematic review, the following 3 types of analyses were used: a meta-analysis (including a subgroup analysis), linear metaregression (with assumption of a straight-line relation between the factor and the biomarker), and β metaregression (with assumption of a curved relation between the factor and biomarker). These analyses were used to investigate the influence of iron status at baseline, the duration of supplementation, and the supplementation form and dose on the response of hemoglobin, SF, and sTfR. We used the 3 methods because the full nature of any relations (whether straight-line or curved) between the factor and each biomarker is not known. Subgrouping allows major groupings to be investigated, and metaregression makes better use of the data for continuous variables (especially important when data are limited). A lack of a significant straight-line relation may result from a highly curved relation. However, it should be remembered that a lack of significant straight-line or curved relations does not necessarily mean a lack of a relation. It may simply mean that the relation is different, perhaps a normal curve, or that the flat part (saturation) of a sinusoidal curve has been entered. The results obtained suggested that the straight-line model explained as much heterogeneity as the curved model

TABLE 5
Effect of sex, menopausal status, duration, supplement form, and baseline iron status on response of sTfR to iron supplementation, including all relevant studies in subgrouping[1]

Factor and status	Difference[2]	Studies; participants[3]	I^2	*P*-difference between subgroups
	g/dL	*n*	%	
Overall analysis (all)	−0.46 (−0.68, −0.23)	22; 1472	75	—
Sex				—
Men	—	0; 0	—	
Women (all)	−0.51 (−0.77, −0.26)	20; 1395	76	
Menopausal status				—
Premenopausal	−0.51 (−0.77, –0.26)	20; 1395	76	
Postmenopausal	—	0; 0	—	
Duration				0.13
≤13 wk	−0.13 (−0.30, 0.03)	11; 454	36	
>13–26 wk	−0.31 (−0.56, −0.06)	8; 561	23	
>26 wk	−2.05 (−4.28, 0.17)	3; 457	94	
Supplement form				0.16
Tablet (nonheme)	−0.32 (−0.54, −0.10)	12; 480	37	
Meat or heme	0.50 (−0.65, 1.65)	1; 32	—	
Fortified foods	−0.61 (−1.04, −0.18)	9; 960	88	
Baseline iron status				0.007
IDA	−1.71 (−3.61, 0.19)	4; 220	91	
ID	−0.69 (−1.06, −0.32)	10; 674	0	
Normal	−0.13 (−0.27, 0.00)	8; 578	51	
Dose				<0.00001
≤10 g el Fe/d	−4.50 (−5.66, −3.34)	1; 136	—	
>10–50 g el Fe/d	−0.17 (−0.30, −0.05)	17; 1186	29	
>50–100 g el Fe/d	−1.14 (−1.88, −0.40)	3; 113	0	
>100 g el Fe/d	−1.32 (−2.87, 0.23)	1; 37	—	

[1] All meta-analyses were conducted by using a random-effects methodology. el Fe, elemental iron; ID, iron deficient; IDA, iron-deficiency anemia; sTfR, soluble transferrin receptor.
[2] All values are means; 95% CIs in parentheses.
[3] Number of comparisons do not always add up to 22 (including study subgroups) because some studies were not classifiable for some criteria or were mixed.

did and allowed the size of the relations between some factors and SF to be quantified. None of the models clearly described relations between factors and hemoglobin or sTfR, which illustrated the difficulty in the determination of the relation between iron intake and the response of biomarkers of status. Within this review, as many potential modifiers as possible were removed (through strict inclusion criteria), such as inflammation and high levels of physical activity, and by limiting the included studies to RCTs, which thereby minimized the disparity between study groups. However, other factors, such as ethnicity or body weight, could not be assessed because of the lack of information reported in most of the included studies. The restriction of study

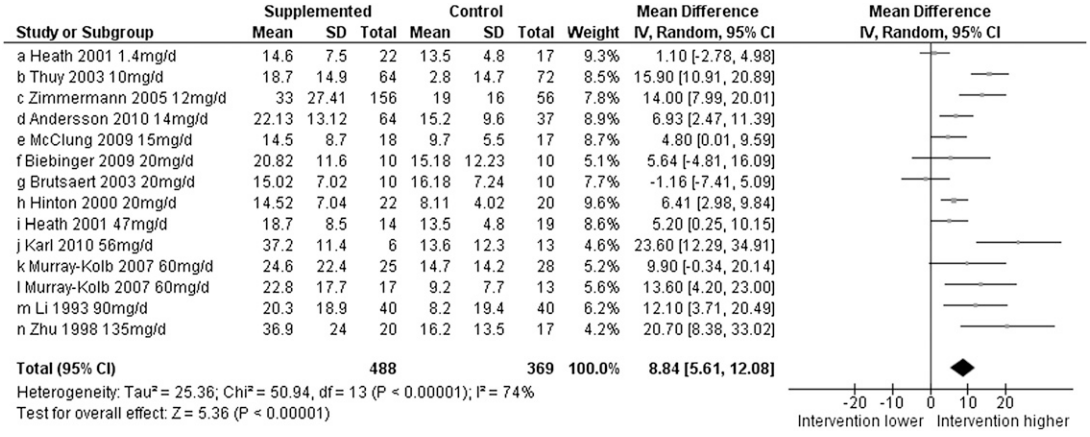

FIGURE 4. Forest plot of the effect of supplementation ordered by increasing supplemental dose on serum ferritin status after intervention. For each study, the mean difference is represented by a square and the 95% CI by a line; the overall effect (for each subgroup and all studies) is represented by a diamond. IV, inverse variance.

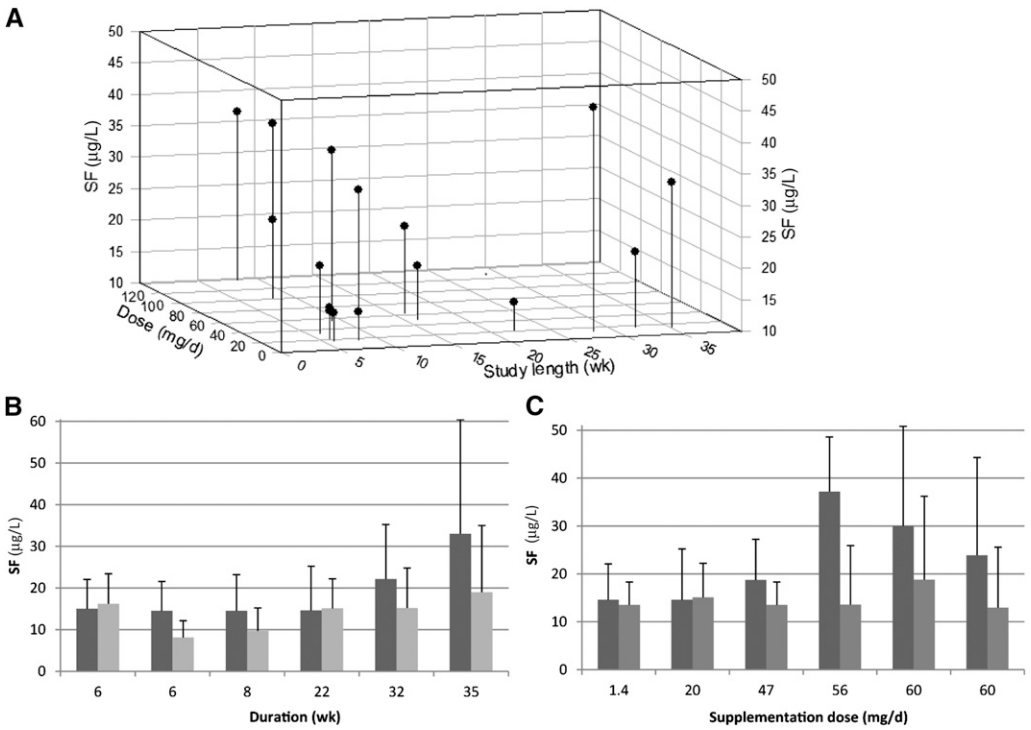

FIGURE 5. A: Three-dimensional scatter plot that shows the effect of dose and duration of supplementation on SF response. To observe the effect of dose and duration alone, only studies in premenopausal women with ID are included in the graph. B: Effects of the duration of intervention in 6 studies with similar iron doses [references 16, 36, and 43 (IDA arm) and 13, 12, and 54 in columns from left to right, respectively) on mean (±SD) SF concentrations presented for each study. C: Effects of increasing dose in 6 studies of similar duration [references 33 (diet arm), 13 and 33 (supplement arm), 39 (IDA arm; shortest study), and 40 and 45 (both ID and anemic arms combined) in columns from left to right, respectively] on mean (±SD) SF concentrations presented for each study. Dark-gray columns denote the supplemented group, and light-gray columns denote the placebo group. ID, iron deficiency; IDA, iron-deficiency anemia; SF, serum ferritin.

inclusion by using a number of modifiers meant that many studies were excluded from this review. However, because of the high heterogeneity of the data, it was judged that such tight inclusion criteria were necessary to assess the intake-status relation in the context of this review.

It is difficult to design effective interventions to address ID in populations because of the numerous factors that affect iron absorption (5). However, the results of our meta-analyses can inform strategies to improve the iron status of populations and of individuals in the clinical setting, in particular, the dose of iron and the length of time required to increase SF, with due allowance for the effect of baseline iron status.

The original conception of the systematic review was undertaken by the EURRECA Network of Excellence and coordinated by partners who are based at Wageningen University (WU) and the University of East Anglia (UEA). Lisette de Groot (WU), Kate Ashton (UEA), Adriënne Cavelaars (WU), Rosalie Dhonukshe-Rutten (WU), and Esmée Doets (WU) contributed to the design and development of the review protocol and search strategy. We thank authors who have kindly provided data when requested and Richard Hurrell and Maria Andersson, who reviewed the list of included studies. We also thank Jennifer Anssett and Ben Thompson, who contributed to the data extraction and duplicate assessment processes; Olga Soverein and Carla Dullemeijer for calculation of β values; and Leland Miller for Figure 5A.

The authors' responsibilities were as follows—AC and RC: conducted electronic searches; AC, RC, and LJH: assessed the studies for inclusion, extracted data, and assessed validity; AC and LH: conducted the analyses and tabulated data; AC: wrote the first draft of the manuscript; RC, LJH, LH, and SJF-T: significantly contributed to the writing of the manuscript and approved the final version; and all authors: contributed to the design and development of the review protocol and search strategy. None of the authors had a conflict of interest.

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