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Complete List of Authors:	Houston, Brett; University of Manitoba / CancerCare Manitoba, Hematology and Medical Oncology Hurrie, Daryl; University of Manitoba Graham, Jeff; University of Manitoba / CancerCare Manitoba, Hematology and Medical Oncology Perija, Brittany; University of Manitoba, Department of Internal Medicine Rimmer, Emily; University of Manitoba / CancerCare Manitoba, Hematology and Medical Oncology Rabbani, Rasheda; University of Manitoba and George & Fay Yee Center for Healthcare Innovation Bernstein, Charles; University of Manitoba, Gastroenterology Turgeon, Alexis; Centre de Recherche du Centre Hospitalier Affilié Universitaire de Québec (CHA), Axe Traumatologie-urgence-soins intensifs, CHA-Hôpital de l'Enfant-Jésus, Université Laval, Anesthesia and Critical Care Medicine Fergusson, Dean; Ottawa Hospital Research Institute Houston, Donald; University of Manitoba / CancerCare Manitoba, Hematology and Medical Oncology Abou-Setta, Ahmed; University of Manitoba and George & Fay Yee Center for Healthcare Innovation Zarychanski, Ryan ; University of Manitoba / CancerCare Manitoba, Hematology and Medical Oncology
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Efficacy of iron therapy on fatigue and work capacity in non-anemic iron deficient adults: a systematic review of randomized controlled trials

Brett L. Houston[1,2], Daryl Hurrie[3], Jeff Graham[1,2], Brittany Perija[4], Emily Rimmer[1,2], Rasheda Rabbani[5,6], Charles N. Bernstein[7], Alexis Turgeon[8], Dean Fergusson[9], Donald S. Houston[1,2], Ahmed M. Abou-Setta[5,6], Ryan Zarychanski[1,2,5,6]

[1]Department of Internal Medicine, Section of Medical Oncology and Haematology, University of Manitoba, Winnipeg, Manitoba, Canada
[2]Department of Medical Oncology and Haematology, CancerCare Manitoba, Winnipeg, Manitoba, Canada.
[3]Applied Health Sciences, Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Manitoba, Canada
[4]Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
[5]George & Fay Yee Center for Healthcare Innovation, University of Manitoba/Winnipeg Regional Health Authority, Winnipeg, Manitoba, Canada
[6]Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[7]Department of Internal Medicine, Section of Gastroenterology, University of Manitoba, Winnipeg, Manitoba, Canada
[8]Centre de recherche du CHU de Québec – Université Laval, Population Health and Optimal Health Practices Research Unit, Trauma - Emergency - Critical Care Medicine; Department of Anesthesiology and Critical Care Medicine, Division of Critical Care Medicine, Faculty of Medicine, Université Laval, Québec City, Québec, Canada.
[9]Clinical Epidemiology Program, Ottawa Hospital Research Institute (OHRI); Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Corresponding author: Ryan Zarychanski
ON 2051-675 McDermot Avenue
CancerCare Manitoba
Winnipeg, Manitoba, R3E OV9
T: 204-787-2108
F: 204-786-0196
rzarychanski@cancercare.mb.ca

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OBJECTIVE: Iron supplementation in iron deficiency anemia is standard practice, but the benefits of iron supplementation in iron deficient non-anemic (IDNA) individuals remains controversial. Our objective is to identify the effects of iron therapy on fatigue and work capacity in iron deficient non-anemic adults.

DESIGN: Systematic review and meta-analysis of randomized controlled trials (RCTs)

SETTING: Primary care

PARTICIPANTS: Adults (≥18 years) who were iron deficient but non-anemic

INTERVENTIONS: Oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included. Comparators included placebo or active therapy.

RESULTS: We identified RCTs in Medline, Embase, CENTRAL, CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016. We searched the World Health Organization's ICTRP for relevant ongoing trials and performed forward searches of included trials and relevant reviews in Web of Science. We assessed internal validity of included trials using the Cochrane Risk of Bias tool, and the external validity using the GRADE methodology. From 11580 citations we included 18 unique trials, and 2 companion papers enrolling 1162 patients. Iron supplementation was associated with reduced self-reported fatigue (standardized mean difference (SMD) -0.38; 95% CI -0.52 to -0.23; I^2 0%; 4 trials; 714 participants), but was not associated with differences in objective measures of work capacity, including maximal oxygen consumption (VO_2 max) (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%; 9 trials; 235 participants), and timed methods of exercise testing.

CONCLUSION: In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in work capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider a course of iron supplementation to improve symptoms of fatigue in presence or absence of documented anemia.

SYSTEMATIC REVIEW REGISTRATION: PROSPERO (CRD42014007085)

STUDY STRENGTHS and LIMITATIONS:

Strengths:

- We used a comprehensive search strategy, an *a priori* protocol, and adhered to established methodological (e.g. PRISMA, GRADE) guidelines
- We identified an at-risk patient population, for whom iron deficiency is highly prevalent, but treatment is unknown
- Our outcomes are clinically relevant and patient centered.

Limitations:

- In our selected population of iron deficient but not anemic individuals, the majority of studies evaluated healthy females
- In the included trials, tolerability and adverse events of iron therapy was incompletely captured

INTRODUCTION

Iron deficiency (ID) is estimated to affect two billion people, and is the leading cause of anemia worldwide[1,2]. Iron is necessary for cellular immune responses, oxidative metabolism within the mitochondria, and production of hemoglobin and myoglobin. When iron losses exceed dietary iron absorption, iron stores become depleted resulting in impaired hemoglobin production and decreased red blood cell hemoglobin content[3]. Reduction in hemoglobin concentration below a threshold (conventionally defined by the World Health Organization (WHO) as 120g/L for females and 130g/L for males) signifies anemia[4].

It is well established that anemia results in decreased physical work capacity and increased fatigue proportional to anemia severity[5-9]. Unfortunately, patient-reported fatigue is common in community and primary care settings with a prevalence ranging

from 7 to 45%[10]. It is estimated that the indirect annual economic consequence of chronic fatigue in the United States is 9.1 billion dollars[11].

The clinical relevance of iron deficiency in the absence of anemia is poorly understood, but may impact well-being, perceptions of fatigue, or contribute to decrements in physical performance through impairment in biochemical processes including tissue and mitochondrial oxidative capacity[8]. While iron replacement can normalize hemoglobin concentration, restore work capacity and improve fatigue in iron deficiency anemia, it is unclear if supplementation affects fatigue and physical work capacity in iron deficient but non-anemic (IDNA) individuals. In the absence of compelling efficacy data on well-being or muscle function, the use of iron supplements are common in the general population and are routinely recommended to high performance athletes to enhance performance.

Given the global prevalence of iron deficiency and impact of fatigue, the purpose of this systematic review is to identify, critically appraise and meta-analyse data from prospective randomized trials evaluating iron therapy in adults with IDNA.

METHODS

Using an *a priori* published protocol (CRD42014007085; available at https://www.crd.york.ac.uk/PROSPERO/)[12], we conducted a systematic review using methodological approaches outlined in the *Cochrane Handbook for Systematic Reviewers* and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria[12-14]. A panel of experts from multiple fields (e.g. internal medicine, hematology, kinesiology, gastroenterology, research methodology)

formulated the research question, reviewed search strategies and methods, and provided input throughout the review process.

Populations, Interventions, Comparators, Outcome Measures, Setting and Study Designs

Our research question was "In iron depleted but non-anemic adults, does iron supplementation improve fatigue and work capacity." We included randomized controlled trials of adults (≥18 years) who were iron deficient but non-anemic (**Appendix 1**). Interventions included oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included. We included trials that evaluated outcomes at least 1 month from the initiation of iron therapy. Comparators included placebo or active therapy. Our exclusion criteria are presented in **Appendix 2**.

Our primary outcome measures were self-reported fatigue and objective measures of work capacity. Secondary outcomes included the incidence of anemia, change in hemoglobin concentration and serum ferritin, and the incidence of adverse outcomes including iron toxicity, constipation, diarrhea, gastrointestinal intolerance and nausea.

Search Strategy for Identification of Studies

We searched Medline, Embase, CENTRAL (Cochrane Library), CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016 to identify relevant citations of published trials, using individualized systematic search strategies for each database. The MEDLINE strategy is presented in **Appendix 3**. We searched the World Health Organization's (WHO) International Clinical Trials Registry Platform (ICTRP),

clinicaltrials.gov and relevant conference proceedings to identify planned, ongoing, or recently completed but unpublished trials. We performed forward searches of included trials and relevant reviews in Web of Science to identify additional citations, and contacted study authors to request pertinent unpublished data or provide clarifications on study methods or results. Reference lists of narrative and systematic reviews and of the included trials were searched for additional citations. We performed reference management in EndNote™ (Version X7, Thomson Reuters, Philadelphia, PA, USA).

Study Selection, Data Extraction and Quality Assessment

We screened citations, selected studies and extracted data from included trials using standardized and piloted screening and data extraction forms. Citation screening, study selection and data extraction were performed in duplicate. We assessed the internal validity of included trials using the Cochrane Collaboration Risk of Bias tool[13]. Discrepancies between the two reviewers were resolved by consensus or by a third reviewer (RZ), as required. Data extraction and descriptive statistics were performed using Microsoft Excel 2016 (Excel version 15, Microsoft Corp).

Data Analysis

Data analysis was performed using Review Manager (RevMan v5.3.5, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). Study level comparisons of dichotomous data were presented as risk ratios (RR) with 95% confidence intervals (CI). Pooled continuous data were expressed as the mean difference

(MD), or standardized mean difference (SMD). Change scores or post-treatment means were extracted to inform summary estimates for continuous data. Pooled risk ratios and 95% confidence intervals were conducted using Mantel-Haenszel random-effects model. Pooled MDs or SMDs were calculated using a random-effects model. For the primary outcome of fatigue, if multiple scales were reported, fatigue-specific scores were preferred over general scores and the most commonly reported and clinically meaningful score was used to generate summary effect measures. In studies evaluating exercise capacity, weight-based VO_2 max values were utilized preferentially if both absolute and weight-based VO_2 max results were provided. Statistical heterogeneity was quantified using the I^2 statistic[15]. For the primary outcomes of fatigue and work capacity, we evaluated potential publication bias using funnel plot analysis[16]. All tests of statistical inference reflect a 2-sided α of 0.05.

Subgroup Analyses

We performed subgroup analyses for fatigue and exercise capacity outcomes according to biologic sex, athletic status (athlete or non-athlete), method of iron administration, duration of therapy, duration of study follow up, and risk of bias.

Grading the Evidence

We graded the strength of evidence for our primary outcomes using the GRADE methodology. This approach classifies the strength of evidence as "*high", "moderate", "low"* or *"very low."*

RESULTS

Trial Characteristics & Study Populations

Of the 11,580 citations identified, we included 18 unique trials and two companion papers[17,18], enrolling 1162 subjects (**Figure 1; Table 1**). Trials were published between 1989 and 2015, and all trials were published in peer-reviewed journals. Eight trials[19-26] were from North America, seven trials[27-33] were from Europe, two trials[18,34] were from Australia, and one trial[35] was from Asia.

Exclusively healthy females (aged 17 to 55 years old) with varying levels of fitness (sedentary to well-trained) were enrolled in all but three studies[22,27,29]. The WHO cutoff for anemia [hemoglobin concentration ≥130 g/L (males) and ≥120 g/L (females)] was used by 9 studies[19,22-25,30-32,35], whereas 7 studies used lower values ranging from ≥110 to <120 g/l[20,21,27,28,33,34,36], and baseline hemoglobin concentration was not provided in 2 trial reports[26,29].

All trials were placebo-controlled. In 13 of 18 trials (72%), we considered the blinding of participants and personnel to be adequate. Likewise, 10 trials (55%) adequately incorporated blinded outcome assessment. One trial[29] was considered to have a low risk of bias (**Table 2**). The remainder of the trials were considered unclear risk of bias, due to unclear processes of randomization (12 trials[19-27,30,33,34]) or allocation concealment (13 trials[19-27,30,31,34,35]).

Interventions

Iron interventions consisted of iron supplementation administered orally, intramuscularly or intravenously. Of the trials evaluating oral supplements, all but one[29]

used ferrous sulfate (13 trials[19-26,30,32,33,35,36], 721 participants). Intravenous iron was administered in three trials[27,28,31] (395 participants), and intramuscular iron in one trial[34] (16 participants). In trials using oral iron[19-26,29,30,32,33,35,36], the mean daily elemental iron dose was 86.9mg (± 49.1mg; range: 16 to 200mg). In trials reporting intravenous iron[27,28,31], the mean daily elemental iron dose was 566mg (± 330mg; range 200 to 1000mg) and mean total elemental iron dose 767mg (± 206mg; range 500 to 1000mg). Among all studies, the mean duration of iron therapy was 46 days (± 30 days; range 1 to 112 days), and mean duration of follow-up was 57 days (± 24 days; range 28 to 112 days).

Primary Outcomes

Fatigue

Four trials[28,31-33] enrolling 714 participants were eligible for meta-analysis. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS),[28] the Current and Past Psychological State scale (CAPPS),[32] visual analog scale[33] or Brief Fatigue Inventory questionnaire (BFI)[31] (SMD -0.38; 95% CI -0.52 to -0.23; I^2 0%) (**Figure 2)**. In one trial using the BFI score, fatigue was not significantly different between groups after 12 weeks, although it was improved in the subgroup of participants with the lowest iron stores (ferritin ≤ 15 ng/ml or transferrin saturation ≤ 20%)[31]. Evaluation of publication bias was not possible due to the low number of included trials. Given that the majority of trials were of unclear risk of bias, we graded the overall strength of evidence as moderate.

Work Capacity

Work capacity was reported in 10 trials[19,20,22-27,30,34] (291 participants); all but one[20] of the trials employed at least one of three common aerobic tests of work capacity: time trial[19,25], time to exhaustion[23,26,27,34], or VO_2 max[19,22-27,30,34] performance from a graded exercise test. In two trials (79 participants) that used 15 km time trials[19,25], iron supplementation was not associated with improved exercise capacity (SMD -0.09; 95% CI -0.53 to 0.35; I^2 0%) **(Appendix 4A)**. In four trials[23,26,27,34] (69 participants) that used time-to-exhaustion tests, iron supplementation did not significantly improve exercise capacity (SMD 0.25; 95% CI -0.22 to 0.73; I^2 0%) **(Appendix 4B)**. Nine trials[19,22-27,30,34] (235 participants) reported VO_2 max as a surrogate measure of work capacity. Iron supplementation did not increase VO_2 max (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%) (**Appendix 4C**). We found no evidence of funnel plot asymmetry to suggest publication bias for this outcome **(Appendix 5).** The overall strength of the evidence for time trial, time to exhaustion and VO2 max outcomes were low, given the imprecision of effect estimates and that the majority of trials were of unclear risk of bias.

One trial[20] (20 participants) used dynamic knee extension exercise to evaluate changes in work capacity. In this trial, the decline in maximum voluntary contraction after 6 minutes of exercise was significantly less in participants randomized to receive iron. Among 16 other unique measures of work capacity, 19% (3 of 16) found statistically significant increases in measures of work capacity with iron supplementation (**Appendix 6**).

Subgroup analysis

Subgroup analyses based on method of iron administration and duration of follow-up demonstrated no statistically significant differences in subjective fatigue. Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias could not be evaluated as all trials contributing data to the meta-analyses enrolled females of uncharacterized athletic status, and were of unclear risk of bias[28,31-33]. Subgroup analyses evaluating athletic status and method of iron administration demonstrated no statistically significant differences in objective work capacity. Biologic sex, duration of follow-up and risk of bias were unevaluable as all trials enrolled females with follow-up of less than 2 months, and all were of unclear risk of bias[19,22-27,30,34] **(Appendix 7 and 8)**.

Secondary Outcomes and Adverse Events

Despite the absence of baseline anemia, iron supplementation significantly increased serum hemoglobin concentration (MD 3.91 g/L; 95% CI 1.64 to 6.18; I^2 = 44%; 13 trials; 496 participants)[18-27,30,32,34]. In two trials[25,28] reporting incident anemia, a new diagnosis of anemia at trial completion was less common in patients randomized to receive iron supplementation. Iron supplementation also significantly increased serum ferritin (MD 9.23 µmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants).

Adverse events were sparsely reported. Gastrointestinal intolerance was reported in three trials[23,29,32], and was significantly increased in one trial[29] using intramuscular iron administration, but not in the two trials[23,32] that used oral administration. Nausea was reported in four trials[18,28,31,33]; two trials[28,31] using intravenous administration of iron reported significantly increased nausea, whereas nausea was not increased in patients who received iron by oral administration[18,33]. Constipation was reported in one trial[18], and

diarrhea in two trials[18,31] (**Appendix 9**). Adherence with the study intervention was reported in 13 trials[18,19,22-29,32,33,35]. Iron supplementation was not associated with differential rates of medication adherence (RR 1.0; CI 95% 0.99 to 1.01; I^2 0%; 12 trials; 958 participants). The route of administration of the study intervention was also not associated with differences in adherence (**Appendix 10**).

DISCUSSION

In iron deficient but non-anemic adults, we found iron supplementation was associated with reduced subjective measures of fatigue but had no significant impact on objective work capacity. Given iron deficiency is the most prevalent micronutrient deficiency worldwide[2], there is a discrepant lack of robust evidence evaluating iron supplementation in the absence of anemia across important patient populations. Despite rigorous and systematic methodology, we were only able to identify 18 trials enrolling 1162 adults, representing a minute fraction of affected individuals.

While treatment of iron deficiency in the absence of anemia is associated with reduced subjective fatigue, whether this translates to clinically meaningful outcomes, including quality of life, work absenteeism, job or athletic performance is uncertain. Contrary to iron deficiency with established anemia, lack of robust data in iron deficient but non-anemic individuals is reflected in the under-representation of guideline recommendations pertaining to this larger population. The proportion of iron deficient, non-anemic individuals who receive supplementation is further unknown.

Our systemic review builds on the results of two published evidence syntheses evaluating iron supplementation[37,38]. In a systematic review of healthy menstruating

women, iron supplementation, irrespective of iron status or anemia, improved hemoglobin and measures of iron stores[37]. A second systematic review that included studies of pregnant women, blood donors and children, and included data from both randomized and non-randomized trials concluded benefit of iron supplementation[38]. Despite the high prevalence of iron deficiency, significant heterogeneity in patient populations and study designs, and absence of data pertaining to objective muscle performance limits the generalizability of these findings.

In trials where a proportion of participants were anemic at enrollment, and with the knowledge that anemia results in decreased physical work capacity, iron supplementation has previously been associated with improved maximal and submaximal exercise performance[5-8]. We found insufficient evidence to suggest that iron supplementation improves exercise capacity in iron-depleted non-anemic adults, differing from the results of physiologic experiments that describe VO_2 max improvements with iron supplementation, independent of hemoglobin[39]. These findings were postulated to be secondary to iron-mediated improvements in muscle oxidative capacity and improved mitochondrial function, the validity of which is unclear[39].

A potential weakness our systematic review is the difficulty masking oral iron due to predictable gastrointestinal side effects and changes in stool color, and the impact of imperfect blinding on subjective measures of fatigue. However, despite this, fatigue was consistently reduced in trials evaluating both oral (n = 2) and intravenous (n = 2) iron preparations. Healthy females comprised the study population in 15 of 18 included trials; subjective measures of fatigue may not consistently apply to other at-risk populations. The duration of follow was relatively short (57 days; range 28-112) and perhaps too brief

to expect significant changes in muscle metabolism or function. Finally, the lack of systematic reporting of adverse events impairs our ability to draw conclusions regarding the incidence of these events and tolerability of iron therapy.

The strengths of this review include the comprehensiveness of the search strategy, which included electronic databases, trial registries, and forward searches. We used an *a priori* published protocol and followed established methodological guidelines concerning the conduct and reporting of this review. We synthesized patient-centered outcomes and evaluated efficacy in the context of relevant safety outcomes and adverse events. In contrast to the systematic review of *Low et al*, we excluded studies that enrolled patients with anemia at baseline[37]. While cut-offs for anemia varied slightly among included trials, this important inclusion criteria reduces (but may not eliminate) the probability that changes in fatigue or muscle function are due to correction of anemia or independent of oxygen carrying capacity reflecting increased red cell mass. While the duration of follow up in most studies was modest, the mean daily elemental iron dose (86.9 ± 49.1mg) reflects a recommended 'treatment' for patients with iron deficiency anemia[40].

In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in work capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider a course of iron supplementation to improve symptoms of fatigue in presence or absence of documented anemia.

Contribution:

Two researchers (BH and DH) lead and coordinated all aspects of the review, including but not limited to preparation of the literature search, screening relevant material, data analysis and extraction, interpretation of the results of the meta-analytic procedures, bias investigation, and preparation of the final report; three second reviewers (JG, ER, BP) conducted independent screening of relevant material, extracted and analyzed data and aided in report preparation; one hematologist/ intensivist (RZ), methodologist (DAF), and anesthetist/intensivist (AFT) with expertise in systematic reviews provided content expertise and methodological input, and resolved disagreement among reviewers; one systematic review expert (AMAS) provided methodological input; two hematologists (DSH and ER), one gastroenterologist (CB) and one statistician (RR) provided content expertise. All authors were involved in the process of study design and manuscript review.

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Data sharing: We are submitting (in our manuscript and supplementary files) all planned data analyses.

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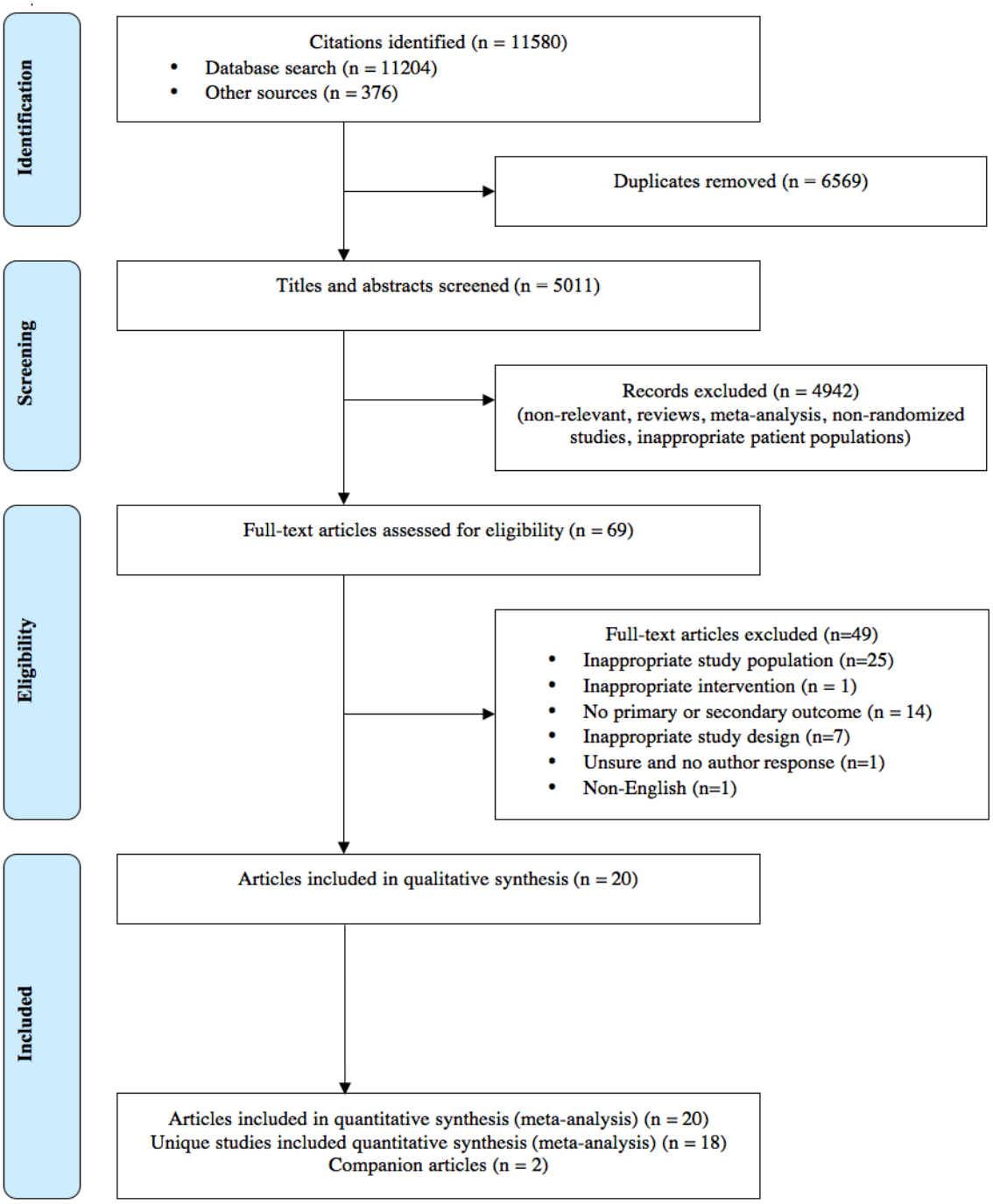

Figure 1. Study flow diagram following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA)[14] with modifications.

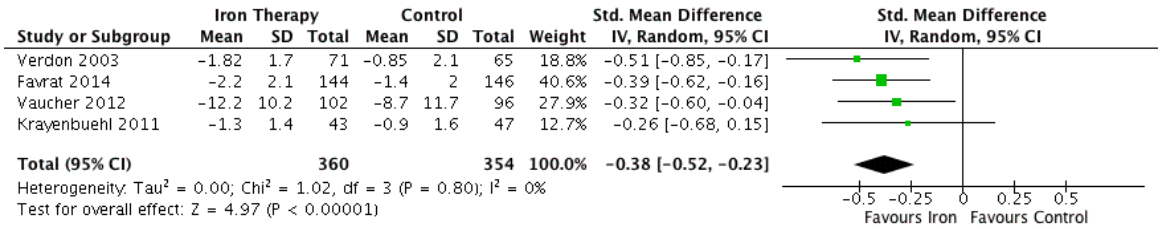

Figure 2. Validated fatigue scores

Table 1. Characteristics of individual trials, patient populations and interventions

Source	Population	No. of patients (iron)	No. of patients (control)	Control	Age (range)	Min Hb (g/L)	Max ferritin (ug/L)	Iron type	Daily iron dose (mg)	Iron route	Iron duration (days)	Follow -up (days)
Brownlie[17,19]	Physically active untrained women	22	19	Placebo	18-33	120	16	Ferrous sulfate	16	PO	42	42
Brutsaert[20]	Untrained women	10	10	Placebo	18-45	110	20	Ferrous sulfate	18.1	PO	42	42
Burden[27]	University endurance runners	7	8	Saline		120	30 (F); 40 (M)	Ferric carboxymaltose	500	IV	1	28
Donangelo[21]	Young women	12	11	Zinc gluconate	20-28	110	20	Ferrous sulfate	100	PO	56	70
Favrat[28]	Premenopausal women with fatigue	144	146	Saline		115	15	Ferric carboxymaltose	1000	IV	1	56
Flink[29]	Individuals with low unstimulated salivary flow	25	21	Placebo	15-46		30 (F); 50 (M)	Ferrous fumarate	120	PO	90	90
Fogelholm[30]	Female athletes	17	14	Placebo	17-31	120	25	Ferrous sulfate	100	PO	56	56
Hinton[22]	Recreationally trained individuals	9	8	Placebo	18-41	120 (F); 130 (M)	16	Ferrous sulfate	30	PO	42	42
Klingshirn[23]	Female endurance runners	9	9	Placebo	22-39	120	20	Ferrous sulfate	100	PO	56	56
Krayenbueh[31]	Premenopausal women with fatigue	43	47	Saline		120	50	Venofer	200	IV	4	84
LaManca[26]	Healthy females	28	28	Placebo			20	Ferrous sulfate	100	PO	56	56
Leonard[18,36]*	Young women	16*	8	Placebo	18-35	115	20	Ferrous sulfate	60/80	PO	112	112
Moafi[35]	Female students	36	36	Placebo	18-35	120	20	Ferrous sulfate	50	PO	42	42
Newhouse[24]	Young women	19	21	Placebo	18-40	120	20	Ferrous sulfate	200	PO	56	56
Peeling[34]	Well-trained female athletes	8	8	Saline		115	35	Ferrum H	100	IM	5	28
Vaucher[32]	Women with fatigue from clinic	102	96	Placebo	18-50	120	50	Ferrous sulfate	80	PO	84	84
Verdon[33]	Women with fatigue from clinic	75	69	Placebo	18-55	117		Ferrous sulfate	80	PO	28	28
Zhu[25]	Physically active women	20	17	Placebo	19-36	120	16	Ferrous sulfate	135.3	PO	56	56

*Two intervention arms - weighted averaged between two iron treatment groups; Max = maximum; Min = minimum; Hb = hemoglobin; F = females; M = males; PO = oral; IM = intramuscular; IV = intravenous

Table 2. Cochrane Risk of Bias Summary. Green = low risk of bias; Yellow = unclear risk of bias

	OVERALL	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Brownlie[17,19]								
Brutsaert[20]								
Burden[27]								
Donangelo[21]								
Favrat[28]								
Flink[29]								
Fogelholm[30]								
Hinton[22]								
Klingshirn[23]								
Krayenbueh[31]								
LaManca[26]								
Leonard[18,36]								
Moafi[35]								
Newhouse[24]								
Peeling[34]								
Vaucher[32]								
Verdon[33]								
Zhu[25]								

Appendix 1. Inclusion Criteria

1. Non-anemic (≥80%): Indicated by study cutoff values, or hemoglobin concentration [Hb] ≥130 g/L (males), ≥120 g/L (females);
2. Adults (≥18 yrs); (≥80%)
3. Iron Depleted (≥80%): According to study specific definition
4. Iron therapy administered as oral / intramuscular / intravenous therapy, all therapy durations, doses and frequencies of administration will be included
5. Studies where outcomes are assessed 1 or more months from the initiation of oral iron therapy
6. Only prospective randomized trials will be considered.

Appendix 2. Exclusion Criteria

1. Studies involving animals;
2. Females who were pregnant or breastfeeding;
3. Individuals with fatigue (≥20%) identified as being the result of some other pathology (i.e. psychiatric diagnosis, thyroid, liver, rheumatic, renal, cardiovascular, pulmonary, or oncologic cause)
4. Studies involving surgical patients
5. Studies involving author identified blood donors or phlebotomy
6. Studies assessing the pharmacokinetic properties of iron compounds in healthy volunteers where the short term outcomes are expressed as the objective (<1 month)
7. Non-English studies
8. Observational study designs, quasi-randomized, cross-over, or cluster randomized trials will not be considered for this review.
9. Studies where no relevant primary or secondary outcomes of interest are reported
10. Dietary fortification studies

Appendix 3. Search Strategy

Ovid Multifile (MEDLINE & Embase)

Database: Embase <1974 to 2015 Week 47>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

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1 Iron/df (4519)
2 exp Ferritins/df (87)
3 exp Ferrous Compounds/df (1)
4 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
5 or/1-4 (57094)
6 Anemia/pc [Prevention & Control] (3144)
7 Anemia, Iron-Deficiency/pc [Prevention & Control] (2061)
8 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
9 Deficiency Diseases/dt (968)
10 Iron/ad, tu (8860)
11 exp Ferritins/ad, tu (185)
12 exp Ferrous Compounds/ad, tu (2094)
13 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
14 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
15 Iron/ and Dietary Supplements/ (4252)
16 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
17 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
18 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
19 or/6-18 (55320)
20 5 and 19 (17537)
21 (controlled clinical trial or randomized controlled trial).pt. (504747)
22 clinical trials as topic.sh. (180086)
23 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
24 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
25 trial.ti. (344352)
26 or/21-25 (2079234)
27 20 and 26 (2880)
28 exp Animals/ not (exp Animals/ and Humans/) (9776432)
29 27 not 28 (2641)
30 (comment or editorial or interview or news).pt. (1640361)
31 (letter not (letter and randomized controlled trial)).pt. (1871051)
32 29 not (30 or 31) (2636)
33 32 use prmz (1373)

34 iron deficiency/ (11754)
35 iron deficiency anemia/ (28987)
36 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
37 or/34-36 (71135)
38 anemia/pc [Prevention] (3144)
39 iron deficiency anemia/pc [Prevention] (2269)
40 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
41 iron deficiency/dt [Drug Therapy] (1631)
42 iron therapy/ (5814)
43 iron/ad, dt, th [Drug Administration, Drug Therapy, Therapy] (13258)
44 ferritin/ad, dt [Drug Administration, Drug Therapy] (211)
45 ferrous ion/ad, dt [Drug Administration, Drug Therapy] (413)
46 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
47 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
48 iron/ and diet supplementation/ (3538)
49 ferrous sulfate/ (6637)
50 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
51 ferrous fumarate/ (831)
52 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
53 ferrous gluconate/ (1490)
54 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
55 or/38-54 (62415)
56 37 and 55 (22461)
57 randomized controlled trial/ or controlled clinical trial/ (1035658)
58 exp "clinical trial (topic)"/ (172053)
59 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
60 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
61 trial.ti. (344352)
62 or/57-61 (2260510)
63 56 and 62 (3652)
64 exp animal experimentation/ or exp models animal/ or exp animal experiment/ or nonhuman/ or exp vertebrate/ (40209729)
65 exp humans/ or exp human experimentation/ or exp human experiment/ (31154163)
66 64 not 65 (9057215)
67 63 not 66 (3517)
68 editorial.pt. (898431)
69 letter.pt. not (letter.pt. and randomized controlled trial/) (1866594)
70 67 not (68 or 69) (3492)
71 70 use oemez (2075)
72 33 or 71 (3448)

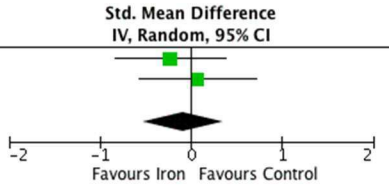
73 remove duplicates from 72 (2408) [TOTAL UNIQUE RECORDS]
74 73 use prmz (1319) [MEDLINE UNIQUE RECORDS]
75 73 use oemez (1089) [EMBASE UNIQUE RECORDS]

Appendix 4. Measures of Work Capacity

4A. 15 km Time Trial

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Hinton 2007	29.6	2.8	22	30.3	3.1	20	53.1%	-0.23 [-0.84, 0.37]
Zhu 1998	30.2	4	20	29.9	3.9	17	46.9%	0.07 [-0.57, 0.72]
Total (95% CI)			**42**			**37**	**100.0%**	**-0.09 [-0.53, 0.35]**

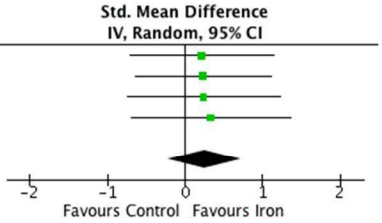
Heterogeneity: Tau² = 0.00; Chi² = 0.46, df = 1 (P = 0.50); I² = 0%
Test for overall effect: Z = 0.39 (P = 0.69)



4B. Time to Exhaustion

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Klingshirn 1992	83.2	13.6	9	80.4	10.8	9	26.2%	0.22 [-0.71, 1.14]
LaManca 1993	51.4	23.6	10	45.85	22.04	10	29.1%	0.23 [-0.65, 1.11]
Peeling 2007	3.36	0.54	8	3.22	0.57	8	23.2%	0.24 [-0.75, 1.22]
Burden 2015	373.3	48.1	7	352.9	64.1	8	21.5%	0.34 [-0.69, 1.36]
Total (95% CI)			**34**			**35**	**100.0%**	**0.25 [-0.22, 0.73]**

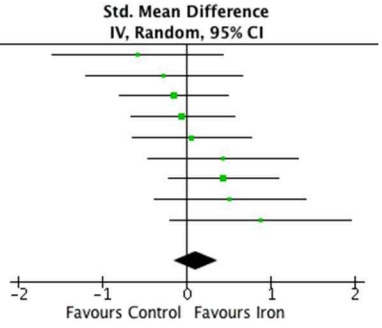
Heterogeneity: Tau² = 0.00; Chi² = 0.03, df = 3 (P = 1.00); I² = 0%
Test for overall effect: Z = 1.04 (P = 0.30)



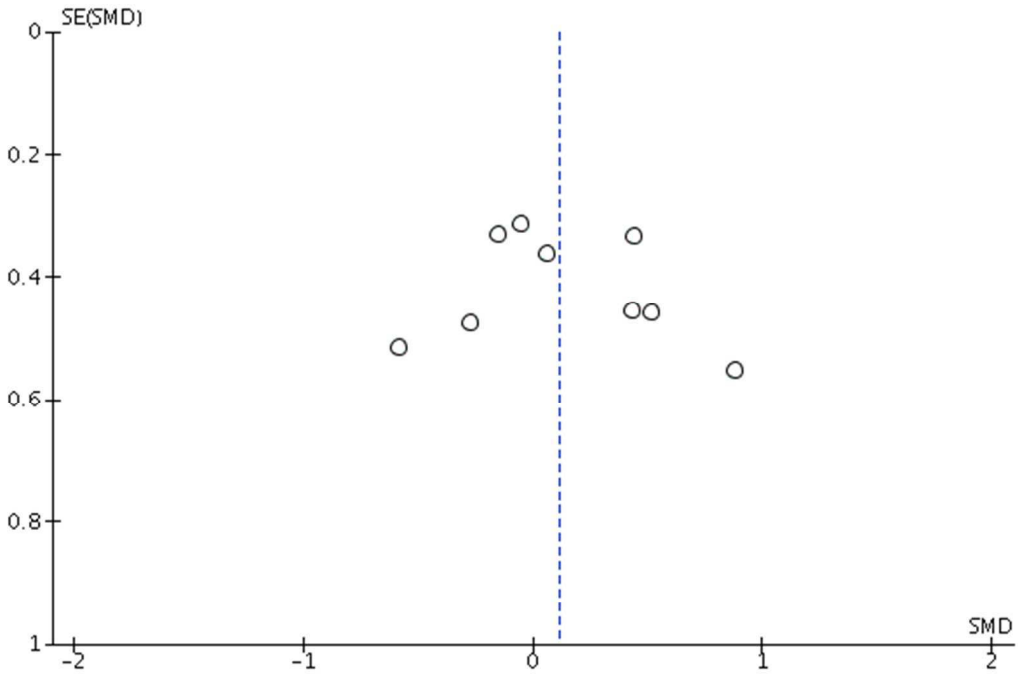
4C. Oxygen Consumption (VO_2 max)

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Peeling 2007	46.9	7.6	8	50.7	4.2	8	6.6%	-0.59 [-1.59, 0.42]
Klingshirn 1992	50.47	4.6	9	51.72	4.15	9	7.8%	-0.27 [-1.20, 0.66]
Zhu 1998	2.535	0.482	20	2.608	0.443	17	16.0%	-0.15 [-0.80, 0.49]
Brownlie 2002	57.6	8.4	22	58.1	10.5	19	17.8%	-0.05 [-0.67, 0.56]
Fogelholm 1992	45.7	7	14	45.3	6	17	13.4%	0.06 [-0.65, 0.77]
LaManca 1993	41.72	3.2	10	39.48	6.3	10	8.5%	0.43 [-0.46, 1.32]
Newhouse 1989	52.7	3.8	19	50.6	5.5	18	15.7%	0.44 [-0.22, 1.09]
Hinton 2007	42.41	8.54	10	37.92	8.23	10	8.4%	0.51 [-0.38, 1.41]
Burden 2015	70.3	5.29	7	64.27	7.35	8	5.8%	0.88 [-0.20, 1.95]
Total (95% CI)			**119**			**116**	**100.0%**	**0.11 [-0.15, 0.37]**

Heterogeneity: Tau² = 0.00; Chi² = 7.57, df = 8 (P = 0.48); I² = 0%
Test for overall effect: Z = 0.86 (P = 0.39)



Appendix 5. Funnel Plot of Studies which captured VO_2 max



Appendix 6. Work capacity tests investigated among the trials reporting measures of work capacity

Study	Population details	Work capacity tests	Intervention outcomes
Brownlie[19]	Physically active untrained women	VO_2 max RER, HRmax 15km time trial TT RER, TT VO_2max, TT lactates	Significant increases in VO_2 max and decreases in RER in iron treated group compared to placebo; no difference in HRmax Significant reduction in TT in iron group compared to placebo; no differences between TT RER, TT %VO_2 max, TT lactates
Brutsaert[20]	Untrained women	Dynamic knee extension to fatigue	Significant reduction in MVC decline in iron group compared to placebo
Burden[27]	University endurance runners	VO_2 Time to exhaustion RPE	No significant difference in VO_2 max, time to exhaustion or RPE in iron group compared to placebo.
Fogelholm[30]	Female athletes	VO_2 max Lactate levels	No significant difference in VO_2 max and lactate levels between iron and placebo group
Hinton[22]	Recreationally trained individuals	VO_2 max Submaximal test Ventilatory threshold	Significant improvements in gross energetic efficiency and VT among iron groups compared to placebo; no significant difference in VO_2 max between groups
Klingshirn[23]	Female endurance runners	VO_2 max Time to exhaustion Lactate threshold	No significant differences in all measures between iron group and placebo
LaManca[26]	Healthy females	VO_2 max Time to exhaustion RER, HR, lactate	Significant increases in VO_2 max in iron group compared to placebo; no difference in time to exhaustion, RER, HR or lactate.
Newhouse[24]	Young women	VO_2 max Wingate anaerobic test Anaerobic speed test	No significant differences were observed between iron group and placebo

		Ventilatory threshold Muscle enzyme assessments	
Peeling[34]	Well-trained female athletes	VO_2 max Submaximal economy test Time to exhaustion	No significant differences were observed between iron and placebo group
Zhu[25]	Physically active women	VO_2 max 15km time trial TT lactates	No significant differences were observed between iron and placebo group

RER = respiratory exchange ratio; HRmax = maximum heart rate; km = kilometer; TT = time trial; RPE = rated perceived exertion; MVC = maximum ventilator capacity

Appendix 7. Subgroup Analysis for Fatigue

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Method of iron administration						
Oral	[32,33]	173	161	SMD -0.39 (-0.61, -0.18)	0%	p = 0.82
Intravenous	[28,31]	187	193	SMD -0.36 (-0.56, -0.16)	0%	I^2 = 0%
Duration of study follow-up						
<2 months	[28,33]	215	211	SMD -0.43 (-0.62, -0.23)	0%	p = 0.41
>2 months	[31,32]	145	143	SMD -0.30 (-0.53, -0.07)	0%	I^2 = 0%

Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias subgroup analyses were unevaluable in subgroup analyses as all participants were females, of uncategorized athletetic status. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference

Appendix 8. Subgroup Analysis for Exercise Capacity

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Population						
Athlete	[22-24,26,27,30,34]	77	80	SMD 0.22 (-0.10, 0.55)	3%	p = 0.25
Non-athlete	[19,25]	42	36	SMD -0.10 (-0.55, 0.35)	0%	I^2 = 24%
Method of iron administration						
Oral	[19,22-26,30]	104	100	SMD 0.12 (-0.16, -0.39)	0%	p = 0.15
IV	[27]	7	8	SMD 0.88 (-0.20, 1.95)	NA	I^2 = 47%
IM	[34]	8	8	SMD -0.59 (-1.59, 0.42)	NA	

Biologic sex, duration of follow-up and risk of bias subgroup analyses were unevaluable in subgroup analyses as all trials enrolled females and had a follow-up period of less than two months. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference; IV = intravenous; IM = intramuscular

Appendix 9. Adverse effects in trials of iron supplementation in iron-deficient, non anemic individuals

Study	Constipation	Diarrhea	Nausea	GI intolerance
Intravenous				
Burden[27]	NR	NR	NR	NR
Favrat[28]	NR	NR	Iron: 8; Control: 2	NR
Krayenbuehl[31]	NR	Iron: 0; Control: 1	Iron: 6; Control: 1	NR
Intramuscular				
Flink[29]	NR	NR	NR	Iron: 14; Control: 2
Oral				
Brownlie[19]	NR	NR	NR	NR
Brutsaert[20]	"Frequency and severity of reported side effects due to supplementation was very low and did not differ significantly between groups"			
Donangelo[21]	NR	NR	NR	NR
Fogelholm[30]	NR	NR	NR	NR
Hinton[22]	NR	NR	NR	NR
Klingshirn[23]	NR	NR	NR	Iron: 1; Control: 0
LaManca[26]	NR	NR	NR	NR
Leonard[18]	Iron: 1; Control: 0	Iron: 2; Control: 2	Iron: 2; Control: 1	NR
Moafi[35]	"When symptoms occurring immediately before or during menses were excluded, there were no significant differences either in frequency or severity of symptoms experienced"			
Newhouse[24]	NR	NR	NR	NR
Peeling[34]	NR	NR	NR	NR
Vaucher[32]	NR	NR	NR	Iron: 12; Control: 10
Verdon[33]	NR	NR	Iron: 0; control: 1	NR
Zhu[25]	NR	NR	NR	NR

NR, not reported; GI, gastrointestinal

Appendix 10. Compliance with the study intervention

	Iron (%)	Control (%)
Intravenous		
Burden[27]	100	100
Favrat[28]	100	100
Krayenbuehl[31]	NR	NR
Intramuscular		
Peeling[34]	NR	NR
Oral		
Brownlie[19]	91	89
Brutsaert[20]	NR	NR
Donangelo[21]	NR	NR
Flink[29]	71	82
Fogelholm[30]	NR	NR
Hinton[22]	98	99
Klingshirn[23]	89	91
LaManca[26]	82	85
Leonard[18*]	89	92
Moafi[35]	89	92
Newhouse[24]	>75	>75
Vaucher[32]	93	94
Verdon[33]	95	98
Zhu[25]	88	87

*weighted averaged between two iron treatment groups; NR = not reported

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp 3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9, Fig 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10, 11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

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Efficacy of iron supplementation on fatigue and physical capacity in non-anemic iron deficient adults: a systematic review of randomized controlled trials

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Efficacy of iron supplementation on fatigue and physical capacity in non-anemic iron deficient adults: a systematic review of randomized controlled trials

Brett L. Houston[1,2], Daryl Hurrie[3], Jeff Graham[1,2], Brittany Perija[4], Emily Rimmer[1,2], Rasheda Rabbani[5,6], Charles N. Bernstein[7], Alexis Turgeon[8], Dean Fergusson[9], Donald S. Houston[1,2], Ahmed M. Abou-Setta[5,6], Ryan Zarychanski[1,2,5,6]

[1]Department of Internal Medicine, Section of Medical Oncology and Haematology, University of Manitoba, Winnipeg, Manitoba, Canada
[2]Department of Medical Oncology and Haematology, CancerCare Manitoba, Winnipeg, Manitoba, Canada
[3]Applied Health Sciences, Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Manitoba, Canada
[4]Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
[5]George & Fay Yee Center for Healthcare Innovation, University of Manitoba/Winnipeg Regional Health Authority, Winnipeg, Manitoba, Canada
[6]Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[7]Department of Internal Medicine, Section of Gastroenterology, University of Manitoba, Winnipeg, Manitoba, Canada
[8]Centre de recherche du CHU de Québec – Université Laval, Population Health and Optimal Health Practices Research Unit, Trauma - Emergency - Critical Care Medicine; Department of Anesthesiology and Critical Care Medicine, Division of Critical Care Medicine, Faculty of Medicine, Université Laval, Québec City, Québec, Canada.
[9]Clinical Epidemiology Program, Ottawa Hospital Research Institute (OHRI); Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Corresponding author: Ryan Zarychanski
ON 2051-675 McDermot Avenue
CancerCare Manitoba
Winnipeg, Manitoba, R3E OV9
T: 204-787-2108
F: 204-786-0196
rzarychanski@cancercare.mb.ca

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Systematic Review Registration: PROSPERO (CRD42014007085)

OBJECTIVE: Iron supplementation in iron deficiency anemia is standard practice, but the benefits of iron supplementation in iron deficient non-anemic (IDNA) individuals remains controversial. Our objective is to identify the effects of iron therapy on fatigue and physical capacity in iron deficient non-anemic adults.

DESIGN: Systematic review and meta-analysis of randomized controlled trials (RCTs)

SETTING: Primary care

PARTICIPANTS: Adults (≥18 years) who were iron deficient but non-anemic

INTERVENTIONS: Oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included

COMPARATORS: Placebo or active therapy

RESULTS: We identified RCTs in Medline, Embase, CENTRAL, CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016. We searched the World Health Organization's ICTRP for relevant ongoing trials and performed forward searches of included trials and relevant reviews in Web of Science. We assessed internal validity of included trials using the Cochrane Risk of Bias tool, and the external validity using the GRADE methodology. From 11580 citations we included 18 unique trials, and 2 companion papers enrolling 1170 patients. Using a Mantel-Haenszel random-effects model, iron supplementation was associated with reduced self-reported fatigue (standardized mean difference (SMD) -0.38; 95% CI -0.52 to -0.23; I^2 0%; 4 trials; 714 participants), but was not associated with differences in objective measures of physical capacity, including maximal oxygen consumption (VO_2 max) (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%; 9 trials; 235 participants), and timed methods of exercise testing. Iron supplementation significantly increased serum hemoglobin concentration (MD 4.01 g/L;

95% CI 1.22 to 6.81; I^2 = 48%; 12 trials; 298 participants) and serum ferritin (MD 9.23 µmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants).

CONCLUSION: In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in physical capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider consumption of iron-rich foods or iron supplementation to improve symptoms of fatigue in the absence of documented anemia.

SYSTEMATIC REVIEW REGISTRATION: PROSPERO (CRD42014007085)

STUDY STRENGTHS and LIMITATIONS:

Strengths:

- We used a comprehensive search strategy, an *a priori* protocol, and adhered to established methodological (e.g. PRISMA, GRADE) guidelines
- We identified an at-risk patient population, for whom iron deficiency is highly prevalent, but treatment is unknown
- Our outcomes are clinically relevant and patient centered

Limitations:

- In our selected population of iron deficient but not anemic individuals, the majority of studies evaluated healthy females
- In the included trials, tolerability and adverse events of iron therapy was incompletely captured

INTRODUCTION

Iron deficiency (ID) is estimated to affect two billion people, and is the leading cause of anemia worldwide[1,2]. Iron is necessary for cellular immune responses, oxidative metabolism within the mitochondria, and production of hemoglobin and myoglobin. When iron losses exceed dietary iron absorption, iron stores become depleted resulting in impaired hemoglobin production and decreased red blood cell hemoglobin content[3]. Reduction in hemoglobin concentration below a threshold (conventionally defined by the World Health Organization (WHO) as 120g/L for females and 130g/L for males) signifies anemia[4].

It is well established that anemia results in decreased physical capacity and increased fatigue proportional to anemia severity[5-9]. Unfortunately, patient-reported fatigue is common in community and primary care settings with a prevalence ranging

from 7 to 45%[10]. It is estimated that the indirect annual economic consequence of chronic fatigue in the United States is 9.1 billion dollars[11].

The clinical relevance of iron deficiency in the absence of anemia is poorly understood, but may impact well-being, perceptions of fatigue, or contribute to decrements in physical performance through impairment in biochemical processes including tissue and mitochondrial oxidative capacity[8]. While iron replacement can normalize hemoglobin concentration, restore work capacity and improve fatigue in iron deficiency anemia, it is unclear if supplementation affects fatigue and physical capacity in iron deficient but non-anemic (IDNA) individuals. In the absence of compelling efficacy data on well-being or muscle function, the use of iron supplements are common in the general population and are routinely recommended to high performance athletes to enhance performance.

Given the global prevalence of iron deficiency and impact of fatigue, the purpose of this systematic review is to identify, critically appraise and meta-analyse data from prospective randomized trials evaluating iron therapy in adults with IDNA.

METHODS

Using an *a priori* published protocol (CRD42014007085; available at https://www.crd.york.ac.uk/PROSPERO/)[12], we conducted a systematic review using methodological approaches outlined in the *Cochrane Handbook for Systematic Reviewers* and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria[12-14]. A panel of experts from multiple fields (e.g. internal medicine, hematology, kinesiology, gastroenterology, research methodology)

formulated the research question, reviewed search strategies and methods, and provided input throughout the review process.

Populations, Interventions, Comparators, Outcome Measures, Setting and Study Designs

Our research question was "In iron depleted but non-anemic adults, does iron supplementation improve fatigue and physical capacity." We included randomized controlled trials of adults (≥18 years) who were iron deficient but non-anemic (Appendix 1). Interventions included oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included. We included trials that evaluated outcomes at least 28 days from the initiation of iron therapy. Comparators included placebo or active therapy. Our exclusion criteria are presented in Appendix 2.

Our primary outcome measures were self-reported fatigue and objective measures of physical capacity. Secondary outcomes included the incidence of anemia, change in hemoglobin concentration and serum ferritin, and the incidence of adverse outcomes including iron toxicity, constipation, diarrhea, gastrointestinal intolerance and nausea.

Search Strategy for Identification of Studies

We searched Medline, Embase, CENTRAL (Cochrane Library), CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016 to identify relevant citations of published trials, using individualized systematic search strategies for each database. The MEDLINE strategy is presented in Appendix 3. We searched the World Health Organization's (WHO) International Clinical Trials Registry Platform (ICTRP), clinicaltrials.gov and relevant conference proceedings to identify planned, ongoing, or

recently completed but unpublished trials. We performed forward searches of included trials and relevant reviews in Web of Science to identify additional citations, and contacted study authors to request pertinent unpublished data or provide clarifications on study methods or results. Reference lists of narrative and systematic reviews and of the included trials were searched for additional citations. We performed reference management in EndNote™ (Version X7, Thomson Reuters, Philadelphia, PA, USA).

Study Selection, Data Extraction and Quality Assessment

We screened citations, selected studies and extracted data from included trials using standardized and piloted screening and data extraction forms. Citation screening, study selection and data extraction were performed in duplicate. The following data were extracted from each trial: author identification, publication year, publication language, trial location, source of trial funding, participant characteristics (age, sex, weight), intervention/comparator (drug utilized, dose (elemental iron), route of administration, duration), as well as results for the primary and secondary outcomes. We assessed the internal validity of included trials using the Cochrane Collaboration Risk of Bias tool[13]. Discrepancies between the two reviewers were resolved by consensus or by a third reviewer (RZ), as required. Data extraction and descriptive statistics were performed using Microsoft Excel 2016 (Excel version 15, Microsoft Corp).

Data Analysis

Data analysis was performed using Review Manager (RevMan v5.3.5, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). Study level

comparisons of dichotomous data were presented as risk ratios (RR) with 95% confidence intervals (CI). Pooled continuous data were expressed as the mean difference (MD), or standardized mean difference (SMD). Change scores or post-treatment means were extracted to inform summary estimates for continuous data. Pooled risk ratios and 95% confidence intervals were calculated using Mantel-Haenszel random-effects model. Pooled MDs or SMDs were calculated using a random-effects model. For the primary outcome of fatigue, if multiple scales were reported, fatigue-specific scores were preferred over general scores and the most commonly reported and clinically meaningful score was used to generate summary effect measures. In studies evaluating exercise capacity, weight-based VO_2 max values were utilized preferentially if both absolute and weight-based VO_2 max results were provided. Statistical heterogeneity was quantified using the I^2 statistic[15]. For the primary outcomes of fatigue and work capacity, we evaluated potential publication bias using funnel plot analysis[16]. All tests of statistical inference reflect a 2-sided α of 0.05.

Subgroup Analyses

We performed subgroup analyses for fatigue and exercise capacity outcomes according to biologic sex, athletic status (athlete or non-athlete), method of iron administration, duration of therapy, duration of study follow up, and risk of bias.

Grading the Evidence

We graded the strength of evidence for our primary outcomes using the GRADE methodology. This approach classifies the strength of evidence as "*high", "moderate", "low"* or *"very low."*

RESULTS

Trial Characteristics & Study Populations

Of the 11,580 citations identified, we included 18 unique trials and two companion papers[17,18], enrolling 1170 subjects (Figure 1; Table 1). Trials were published between 1989 and 2015, and all trials were published in peer-reviewed journals. Eight trials[19-26] were from North America, seven trials[27-33] were from Europe, two trials[18,34] were from Australia, and one trial[35] was from Asia.

Exclusively healthy females (aged 17 to 55 years old) with varying levels of fitness (sedentary to well-trained) were enrolled in all but three studies[22,27,29]. The WHO cutoff for anemia [hemoglobin concentration ≥130 g/L (males) and ≥120 g/L (females)] was used by 9 studies[19,22-25,30-32,35], whereas 7 studies used lower values ranging from ≥110 to <120 g/l[20,21,27,28,33,34,36], and baseline hemoglobin concentration was not provided in 2 trial reports[26,29].

All trials were placebo-controlled. In 13 of 18 trials (72%), we considered the blinding of participants and personnel to be adequate. Likewise, 10 trials (55%) adequately incorporated blinded outcome assessment. One trial[29] was considered to have a low risk of bias (Table 2). The remainder of the trials were considered unclear risk of bias, due to unclear processes of randomization (12 trials[19-27,30,33,34]) or allocation concealment (13 trials[19-27,30,31,34,35]).

Table 1. Characteristics of individual trials, patient populations and interventions

Trial #	Source	Population	No. of patients (iron)	No. of patients (control)	Control	Age (range)	Min Hb (g/L)	Max ferritin (ug/L)	Iron type	Daily iron dose (mg)	Iron route	Iron duration (days)	Follow-up (days)
1	Brownlie[17,19]	Physically active untrained women	22	19	Placebo	18-33	120	16	Ferrous sulfate	16	PO	42	42
2	Brutsaert[20]	Untrained women	10	10	Placebo	18-45	110	20	Ferrous sulfate	18.1	PO	42	42
3	Burden[27]	University endurance runners	7	8	Saline		120	30 (F); 40 (M)	Ferric carboxymaltose	500	IV	1	28
4	Donangelo[21]	Young women	12	11	Zinc gluconate	20-28	110	20	Ferrous sulfate	100	PO	56	70
5	Favrat[28]	Premenopausal women with fatigue	144	146	Saline		115	15	Ferric carboxymaltose	1000	IV	1	56
6	Flink[29]	Individuals with low unstimulated salivary flow	25	21	Placebo	15-46		30 (F); 50 (M)	Ferrous fumarate	120	PO	90	90
7	Fogelholm[30]	Female athletes	17	14	Placebo	17-31	120	25	Ferrous sulfate	100	PO	56	56
8	Hinton[22]	Recreationally trained individuals	9	8	Placebo	18-41	120 (F); 130 (M)	16	Ferrous sulfate	30	PO	42	42
9	Klingshirn[23]	Female endurance runners	9	9	Placebo	22-39	120	20	Ferrous sulfate	100	PO	56	56
10	Krayenbuehl[31]	Premenopausal women with fatigue	43	47	Saline		120	50	Venofer	200	IV	4	84
11	LaManca[26]	Healthy females	28	28	Placebo			20	Ferrous sulfate	100	PO	56	56
12	Leonard[18,36]*	Young women	16*	8	Placebo	18-35	115	20	Ferrous sulfate	60/80	PO	112	112
13	Moafi[35]	Female students	36	36	Placebo	18-35	120	20	Ferrous sulfate	50	PO	42	42
14	Newhouse[24]	Young women	19	21	Placebo	18-40	120	20	Ferrous sulfate	200	PO	56	56
15	Peeling[34]	Well-trained female athletes	8	8	Saline		115	35	Ferrum H	100	IM	5	28
16	Vaucher[32]	Women with fatigue from clinic	102	96	Placebo	18-50	120	50	Ferrous sulfate	80	PO	84	84
17	Verdon[33]	Women with fatigue from clinic	71	65	Placebo	18-55	117		Ferrous sulfate	80	PO	28	28
18	Zhu[25]	Physically active women	20	17	Placebo	19-36	120	16	Ferrous sulfate	135.3	PO	56	56
TOTAL:			**598**	**572**									

*Trial included two intervention arms, with 8 patients enrolled in each arm; represents weighted averages between two iron treatment groups; Max = maximum; Min = minimum; Hb = hemoglobin; F = females; M = males; PO = oral; IM = intramuscular; IV = intravenous

Table 2. Cochrane Risk of Bias summary. Green (+) = low risk of bias; Yellow (?) = unclear risk of bias

	OVERALL	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Brownlie[17,19]	?	?	?	+	?	?	+	+
Brutsaert[20]	?	?	?	+	?	?	+	+
Burden[27]	?	?	?	+	+	+	+	+
Donangelo[21]	?	?	?	?	?	+	+	+
Favrat[28]	?	+	+	?	?	?	+	+
Flink[29]	+	+	+	+	+	+	+	+
Fogelholm[30]	?	?	?	+	+	+	+	+
Hinton[22]	?	?	?	?	?	+	+	+
Klingshirn[23]	?	?	?	+	?	+	+	+
Krayenbuehl[31]	?	+	?	+	+	+	?	+
LaManca[26]	?	?	?	?	?	+	+	+
Leonard[18,36]	?	+	+	+	+	?	+	+
Moafi[35]	?	+	?	+	+	+	+	+
Newhouse[24]	?	?	?	+	+	?	+	+
Peeling[34]	?	?	?	?	?	+	+	+
Vaucher[32]	?	+	+	+	+	?	+	+
Verdon[33]	?	?	+	+	+	+	+	+
Zhu[25]	?	?	?	+	+	+	+	+

Interventions

Iron interventions consisted of iron supplementation administered orally, intramuscularly or intravenously. Of the trials evaluating oral supplements, all but one[29] used ferrous sulfate (13 trials[19-26,30,32,33,35,36], 713 participants). Intravenous iron was administered in three trials[27,28,31] (395 participants), and intramuscular iron in one trial[34] (16 participants). In trials using oral iron[19-26,29,30,32,33,35,36], the mean daily elemental iron dose was 86.9mg (± 49.1mg; range: 16 to 200mg). In trials reporting intravenous iron[27,28,31], the mean daily elemental iron dose was 566mg (± 330mg; range 200 to 1000mg) and mean total elemental iron dose 767mg (± 206mg; range 500 to 1000mg). Among all studies, the mean duration of iron therapy was 46 days (± 30 days; range 1 to 112 days), and mean duration of follow-up was 57 days (± 24 days; range 28 to 112 days).

Primary Outcomes

Fatigue

Four trials[28,31-33] enrolling 714 participants were eligible for meta-analysis. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS),[28] the Current and Past Psychological State scale (CAPPS),[32] visual analog scale[33] or Brief Fatigue Inventory questionnaire (BFI)[31] (SMD -0.38; 95% CI -0.52 to -0.23; I^2 0%) (Figure 2). In one trial using the BFI score, fatigue was not significantly different between groups after 12 weeks, although it was improved in the subgroup of participants with the lowest iron stores (ferritin ≤ 15 ng/ml or transferrin saturation ≤ 20%)[31]. Evaluation of publication bias was not possible due to the low number of included trials. Given that the majority of trials were of unclear risk of bias, we graded the overall strength of evidence as moderate.

Physical Capacity

Physical capacity was reported in 10 trials[19,20,22-27,30,34] (291 participants); all but one[20] of the trials employed at least one of three common aerobic tests of physical capacity: time trial[22,25], time to exhaustion[23,26,27,34], or VO_2 max[19,22-27,30,34] performance from a graded exercise test. In two trials (79 participants) that used 15 km time trials[22,25], iron supplementation was not associated with improved exercise capacity (SMD -0.09; 95% CI -0.53 to 0.35; I^2 0%) (Appendix 4A). In four trials[23,26,27,34] (69 participants) that used time-to-exhaustion tests, iron supplementation did not significantly improve exercise capacity (SMD 0.25; 95% CI -0.22 to 0.73; I^2 0%) (Appendix 4B). Nine

trials[19,22-27,30,34] (235 participants) reported VO_2 max as a surrogate measure of physical capacity. Iron supplementation did not increase VO_2 max (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%) (Appendix 4C). We found no evidence of funnel plot asymmetry to suggest publication bias for this outcome (Appendix 5). The overall strength of the evidence for time trial, time to exhaustion and VO_2 max outcomes were low, given the imprecision of effect estimates and that the majority of trials were of unclear risk of bias.

One trial[20] (20 participants) used dynamic knee extension exercise to evaluate changes in physical capacity. In this trial, the decline in maximum voluntary contraction after 6 minutes of exercise was significantly less in participants randomized to receive iron. Among 16 other unique measures of physical capacity, 19% (3 of 16) found statistically significant increases in measures of physical capacity with iron supplementation (Appendix 6).

Subgroup analysis

Subgroup analyses based on method of iron administration and duration of follow-up demonstrated no statistically significant differences in subjective fatigue (Appendix 7). Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias could not be evaluated as all trials contributing data to the meta-analyses enrolled females of uncharacterized athletic status, and were of unclear risk of bias[28,31-33]. Subgroup analyses evaluating athletic status and method of iron administration demonstrated no statistically significant differences in objective physical capacity (Appendix 8). Biologic sex, duration of follow-up and risk of bias were unevaluable as all trials enrolled females with follow-up of less than 2 months, and all were of unclear risk of bias[19,22-27,30,34].

Secondary Outcomes and Adverse Events

Despite the absence of baseline anemia, iron supplementation significantly increased serum hemoglobin concentration (MD 4.01 g/L; 95% CI 1.22 to 6.81; I^2 = 48%; 12 trials; 298 participants)[18-27,30,34] (Appendix 9). In two trials[25,28] reporting incident anemia, a new diagnosis of anemia at trial completion was less common in patients randomized to receive iron supplementation. Iron supplementation also significantly increased serum ferritin (MD 9.23 µmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants) (Appendix 10).

Adverse events were sparsely reported. Gastrointestinal intolerance was reported in three trials[23,29,32], and was significantly increased in one trial[29] using intramuscular iron administration, but not in the two trials[23,32] that used oral administration. Nausea was reported in four trials[18,28,31,33]; two trials[28,31] using intravenous administration of iron reported significantly increased nausea, whereas nausea was not increased in patients who received iron by oral administration[18,33]. Constipation was reported in one trial[18], and diarrhea in two trials[18,31] (Appendix 11). Adherence with the study intervention was reported in 13 trials[18,19,22-29,32,33,35]. Iron supplementation was not associated with differential rates of medication adherence (RR 1.0; CI 95% 0.99 to 1.01; I^2 0%; 12 trials; 958 participants). The route of administration of the study intervention was also not associated with differences in adherence (Appendix 12).

DISCUSSION

In iron deficient but non-anemic adults, we found iron supplementation was associated with reduced subjective measures of fatigue but had no significant impact on objective physical capacity. Given iron deficiency is the most prevalent micronutrient deficiency worldwide[2], there is a discrepant lack of robust evidence evaluating iron supplementation in the absence of anemia across important patient populations. Despite rigorous and systematic methodology, we were only able to identify 18 trials enrolling 1170 adults, representing a minute fraction of affected individuals.

While treatment of iron deficiency in the absence of anemia is associated with reduced subjective fatigue, whether this translates to clinically meaningful outcomes, including quality of life, work absenteeism, job or athletic performance is uncertain. Contrary to iron deficiency with established anemia, lack of robust data in iron deficient but non-anemic individuals is reflected in the under-representation of guideline recommendations pertaining to this larger population. The proportion of iron deficient, non-anemic individuals who receive supplementation is further unknown.

Our systemic review builds on the results of three published evidence syntheses evaluating iron supplementation[37-39]. In a systematic review of healthy menstruating women, iron supplementation, irrespective of iron status or anemia, improved hemoglobin and measures of iron stores[37]. Two systematic reviews included studies of pregnant women, blood donors and children, and included data from both randomized and non-randomized trials[38,39]. These studies concluded benefit of iron supplementation, although in the review by *Yokoi et al*, the benefit was limited to randomized controlled trials. Despite the high prevalence of iron deficiency, significant heterogeneity in patient

populations and study designs, and absence of data pertaining to objective muscle performance limits the generalizability of these findings.

In trials where a proportion of participants were anemic at enrollment, and with the knowledge that anemia results in decreased physical capacity, iron supplementation has previously been associated with improved maximal and submaximal exercise performance[5-8]. We found insufficient evidence to suggest that iron supplementation improves exercise capacity in iron-depleted non-anemic adults, differing from the results of physiologic experiments that describe VO_2 max improvements with iron supplementation, independent of hemoglobin[40]. These findings were postulated to be secondary to iron-mediated improvements in muscle oxidative capacity and improved mitochondrial function, the validity of which is unclear[40].

A potential weakness our systematic review is the difficulty masking oral iron due to predictable gastrointestinal side effects and changes in stool color, and the impact of imperfect blinding on subjective measures of fatigue. However, despite this, fatigue was consistently reduced in trials evaluating both oral (n = 2) and intravenous (n = 2) iron preparations. Healthy females comprised the study population in 15 of 18 included trials; subjective measures of fatigue may not consistently apply to other at-risk populations. The duration of follow was relatively short (57 days; range 28-112 days) and perhaps too brief to expect significant changes in muscle metabolism or function. Finally, the lack of systematic reporting of adverse events impairs our ability to draw conclusions regarding the incidence of these events and tolerability of iron therapy.

The strengths of this review include the comprehensiveness of the search strategy, which included electronic databases, trial registries, and forward searches. We used an *a*

priori published protocol and followed established methodological guidelines concerning the conduct and reporting of this review. We synthesized patient-centered outcomes and evaluated efficacy in the context of relevant safety outcomes and adverse events. In contrast to the systematic review of *Low et al*, we excluded studies that enrolled patients with anemia at baseline[37]. While cut-offs for anemia varied slightly among included trials, this important inclusion criteria reduces (but may not eliminate) the probability that changes in fatigue or muscle function are due to correction of anemia or independent of oxygen carrying capacity reflecting increased red cell mass. While the duration of follow up in most studies was modest, the mean daily elemental iron dose (86.9 ± 49.1mg) reflects a recommended 'treatment' for patients with iron deficiency anemia[41].

In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in physical capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider consumption of iron-rich foods or iron supplementation to improve symptoms of fatigue in the absence of documented anemia.

Figure Legends:

Figure 1. Study flow diagram following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA)[14] with modifications. Of the 11,580 citations identified, we included 18 unique trials and two companion papers.

Figure 2. The effect of iron supplementation on patient-reported fatigue, using validated fatigue scores. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS), the Current and Past Psychological State scale (CAPPS), visual analog scale or Brief Fatigue Inventory questionnaire (BFI).

Contribution:

Two researchers (BH and DH) lead and coordinated all aspects of the review, including but not limited to preparation of the literature search, screening relevant material, data analysis and extraction, interpretation of the results of the meta-analytic procedures, bias investigation, and preparation of the final report; three second reviewers (JG, ER, BP) conducted independent screening of relevant material, extracted and analyzed data and aided in report preparation; one hematologist/ intensivist (RZ), methodologist (DAF), and anesthetist/intensivist (AFT) with expertise in systematic reviews provided content expertise and methodological input, and resolved disagreement among reviewers; one systematic review expert (AMAS) provided methodological input; two hematologists (DSH and ER), one gastroenterologist (CB) and one statistician (RR) provided content expertise. All authors were involved in the process of study design and manuscript review.

Competing interests: the authors declare no competing financial interests.

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Data sharing: We are submitting (in our manuscript and supplementary files) all planned data analyses.

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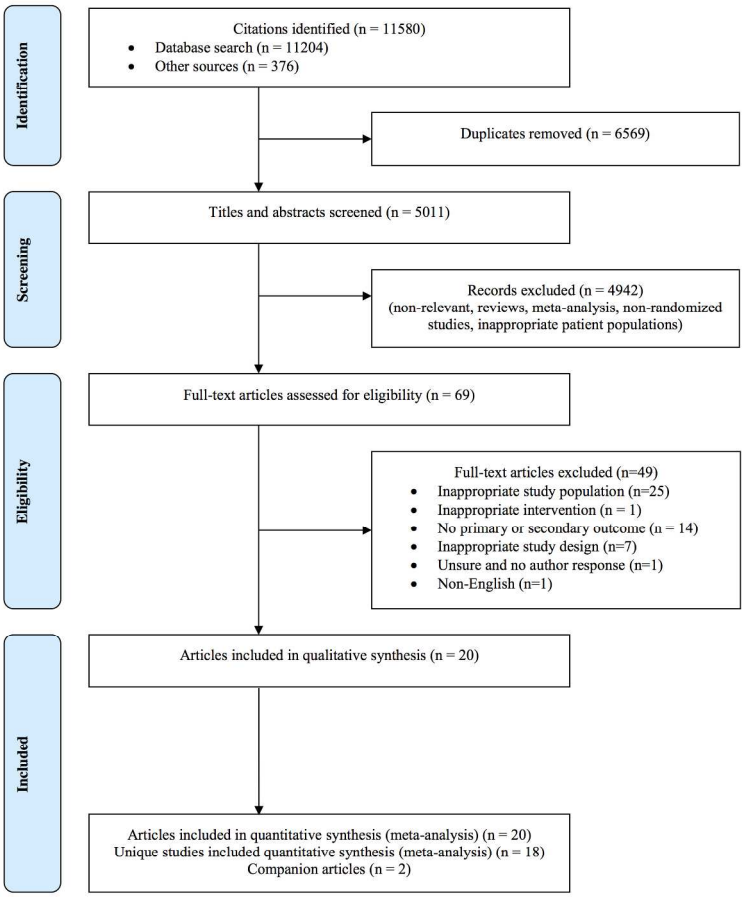

Figure 1. Study flow diagram following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA)14 with modifications. Of the 11,580 citations identified, we included 18 unique trials and two companion papers.

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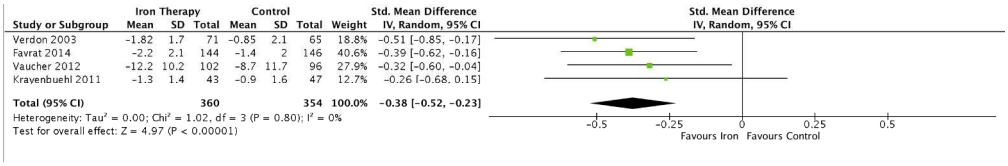


Figure 2. The effect of iron supplementation on patient-reported fatigue, using validated fatigue scores. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS), the Current and Past Psychological State scale (CAPPS), visual analog scale or Brief Fatigue Inventory questionnaire (BFI).

428x67mm (300 x 300 DPI)

Appendix 1. Inclusion criteria

1. Non-anemic (≥80%): Indicated by study cutoff values, or hemoglobin concentration [Hb] ≥130 g/L (males), ≥120 g/L (females);
2. Adults (≥18 yrs); (≥80%)
3. Iron Depleted (≥80%): According to study specific definition
4. Iron therapy administered as oral / intramuscular / intravenous therapy, all therapy durations, doses and frequencies of administration will be included
5. Studies where outcomes are assessed ≥ 28 days from the initiation of oral iron therapy
6. Only prospective randomized trials will be considered.

Appendix 2. Exclusion criteria

1. Studies involving animals;
2. Females who were pregnant or breastfeeding;
3. Individuals with fatigue (≥20%) identified as being the result of some other pathology (i.e. psychiatric diagnosis, thyroid, liver, rheumatic, renal, cardiovascular, pulmonary, or oncologic cause)
4. Studies involving surgical patients
5. Studies involving author identified blood donors or phlebotomy
6. Studies assessing the pharmacokinetic properties of iron compounds in healthy volunteers where the short term outcomes are expressed as the objective (<1 month)
7. Non-English studies
8. Observational study designs, quasi-randomized, cross-over, or cluster randomized trials will not be considered for this review.
9. Studies where no relevant primary or secondary outcomes of interest are reported
10. Dietary fortification studies

Appendix 3. Search strategy

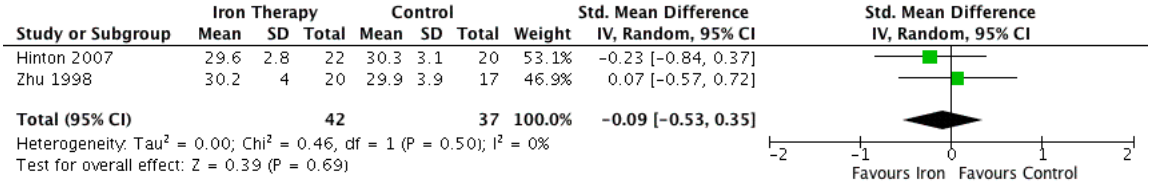
Ovid Multifile (MEDLINE & Embase)
Database: Embase <1974 to 2015 Week 47>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:
---
1 Iron/df (4519)
2 exp Ferritins/df (87)
3 exp Ferrous Compounds/df (1)
4 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
5 or/1-4 (57094)
6 Anemia/pc [Prevention & Control] (3144)
7 Anemia, Iron-Deficiency/pc [Prevention & Control] (2061)
8 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
9 Deficiency Diseases/dt (968)
10 Iron/ad, tu (8860)
11 exp Ferritins/ad, tu (185)
12 exp Ferrous Compounds/ad, tu (2094)
13 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
14 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
15 Iron/ and Dietary Supplements/ (4252)
16 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
17 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
18 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
19 or/6-18 (55320)
20 5 and 19 (17537)
21 (controlled clinical trial or randomized controlled trial).pt. (504747)
22 clinical trials as topic.sh. (180086)
23 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
24 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
25 trial.ti. (344352)
26 or/21-25 (2079234)
27 20 and 26 (2880)
28 exp Animals/ not (exp Animals/ and Humans/) (9776432)
29 27 not 28 (2641)
30 (comment or editorial or interview or news).pt. (1640361)
31 (letter not (letter and randomized controlled trial)).pt. (1871051)
32 29 not (30 or 31) (2636)
33 32 use prmz (1373)

34 iron deficiency/ (11754)
35 iron deficiency anemia/ (28987)
36 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
37 or/34-36 (71135)
38 anemia/pc [Prevention] (3144)
39 iron deficiency anemia/pc [Prevention] (2269)
40 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
41 iron deficiency/dt [Drug Therapy] (1631)
42 iron therapy/ (5814)
43 iron/ad, dt, th [Drug Administration, Drug Therapy, Therapy] (13258)
44 ferritin/ad, dt [Drug Administration, Drug Therapy] (211)
45 ferrous ion/ad, dt [Drug Administration, Drug Therapy] (413)
46 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
47 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
48 iron/ and diet supplementation/ (3538)
49 ferrous sulfate/ (6637)
50 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
51 ferrous fumarate/ (831)
52 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
53 ferrous gluconate/ (1490)
54 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
55 or/38-54 (62415)
56 37 and 55 (22461)
57 randomized controlled trial/ or controlled clinical trial/ (1035658)
58 exp "clinical trial (topic)"/ (172053)
59 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
60 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
61 trial.ti. (344352)
62 or/57-61 (2260510)
63 56 and 62 (3652)
64 exp animal experimentation/ or exp models animal/ or exp animal experiment/ or nonhuman/ or exp vertebrate/ (40209729)
65 exp humans/ or exp human experimentation/ or exp human experiment/ (31154163)
66 64 not 65 (9057215)
67 63 not 66 (3517)
68 editorial.pt. (898431)
69 letter.pt. not (letter.pt. and randomized controlled trial/) (1866594)
70 67 not (68 or 69) (3492)
71 70 use oemez (2075)
72 33 or 71 (3448)

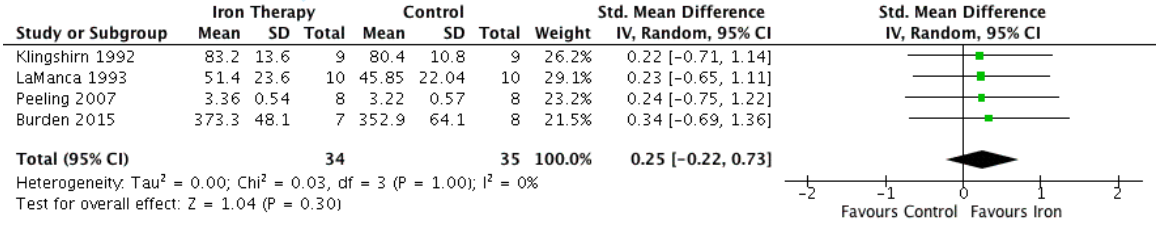
73 remove duplicates from 72 (2408) [TOTAL UNIQUE RECORDS]
74 73 use prmz (1319) [MEDLINE UNIQUE RECORDS]
75 73 use oemez (1089) [EMBASE UNIQUE RECORDS]

Appendix 4. The effect of iron supplementation on measures of physical capacity

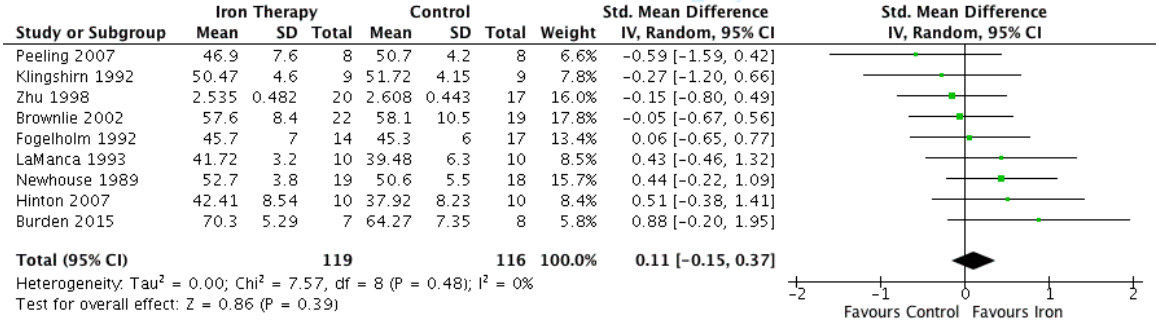
4A. 15 km time trial



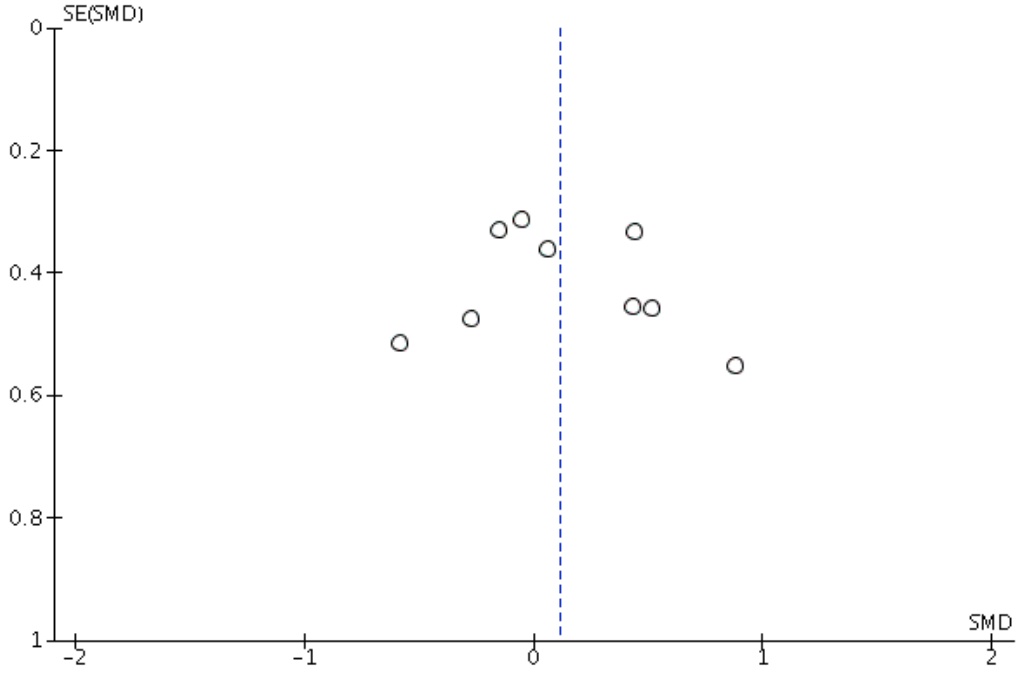
4B. Time to exhaustion



4C. Oxygen consumption (VO_2 max)



Appendix 5. Funnel plot of studies which captured VO_2 max



Appendix 6. Physical capacity tests investigated among the trials reporting measures of physical capacity

Study	Population details	Work capacity tests	Intervention outcomes
Brownlie[19]	Physically active untrained women	VO_2 max RER, HRmax 15km time trial TT RER, TT VO_2max, TT lactates	Significant increases in VO_2 max and decreases in RER in iron treated group compared to placebo; no difference in HRmax Significant reduction in TT in iron group compared to placebo; no differences between TT RER, TT %VO_2 max, TT lactates
Brutsaert[20]	Untrained women	Dynamic knee extension to fatigue	Significant reduction in MVC decline in iron group compared to placebo
Burden[27]	University endurance runners	VO_2 Time to exhaustion RPE	No significant difference in VO_2 max, time to exhaustion or RPE in iron group compared to placebo.
Fogelholm[30]	Female athletes	VO_2 max Lactate levels	No significant difference in VO_2 max and lactate levels between iron and placebo group
Hinton[22]	Recreationally trained individuals	VO_2 max Submaximal test Ventilatory threshold	Significant improvements in gross energetic efficiency and VT among iron groups compared to placebo; no significant difference in VO_2 max between groups
Klingshirn[23]	Female endurance runners	VO_2 max Time to exhaustion Lactate threshold	No significant differences in all measures between iron group and placebo
LaManca[26]	Healthy females	VO_2 max Time to exhaustion RER, HR, lactate	Significant increases in VO_2 max in iron group compared to placebo; no difference in time to exhaustion, RER, HR or lactate.
Newhouse[24]	Young women	VO_2 max Wingate anaerobic test Anaerobic speed test	No significant differences were observed between iron group and placebo

		Ventilatory threshold Muscle enzyme assessments	
Peeling[34]	Well-trained female athletes	VO_2 max Submaximal economy test Time to exhaustion	No significant differences were observed between iron and placebo group
Zhu[25]	Physically active women	VO_2 max 15km time trial TT lactates	No significant differences were observed between iron and placebo group

RER = respiratory exchange ratio; HRmax = maximum heart rate; km = kilometer; TT = time trial; RPE = rated perceived exertion; MVC = maximum ventilator capacity

Appendix 7. Subgroup analysis for fatigue

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Method of iron administration						
Oral	[32,33]	173	161	SMD -0.39 (-0.61, -0.18)	0%	p = 0.82
Intravenous	[28,31]	187	193	SMD -0.36 (-0.56, -0.16)	0%	I^2 = 0%
Duration of study follow-up						
<2 months	[28,33]	215	211	SMD -0.43 (-0.62, -0.23)	0%	p = 0.41
>2 months	[31,32]	145	143	SMD -0.30 (-0.53, -0.07)	0%	I^2 = 0%

Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias subgroup analyses were unevaluable in subgroup analyses as all participants were females, of uncategorized athletic status. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference

Appendix 8. Subgroup analysis for physical capacity

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Population						
Athlete	[22-24,26,27,30,34]	77	80	SMD 0.22 (-0.10, 0.55)	3%	p = 0.25
Non-athlete	[19,25]	42	36	SMD -0.10 (-0.55, 0.35)	0%	I^2 = 24%
Method of iron administration						
Oral	[19,22-26,30]	104	100	SMD 0.12 (-0.16, -0.39)	0%	p = 0.15
IV	[27]	7	8	SMD 0.88 (-0.20, 1.95)	NA	I^2 = 47%
IM	[34]	8	8	SMD -0.59 (-1.59, 0.42)	NA	

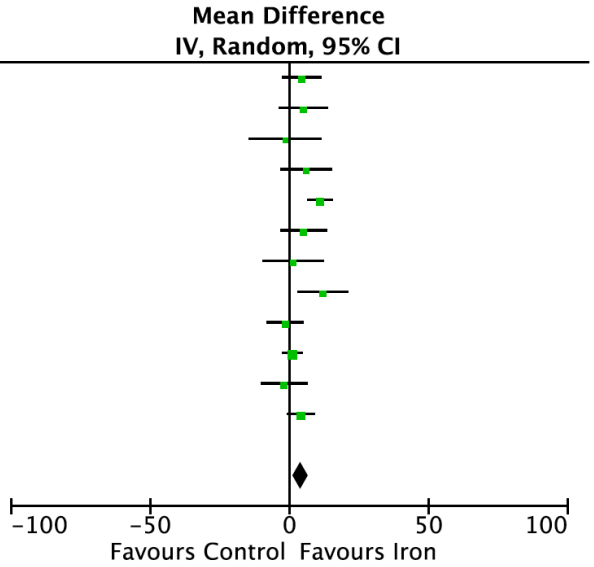
Biologic sex, duration of follow-up and risk of bias subgroup analyses were unevaluable in subgroup analyses as all trials enrolled females and had a follow-up period of less than two months. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference; IV = intravenous; IM = intramuscular

Appendix 9. Effect of iron supplementation on serum hemoglobin

	Iron Therapy			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Brownlie 2002	135.2	9.4	22	130.8	13.1	19	8.7%	4.40 [-2.68, 11.48]
Brutsaert 2003	139	6.3	10	134	12.6	10	6.7%	5.00 [-3.73, 13.73]
Burden 2015	137.2	13.8	7	138.7	11.7	8	3.7%	-1.50 [-14.55, 11.55]
Donangelo 2002	131	7	12	125	14	11	6.3%	6.00 [-3.17, 15.17]
Fogelholm 1992	139	6.7	14	128	5.9	17	12.9%	11.00 [6.51, 15.49]
Hinton 2007	136	10	10	131	9	10	7.1%	5.00 [-3.34, 13.34]
Klingshirn 1992	143.6	12.8	9	142.3	11.1	9	4.8%	1.30 [-9.77, 12.37]
LaManca 1993	141	6.3	10	129	13	10	6.5%	12.00 [3.05, 20.95]
Leonard 2014	130.1	6.1	7	131.6	8.7	13	9.4%	-1.50 [-8.04, 5.04]
Newhouse 1989	135	5	19	134	6	18	14.8%	1.00 [-2.57, 4.57]
Peeling 2007	125	8.5	8	127	8.5	8	7.1%	-2.00 [-10.33, 6.33]
Zhu 1998	136.3	8	20	132.2	7.6	17	11.9%	4.10 [-0.93, 9.13]
Total (95% CI)			**148**			**150**	**100.0%**	**4.01 [1.22, 6.81]**

Heterogeneity: $Tau^2 = 10.46$; $Chi^2 = 21.03$, df = 11 (P = 0.03); $I^2 = 48\%$

Test for overall effect: Z = 2.81 (P = 0.005)

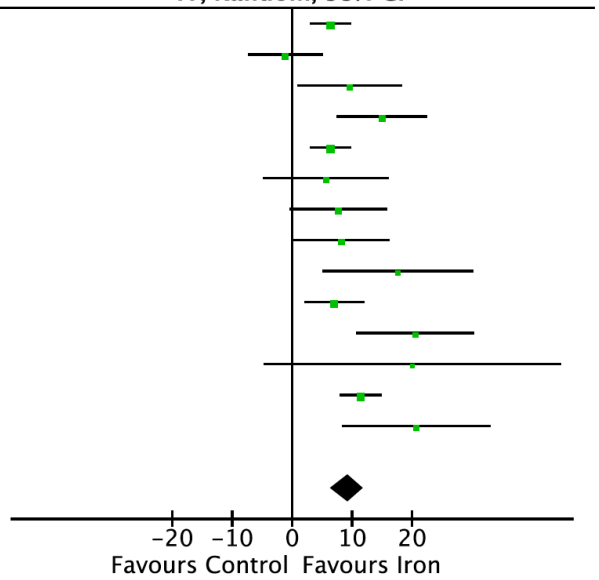


Appendix 10. Effect of iron supplementation on serum ferritin

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Brownlie 2002	14.52	7	22	8.11	3.9	19	12.2%	6.41 [3.00, 9.82]
Brutsaert 2003	15.02	7	10	16.18	7.2	10	8.5%	-1.16 [-7.38, 5.06]
Flink 2006	33	14	24	23.4	15.8	22	6.0%	9.60 [0.94, 18.26]
Fogelholm 1992	26	13.3	14	11	5.9	17	7.1%	15.00 [7.49, 22.51]
Hinton 2000	14.52	7	22	8.11	4	20	12.2%	6.41 [3.00, 9.82]
Hinton 2007	20.82	11.6	10	15.18	12.23	10	4.7%	5.64 [-4.81, 16.09]
Klingshirn 1992	23.44	6.65	9	15.77	10.45	9	6.5%	7.67 [-0.42, 15.76]
LaManca 1993	22.5	10.8	10	14.3	7	10	6.6%	8.20 [0.22, 16.18]
Leonard 2014	32.69	22.1	13	15.1	4	5	3.6%	17.59 [5.08, 30.10]
Moafi 2012	22.39	15.1	36	15.39	1.57	36	10.1%	7.00 [2.04, 11.96]
Newhouse 1989	37.7	19.7	19	17.2	8.9	18	5.2%	20.50 [10.73, 30.27]
Peeling 2007	57	33.9	8	37	11.3	8	1.1%	20.00 [-4.76, 44.76]
Vaucher 2012	11.6	13.7	102	0.2	11	96	12.2%	11.40 [7.95, 14.85]
Zhu 1998	36.9	24	20	16.2	13.5	17	3.7%	20.70 [8.38, 33.02]
Total (95% CI)			**319**			**297**	**100.0%**	**9.23 [6.48, 11.97]**

Heterogeneity: $Tau^2 = 12.99$; $Chi^2 = 30.87$, df = 13 (P = 0.004); $I^2 = 58\%$

Test for overall effect: Z = 6.59 (P < 0.00001)



Appendix 11. Adverse effects in trials of iron supplementation in iron-deficient, non-anemic individuals

Study	Constipation	Diarrhea	Nausea	GI intolerance
Intravenous				
Burden[27]	NR	NR	NR	NR
Favrat[28]	NR	NR	Iron: 8; Control: 2	NR
Krayenbuehl[31]	NR	Iron: 0; Control: 1	Iron: 6; Control: 1	NR
Intramuscular				
Flink[29]	NR	NR	NR	Iron: 14; Control: 2
Oral				
Brownlie[19]	NR	NR	NR	NR
Brutsaert[20]	"Frequency and severity of reported side effects due to supplementation was very low and did not differ significantly between groups"			
Donangelo[21]	NR	NR	NR	NR
Fogelholm[30]	NR	NR	NR	NR
Hinton[22]	NR	NR	NR	NR
Klingshirn[23]	NR	NR	NR	Iron: 1; Control: 0
LaManca[26]	NR	NR	NR	NR
Leonard[18]	Iron: 1; Control: 0	Iron: 2; Control: 2	Iron: 2; Control: 1	NR
Moafi[35]	"When symptoms occurring immediately before or during menses were excluded, there were no significant differences either in frequency or severity of symptoms experienced"			
Newhouse[24]	NR	NR	NR	NR
Peeling[34]	NR	NR	NR	NR
Vaucher[32]	NR	NR	NR	Iron: 12; Control: 10
Verdon[33]	NR	NR	Iron: 0; control: 1	NR
Zhu[25]	NR	NR	NR	NR

NR, not reported; GI, gastrointestinal

Appendix 12. Compliance with the study intervention

	Iron (%)	Control (%)
Intravenous		
Burden[27]	100	100
Favrat[28]	100	100
Krayenbuehl[31]	NR	NR
Intramuscular		
Peeling[34]	NR	NR
Oral		
Brownlie[19]	91	89
Brutsaert[20]	NR	NR
Donangelo[21]	NR	NR
Flink[29]	71	82
Fogelholm[30]	NR	NR
Hinton[22]	98	99
Klingshirn[23]	89	91
LaManca[26]	82	85
Leonard[18*]	89	92
Moafi[35]	89	92
Newhouse[24]	>75	>75
Vaucher[32]	93	94
Verdon[33]	95	98
Zhu[25]	88	87

*weighted averaged between two iron treatment groups; NR = not reported

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp 3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9, Fig 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10, 11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Efficacy of iron supplementation on fatigue and physical capacity in non-anemic iron deficient adults: a systematic review of randomized controlled trials

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Efficacy of iron supplementation on fatigue and physical capacity in non-anemic iron deficient adults: a systematic review of randomized controlled trials

Brett L. Houston[1,2], Daryl Hurrie[3], Jeff Graham[1,2], Brittany Perija[4], Emily Rimmer[1,2], Rasheda Rabbani[5,6], Charles N. Bernstein[7], Alexis Turgeon[8], Dean Fergusson[9], Donald S. Houston[1,2], Ahmed M. Abou-Setta[5,6], Ryan Zarychanski[1,2,5,6]

[1]Department of Internal Medicine, Section of Medical Oncology and Haematology, University of Manitoba, Winnipeg, Manitoba, Canada
[2]Department of Medical Oncology and Haematology, CancerCare Manitoba, Winnipeg, Manitoba, Canada
[3]Applied Health Sciences, Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Manitoba, Canada
[4]Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
[5]George & Fay Yee Center for Healthcare Innovation, University of Manitoba/Winnipeg Regional Health Authority, Winnipeg, Manitoba, Canada
[6]Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[7]Department of Internal Medicine, Section of Gastroenterology, University of Manitoba, Winnipeg, Manitoba, Canada
[8]Centre de recherche du CHU de Québec – Université Laval, Population Health and Optimal Health Practices Research Unit, Trauma - Emergency - Critical Care Medicine; Department of Anesthesiology and Critical Care Medicine, Division of Critical Care Medicine, Faculty of Medicine, Université Laval, Québec City, Québec, Canada.
[9]Clinical Epidemiology Program, Ottawa Hospital Research Institute (OHRI); Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Corresponding author: Ryan Zarychanski
ON 2051-675 McDermot Avenue
CancerCare Manitoba
Winnipeg, Manitoba, R3E OV9
T: 204-787-2108
F: 204-786-0196
rzarychanski@cancercare.mb.ca

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Systematic Review Registration: PROSPERO (CRD42014007085)

OBJECTIVE: Iron supplementation in iron deficiency anemia is standard practice, but the benefits of iron supplementation in iron deficient non-anemic (IDNA) individuals remains controversial. Our objective is to identify the effects of iron therapy on fatigue and physical capacity in iron deficient non-anemic adults.

DESIGN: Systematic review and meta-analysis of randomized controlled trials (RCTs)

SETTING: Primary care

PARTICIPANTS: Adults (≥18 years) who were iron deficient but non-anemic

INTERVENTIONS: Oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included

COMPARATORS: Placebo or active therapy

RESULTS: We identified RCTs in Medline, Embase, CENTRAL, CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016. We searched the World Health Organization's ICTRP for relevant ongoing trials and performed forward searches of included trials and relevant reviews in Web of Science. We assessed internal validity of included trials using the Cochrane Risk of Bias tool, and the external validity using the GRADE methodology. From 11580 citations we included 18 unique trials, and 2 companion papers enrolling 1170 patients. Using a Mantel-Haenszel random-effects model, iron supplementation was associated with reduced self-reported fatigue (standardized mean difference (SMD) -0.38; 95% CI -0.52 to -0.23; I^2 0%; 4 trials; 714 participants), but was not associated with differences in objective measures of physical capacity, including maximal oxygen consumption (VO_2 max) (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%; 9 trials; 235 participants), and timed methods of exercise testing. Iron supplementation significantly increased serum hemoglobin concentration (MD 4.01 g/L;

95% CI 1.22 to 6.81; I^2 = 48%; 12 trials; 298 participants) and serum ferritin (MD 9.23 µmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants).

CONCLUSION: In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in physical capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider consumption of iron-rich foods or iron supplementation to improve symptoms of fatigue in the absence of documented anemia.

SYSTEMATIC REVIEW REGISTRATION: PROSPERO (CRD42014007085)

STUDY STRENGTHS and LIMITATIONS:

Strengths:

- We used a comprehensive search strategy, an *a priori* protocol, and adhered to established methodological (e.g. PRISMA, GRADE) guidelines
- We identified an at-risk patient population, for whom iron deficiency is highly prevalent, but treatment is unknown
- Our outcomes are clinically relevant and patient centered

Limitations:

- Our search was limited to English studies
- In effort to quantify elemental iron administration, we did not include studies evaluating dietary iron fortification

INTRODUCTION

Iron deficiency (ID) is estimated to affect two billion people, and is the leading cause of anemia worldwide[1,2]. Iron is necessary for cellular immune responses, oxidative metabolism within the mitochondria, and production of hemoglobin and myoglobin. When iron losses exceed dietary iron absorption, iron stores become depleted resulting in impaired hemoglobin production and decreased red blood cell hemoglobin content[3]. Reduction in hemoglobin concentration below a threshold (conventionally defined by the World Health Organization (WHO) as 120g/L for females and 130g/L for males) signifies anemia[4].

It is well established that anemia results in decreased physical capacity and increased fatigue proportional to anemia severity[5-9]. Unfortunately, patient-reported

fatigue is common in community and primary care settings with a prevalence ranging from 7 to 45%[10]. It is estimated that the indirect annual economic consequence of chronic fatigue in the United States is 9.1 billion dollars[11].

The clinical relevance of iron deficiency in the absence of anemia is poorly understood, but may impact well-being, perceptions of fatigue, or contribute to decrements in physical performance through impairment in biochemical processes including tissue and mitochondrial oxidative capacity[8]. While iron replacement can normalize hemoglobin concentration, restore work capacity and improve fatigue in iron deficiency anemia, it is unclear if supplementation affects fatigue and physical capacity in iron deficient but non-anemic (IDNA) individuals. In the absence of compelling efficacy data on well-being or muscle function, the use of iron supplements are common in the general population and are routinely recommended to high performance athletes to enhance performance.

Given the global prevalence of iron deficiency and impact of fatigue, the purpose of this systematic review is to identify, critically appraise and meta-analyse data from prospective randomized trials evaluating iron therapy in adults with IDNA.

METHODS

Using an *a priori* published protocol (CRD42014007085; available at https://www.crd.york.ac.uk/PROSPERO/)[12], we conducted a systematic review using methodological approaches outlined in the *Cochrane Handbook for Systematic Reviewers* and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria[12-14]. A panel of experts from multiple fields (e.g.

internal medicine, hematology, kinesiology, gastroenterology, research methodology) formulated the research question, reviewed search strategies and methods, and provided input throughout the review process.

Populations, Interventions, Comparators, Outcome Measures, Setting and Study Designs

Our research question was "In iron depleted but non-anemic adults, does iron supplementation improve fatigue and physical capacity." We included randomized controlled trials of adults (≥18 years) who were iron deficient but non-anemic (Appendix 1). Interventions included oral, intramuscular or intravenous iron supplementation; all therapy doses, frequencies and durations were included. We included trials that evaluated outcomes at least 28 days from the initiation of iron therapy. Comparators included placebo or active therapy. Our exclusion criteria are presented in Appendix 2.

Our primary outcome measures were self-reported fatigue and objective measures of physical capacity. Secondary outcomes included the incidence of anemia, change in hemoglobin concentration and serum ferritin, and the incidence of adverse outcomes including iron toxicity, constipation, diarrhea, gastrointestinal intolerance and nausea.

Search Strategy for Identification of Studies

We searched Medline, Embase, CENTRAL (Cochrane Library), CINAHL, SportDiscus, and CAB Abstracts from inception to October 31, 2016 to identify relevant citations of published trials, using individualized systematic search strategies for each database. The MEDLINE strategy is presented in Appendix 3. We searched the World Health Organization's (WHO) International Clinical Trials Registry Platform (ICTRP),

clinicaltrials.gov and relevant conference proceedings to identify planned, ongoing, or recently completed but unpublished trials. We performed forward searches of included trials and relevant reviews in Web of Science to identify additional citations, and contacted study authors to request pertinent unpublished data or provide clarifications on study methods or results. Reference lists of narrative and systematic reviews and of the included trials were searched for additional citations. We performed reference management in EndNote™ (Version X7, Thomson Reuters, Philadelphia, PA, USA).

Study Selection, Data Extraction and Quality Assessment

We screened citations, selected studies and extracted data from included trials using standardized and piloted screening and data extraction forms. Citation screening, study selection and data extraction were performed in duplicate. The following data were extracted from each trial: author identification, publication year, publication language, trial location, source of trial funding, participant characteristics (age, sex, weight), intervention/comparator (drug utilized, dose (elemental iron), route of administration, duration), as well as results for the primary and secondary outcomes. We assessed the internal validity of included trials using the Cochrane Collaboration Risk of Bias tool[13]. Discrepancies between the two reviewers were resolved by consensus or by a third reviewer (RZ), as required. Data extraction and descriptive statistics were performed using Microsoft Excel 2016 (Excel version 15, Microsoft Corp).

Data Analysis

Data analysis was performed using Review Manager (RevMan v5.3.5, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). Study level comparisons of dichotomous data were presented as risk ratios (RR) with 95% confidence intervals (CI). Pooled continuous data were expressed as the mean difference (MD), or standardized mean difference (SMD). Change scores or post-treatment means were extracted to inform summary estimates for continuous data. Pooled risk ratios and 95% confidence intervals were calculated using Mantel-Haenszel random-effects model. Pooled MDs or SMDs were calculated using a random-effects model. For the primary outcome of fatigue, if multiple scales were reported, fatigue-specific scores were preferred over general scores and the most commonly reported and clinically meaningful score was used to generate summary effect measures. In studies evaluating exercise capacity, weight-based VO_2 max values were utilized preferentially if both absolute and weight-based VO_2 max results were provided. Statistical heterogeneity was quantified using the I^2 statistic[15]. For the primary outcomes of fatigue and work capacity, we evaluated potential publication bias using funnel plot analysis[16]. All tests of statistical inference reflect a 2-sided α of 0.05.

Subgroup Analyses

We performed subgroup analyses for fatigue and exercise capacity outcomes according to biologic sex, athletic status (athlete or non-athlete), method of iron administration, duration of therapy, duration of study follow up, and risk of bias.

Grading the Evidence

We graded the strength of evidence for our primary outcomes using the GRADE methodology. This approach classifies the strength of evidence as "*high", "moderate", "low"* or *"very low."*

RESULTS

Trial Characteristics & Study Populations

Of the 11,580 citations identified, we included 18 unique trials and two companion papers[17,18], enrolling 1170 subjects (Figure 1; Table 1). Trials were published between 1989 and 2015, and all trials were published in peer-reviewed journals. Eight trials[19-26] were from North America, seven trials[27-33] were from Europe, two trials[18,34] were from Australia, and one trial[35] was from Asia.

Exclusively healthy females (aged 17 to 55 years old) with varying levels of fitness (sedentary to well-trained) were enrolled in all but three studies[22,27,29]. The WHO cutoff for anemia [hemoglobin concentration ≥130 g/L (males) and ≥120 g/L (females)] was used by 9 studies[19,22-25,30-32,35], whereas 7 studies used lower values ranging from ≥110 to <120 g/l[20,21,27,28,33,34,36], and baseline hemoglobin concentration was not provided in 2 trial reports[26,29].

All trials were placebo-controlled. In 13 of 18 trials (72%), we considered the blinding of participants and personnel to be adequate. Likewise, 10 trials (55%) adequately incorporated blinded outcome assessment. One trial[29] was considered to have a low risk of bias (Table 2). The remainder of the trials were considered unclear risk of bias, due to unclear processes of randomization (12 trials[19-27,30,33,34]) or allocation concealment (13 trials[19-27,30,31,34,35]).

Table 1. Characteristics of individual trials, patient populations and interventions

Trial #	Source	Population	No. of patients (iron)	No. of patients (control)	Control	Age (range)	Min Hb (g/L)	Max ferritin (ug/L)	Iron type	Daily iron dose (mg)	Iron route	Iron duration (days)	Follow-up (days)
1	Brownlie[17,19]	Physically active untrained women	22	19	Placebo	18-33	120	16	Ferrous sulfate	16	PO	42	42
2	Brutsaert[20]	Untrained women	10	10	Placebo	18-45	110	20	Ferrous sulfate	18.1	PO	42	42
3	Burden[27]	University endurance runners	7	8	Saline		120	30 (F); 40 (M)	Ferric carboxymaltose	500	IV	1	28
4	Donangelo[21]	Young women	12	11	Zinc gluconate	20-28	110	20	Ferrous sulfate	100	PO	56	70
5	Favrat[28]	Premenopausal women with fatigue	144	146	Saline		115	15	Ferric carboxymaltose	1000	IV	1	56
6	Flink[29]	Individuals with low unstimulated salivary flow	25	21	Placebo	15-46		30 (F); 50 (M)	Ferrous fumarate	120	PO	90	90
7	Fogelholm[30]	Female athletes	17	14	Placebo	17-31	120	25	Ferrous sulfate	100	PO	56	56
8	Hinton[22]	Recreationally trained individuals	9	8	Placebo	18-41	120 (F); 130 (M)	16	Ferrous sulfate	30	PO	42	42
9	Klingshirn[23]	Female endurance runners	9	9	Placebo	22-39	120	20	Ferrous sulfate	100	PO	56	56
10	Krayenbuehl[31]	Premenopausal women with fatigue	43	47	Saline		120	50	Venofer	200	IV	4	84
11	LaManca[26]	Healthy females	28	28	Placebo			20	Ferrous sulfate	100	PO	56	56
12	Leonard[18,36]*	Young women	16*	8	Placebo	18-35	115	20	Ferrous sulfate	60/80	PO	112	112
13	Moafi[35]	Female students	36	36	Placebo	18-35	120	20	Ferrous sulfate	50	PO	42	42
14	Newhouse[24]	Young women	19	21	Placebo	18-40	120	20	Ferrous sulfate	200	PO	56	56
15	Peeling[34]	Well-trained female athletes	8	8	Saline		115	35	Ferrum H	100	IM	5	28
16	Vaucher[32]	Women with fatigue from clinic	102	96	Placebo	18-50	120	50	Ferrous sulfate	80	PO	84	84
17	Verdon[33]	Women with fatigue from clinic	71	65	Placebo	18-55	117		Ferrous sulfate	80	PO	28	28
18	Zhu[25]	Physically active women	20	17	Placebo	19-36	120	16	Ferrous sulfate	135.3	PO	56	56
TOTAL:			**598**	**572**									

*Trial included two intervention arms, with 8 patients enrolled in each arm; represents weighted averages between two iron treatment groups; Max = maximum; Min = minimum; Hb = hemoglobin; F = females; M = males; PO = oral; IM = intramuscular; IV = intravenous

Table 2. Cochrane Risk of Bias summary. Green (+) = low risk of bias; Yellow (?) = unclear risk of bias

	OVERALL	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Brownlie[17,19]	?	?	?	+	?	?	+	+
Brutsaert[20]	?	?	?	+	?	?	+	+
Burden[27]	?	?	?	+	+	+	+	+
Donangelo[21]	?	?	?	?	?	+	+	+
Favrat[28]	?	+	+	?	?	?	+	+
Flink[29]	+	+	+	+	+	+	+	+
Fogelholm[30]	?	?	?	+	+	+	+	+
Hinton[22]	?	?	?	?	?	+	+	+
Klingshirn[23]	?	?	?	+	?	+	+	+
Krayenbuehl[31]	?	+	?	+	+	+	?	+
LaManca[26]	?	?	?	?	?	+	+	+
Leonard[18,36]	?	+	+	+	+	?	+	+
Moafi[35]	?	+	?	+	+	+	+	+
Newhouse[24]	?	?	?	+	+	?	+	+
Peeling[34]	?	?	?	?	?	+	+	+
Vaucher[32]	?	+	+	+	+	?	+	+
Verdon[33]	?	?	+	+	+	+	+	+
Zhu[25]	?	?	?	+	+	+	+	+

Interventions

Iron interventions consisted of iron supplementation administered orally, intramuscularly or intravenously. Of the trials evaluating oral supplements, all but one[29] used ferrous sulfate (13 trials[19-26,30,32,33,35,36], 713 participants). Intravenous iron was administered in three trials[27,28,31] (395 participants), and intramuscular iron in one trial[34] (16 participants). In trials using oral iron[19-26,29,30,32,33,35,36], the mean daily elemental iron dose was 86.9mg (± 49.1mg; range: 16 to 200mg). In trials reporting intravenous iron[27,28,31], the mean daily elemental iron dose was 566mg (± 330mg; range 200 to 1000mg) and mean total elemental iron dose 767mg (± 206mg; range 500 to 1000mg). Among all studies, the mean duration of iron therapy was 46 days (± 30 days; range 1 to 112 days), and mean duration of follow-up was 57 days (± 24 days; range 28 to 112 days).

Primary Outcomes

Fatigue

Four trials[28,31-33] enrolling 714 participants were eligible for meta-analysis. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS),[28] the Current and Past Psychological State scale (CAPPS),[32] visual analog scale[33] or Brief Fatigue Inventory questionnaire (BFI)[31] (SMD -0.38; 95% CI -0.52 to -0.23; I^2 0%) (Figure 2). In one trial using the BFI score, fatigue was not significantly different between groups after 12 weeks, although it was improved in the subgroup of participants with the lowest iron stores (ferritin ≤ 15 ng/ml or transferrin saturation ≤ 20%)[31]. Evaluation of publication bias was not possible due to the low number of included trials. Given that the majority of trials were of unclear risk of bias, we graded the overall strength of evidence as moderate.

Physical Capacity

Physical capacity was reported in 10 trials[19,20,22-27,30,34] (291 participants); all but one[20] of the trials employed at least one of three common aerobic tests of physical capacity: time trial[22,25], time to exhaustion[23,26,27,34], or VO_2 max[19,22-27,30,34] performance from a graded exercise test. In two trials (79 participants) that used 15 km time trials[22,25], iron supplementation was not associated with improved exercise capacity (SMD -0.09; 95% CI -0.53 to 0.35; I^2 0%) (Figure 3A). In four trials[23,26,27,34] (69 participants) that used time-to-exhaustion tests, iron supplementation did not significantly improve exercise capacity (SMD 0.25; 95% CI -0.22 to 0.73; I^2 0%) (Figure 3B). Nine trials[19,22-27,30,34] (235

participants) reported VO_2 max as a surrogate measure of physical capacity. Iron supplementation did not increase VO_2 max (SMD 0.11; 95% CI -0.15 to 0.37; I^2 0%) (Figure 3C). We found no evidence of funnel plot asymmetry to suggest publication bias for this outcome (Appendix 4). The overall strength of the evidence for time trial, time to exhaustion and VO_2 max outcomes were low, given the imprecision of effect estimates and that the majority of trials were of unclear risk of bias.

One trial[20] (20 participants) used dynamic knee extension exercise to evaluate changes in physical capacity. In this trial, the decline in maximum voluntary contraction after 6 minutes of exercise was significantly less in participants randomized to receive iron. Among 16 other unique measures of physical capacity, 19% (3 of 16) found statistically significant increases in measures of physical capacity with iron supplementation (Appendix 5).

Subgroup analysis

Subgroup analyses based on method of iron administration and duration of follow-up demonstrated no statistically significant differences in subjective fatigue (Appendix 6). Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias could not be evaluated as all trials contributing data to the meta-analyses enrolled females of uncharacterized athletic status, and were of unclear risk of bias[28,31-33]. Subgroup analyses evaluating athletic status and method of iron administration demonstrated no statistically significant differences in objective physical capacity (Appendix 7). Biologic sex, duration of follow-up and risk of bias were unevaluable as all trials enrolled females with follow-up of less than 2 months, and all were of unclear risk of bias[19,22-27,30,34].

Secondary Outcomes and Adverse Events

Despite the absence of baseline anemia, iron supplementation significantly increased serum hemoglobin concentration (MD 4.01 g/L; 95% CI 1.22 to 6.81; I^2 = 48%; 12 trials; 298 participants)[18-27,30,34] (Appendix 8). In two trials[25,28] reporting incident anemia, a new diagnosis of anemia at trial completion was less common in patients randomized to receive iron supplementation. Iron supplementation also significantly increased serum ferritin (MD 9.23 μmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants) (Appendix 9).

Adverse events were sparsely reported. Gastrointestinal intolerance was reported in three trials[23,29,32], and was significantly increased in one trial[29] using intramuscular iron administration, but not in the two trials[23,32] that used oral administration. Nausea was reported in four trials[18,28,31,33]; two trials[28,31] using intravenous administration of iron reported significantly increased nausea, whereas nausea was not increased in patients who received iron by oral administration[18,33]. Constipation was reported in one trial[18], and diarrhea in two trials[18,31] (Appendix 10). Adherence with the study intervention was reported in 13 trials[18,19,22-29,32,33,35]. Iron supplementation was not associated with differential rates of medication adherence (RR 1.0; CI 95% 0.99 to 1.01; I^2 0%; 12 trials; 958 participants). The route of administration of the study intervention was also not associated with differences in adherence (Appendix 11).

DISCUSSION

In iron deficient but non-anemic adults, we found iron supplementation was associated with reduced subjective measures of fatigue but had no significant impact on objective physical capacity. Given iron deficiency is the most prevalent micronutrient deficiency worldwide[2], there is a discrepant lack of robust evidence evaluating iron supplementation in the absence of anemia across important patient populations. Despite rigorous and systematic methodology, we were only able to identify 18 trials enrolling 1170 adults, representing a minute fraction of affected individuals.

While treatment of iron deficiency in the absence of anemia is associated with reduced subjective fatigue, whether this translates to clinically meaningful outcomes, including quality of life, work absenteeism, job or athletic performance is uncertain. Contrary to iron deficiency with established anemia, lack of robust data in iron deficient but non-anemic individuals is reflected in the under-representation of guideline recommendations pertaining to this larger population. The proportion of iron deficient, non-anemic individuals who receive supplementation is further unknown.

Our systemic review builds on the results of three published evidence syntheses evaluating iron supplementation[37-39]. In a systematic review of healthy menstruating women, iron supplementation, irrespective of iron status or anemia, improved hemoglobin and measures of iron stores[37]. Two systematic reviews included studies of pregnant women, blood donors and children, and included data from both randomized and non-randomized trials[38,39]. These studies concluded benefit of iron supplementation, although in the review by *Yokoi et al*, the benefit was limited to randomized controlled trials. Despite the high prevalence of iron deficiency, significant heterogeneity in patient

populations and study designs, and absence of data pertaining to objective muscle performance limits the generalizability of these findings.

In trials where a proportion of participants were anemic at enrollment, and with the knowledge that anemia results in decreased physical capacity, iron supplementation has previously been associated with improved maximal and submaximal exercise performance[5-8]. We found insufficient evidence to suggest that iron supplementation improves exercise capacity in iron-depleted non-anemic adults, differing from the results of physiologic experiments that describe VO_2 max improvements with iron supplementation, independent of hemoglobin[40]. These findings were postulated to be secondary to iron-mediated improvements in muscle oxidative capacity and improved mitochondrial function, the validity of which is unclear[40].

A potential weakness our systematic review is the difficulty masking oral iron due to predictable gastrointestinal side effects and changes in stool color, and the impact of imperfect blinding on subjective measures of fatigue. However, despite this, fatigue was consistently reduced in trials evaluating both oral (n = 2) and intravenous (n = 2) iron preparations. Healthy females comprised the study population in 15 of 18 included trials; subjective measures of fatigue may not consistently apply to other at-risk populations. The duration of follow was relatively short (57 days; range 28-112 days) and perhaps too brief to expect significant changes in muscle metabolism or function. Finally, the lack of systematic reporting of adverse events impairs our ability to draw conclusions regarding the incidence of these events and tolerability of iron therapy.

The strengths of this review include the comprehensiveness of the search strategy, which included electronic databases, trial registries, and forward searches. We used an *a*

priori published protocol and followed established methodological guidelines concerning the conduct and reporting of this review. We synthesized patient-centered outcomes and evaluated efficacy in the context of relevant safety outcomes and adverse events. In contrast to the systematic review of *Low et al*, we excluded studies that enrolled patients with anemia at baseline[37]. While cut-offs for anemia varied slightly among included trials, this important inclusion criteria reduces (but may not eliminate) the probability that changes in fatigue or muscle function are due to correction of anemia or independent of oxygen carrying capacity reflecting increased red cell mass. While the duration of follow up in most studies was modest, the mean daily elemental iron dose (86.9 ± 49.1mg) reflects a recommended 'treatment' for patients with iron deficiency anemia[41].

In iron deficient non-anemic adults, iron supplementation is associated with reduced subjective measures of fatigue, but not with objective improvements in physical capacity. Given the global prevalence of both iron deficiency and fatigue, patients and practitioners could consider consumption of iron-rich foods or iron supplementation to improve symptoms of fatigue in the absence of documented anemia.

Figure Legends:

Figure 1. Study flow diagram following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA)[14] with modifications. Of the 11,580 citations identified, we included 18 unique trials and two companion papers.

Figure 2. The effect of iron supplementation on patient-reported fatigue, using validated fatigue scores. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS), the Current and Past Psychological State scale (CAPPS), visual analog scale or Brief Fatigue Inventory questionnaire (BFI).

Figure 3. The effect of iron supplementation on measures of physical capacity. Iron supplementation was not associated with a reduction in objective measures of physical capacity when assessed by either maximal oxygen consumption (VO_2 max) and timed methods of exercise testing.

Contribution:

Two researchers (BH and DH) lead and coordinated all aspects of the review, including but not limited to preparation of the literature search, screening relevant material, data analysis and extraction, interpretation of the results of the meta-analytic procedures, bias investigation, and preparation of the final report; three second reviewers (JG, ER, BP) conducted independent screening of relevant material, extracted and analyzed data and aided in report preparation; one hematologist/ intensivist (RZ), methodologist (DAF), and anesthetist/intensivist (AFT) with expertise in systematic reviews provided content expertise and methodological input, and resolved disagreement among reviewers; one systematic review expert (AMAS) provided methodological input; two hematologists (DSH and ER), one gastroenterologist (CB) and one statistician (RR) provided content expertise. All authors were involved in the process of study design and manuscript review.

Competing interests: the authors declare no competing financial interests.

Funding: funding was not obtained for completion of this study.

Data sharing: We are submitting (in our manuscript and supplementary files) all planned data analyses.

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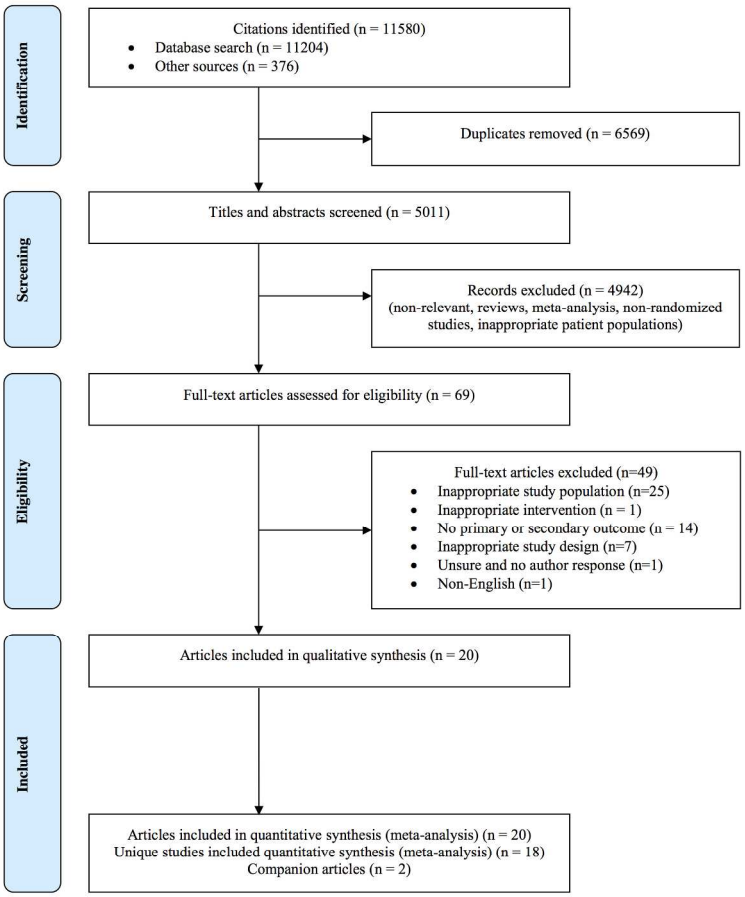

Figure 1. Study flow diagram following the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA)14 with modifications. Of the 11,580 citations identified, we included 18 unique trials and two companion papers.

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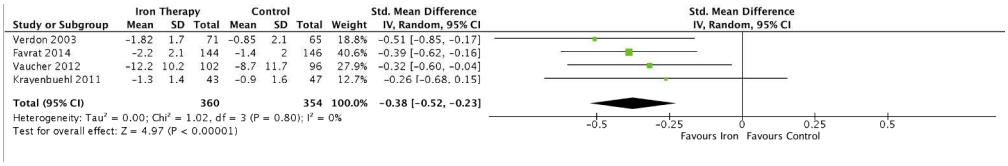

Figure 2. The effect of iron supplementation on patient-reported fatigue, using validated fatigue scores. Iron supplementation was associated with a reduction in subjective measures of fatigue when assessed by either the Piper Fatigue Scale (PFS), the Current and Past Psychological State scale (CAPPS), visual analog scale or Brief Fatigue Inventory questionnaire (BFI).

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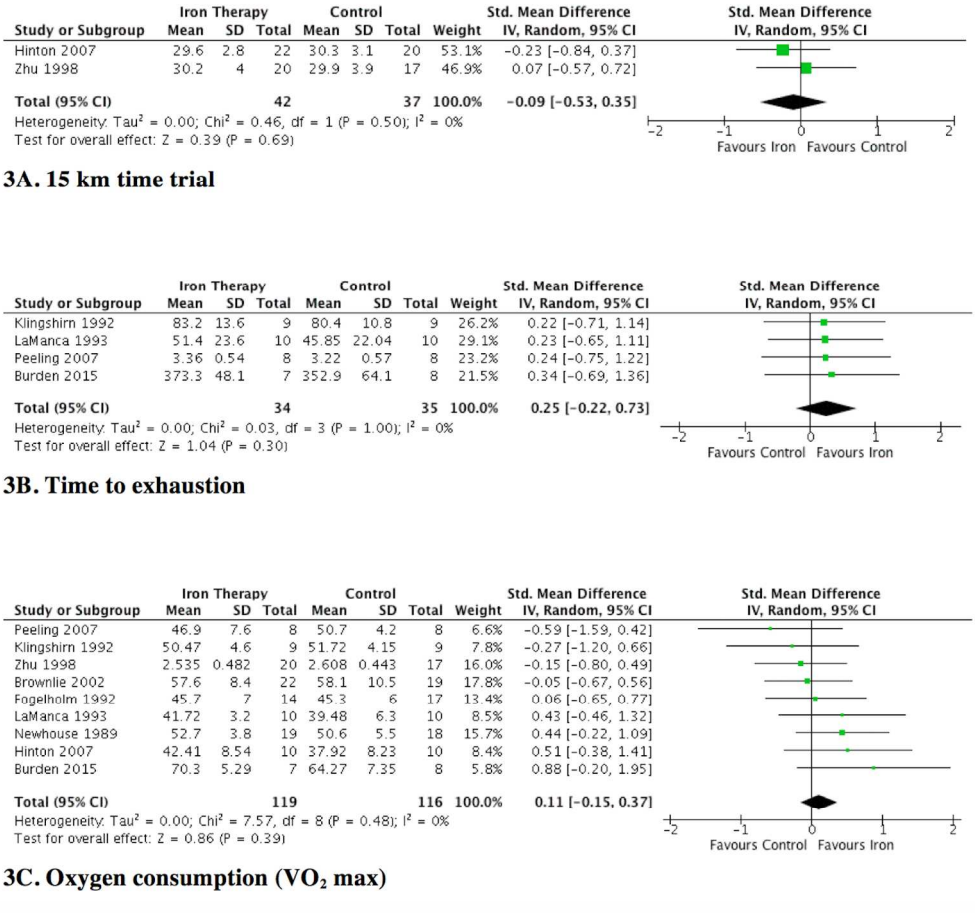

Figure 3. The effect of iron supplementation on measures of physical capacity. Iron supplementation was not associated with a reduction in objective measures of physical capacity when assessed by either maximal oxygen consumption (VO2 max) and timed methods of exercise testing.

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Appendix 1. Inclusion criteria

1. Non-anemic (≥80%): Indicated by study cutoff values, or hemoglobin concentration [Hb] ≥130 g/L (males), ≥120 g/L (females);
2. Adults (≥18 yrs); (≥80%)
3. Iron Depleted (≥80%): According to study specific definition
4. Iron therapy administered as oral / intramuscular / intravenous therapy, all therapy durations, doses and frequencies of administration will be included
5. Studies where outcomes are assessed ≥ 28 days from the initiation of oral iron therapy
6. Only prospective randomized trials will be considered.

Appendix 2. Exclusion criteria

1. Studies involving animals;
2. Females who were pregnant or breastfeeding;
3. Individuals with fatigue (≥20%) identified as being the result of some other pathology (i.e. psychiatric diagnosis, thyroid, liver, rheumatic, renal, cardiovascular, pulmonary, or oncologic cause)
4. Studies involving surgical patients
5. Studies involving author identified blood donors or phlebotomy
6. Studies assessing the pharmacokinetic properties of iron compounds in healthy volunteers where the short term outcomes are expressed as the objective (<1 month)
7. Non-English studies
8. Observational study designs, quasi-randomized, cross-over, or cluster randomized trials will not be considered for this review.
9. Studies where no relevant primary or secondary outcomes of interest are reported
10. Dietary fortification studies

Appendix 3. Search strategy

Ovid Multifile (MEDLINE & Embase)

Database: Embase <1974 to 2015 Week 47>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

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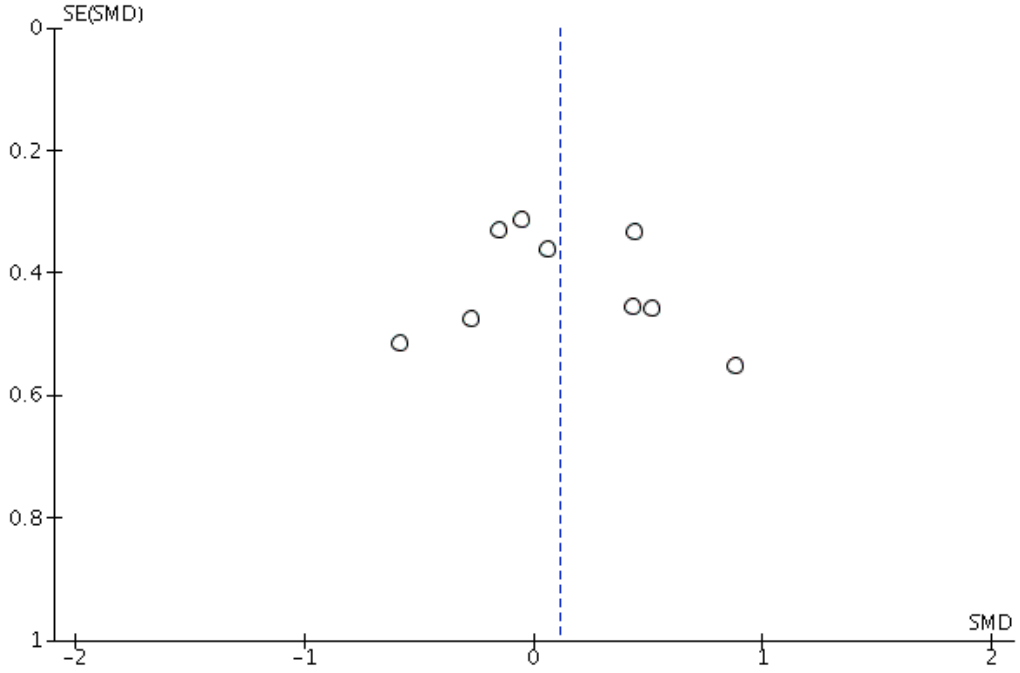
1 Iron/df (4519)
2 exp Ferritins/df (87)
3 exp Ferrous Compounds/df (1)
4 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
5 or/1-4 (57094)
6 Anemia/pc [Prevention & Control] (3144)
7 Anemia, Iron-Deficiency/pc [Prevention & Control] (2061)
8 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
9 Deficiency Diseases/dt (968)
10 Iron/ad, tu (8860)
11 exp Ferritins/ad, tu (185)
12 exp Ferrous Compounds/ad, tu (2094)
13 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
14 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
15 Iron/ and Dietary Supplements/ (4252)
16 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
17 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
18 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
19 or/6-18 (55320)
20 5 and 19 (17537)
21 (controlled clinical trial or randomized controlled trial).pt. (504747)
22 clinical trials as topic.sh. (180086)
23 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
24 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
25 trial.ti. (344352)
26 or/21-25 (2079234)
27 20 and 26 (2880)
28 exp Animals/ not (exp Animals/ and Humans/) (9776432)
29 27 not 28 (2641)
30 (comment or editorial or interview or news).pt. (1640361)
31 (letter not (letter and randomized controlled trial)).pt. (1871051)
32 29 not (30 or 31) (2636)

33 32 use prmz (1373)
34 iron deficiency/ (11754)
35 iron deficiency anemia/ (28987)
36 ((decreased or deficien* or deplet* or inadequa* or insufficien* or low or marginal) adj3 (iron or ferritin*)).tw,kw. (55943)
37 or/34-36 (71135)
38 anemia/pc [Prevention] (3144)
39 iron deficiency anemia/pc [Prevention] (2269)
40 (prevent* adj3 (anemi* or anaemi* or iron deficien*)).tw,kw. (2915)
41 iron deficiency/dt [Drug Therapy] (1631)
42 iron therapy/ (5814)
43 iron/ad, dt, th [Drug Administration, Drug Therapy, Therapy] (13258)
44 ferritin/ad, dt [Drug Administration, Drug Therapy] (211)
45 ferrous ion/ad, dt [Drug Administration, Drug Therapy] (413)
46 ((ferric or ferritin* or ferrous or iron) adj3 (administer* or administration* or dietary or replac* or supplement* or therap* or treatment*)).tw,kw. (33100)
47 (iron adj3 (capsule* or infusion* or injection* or intravenous* or IV or parenteral* or pill or pills or medication* or tablet*)).tw,kw. (10147)
48 iron/ and diet supplementation/ (3538)
49 ferrous sulfate/ (6637)
50 (ferrous sulfate or ferrous sulphate or aktiferrin or apo-ferrous sulfate or auryxia or bifera or biofer or ceferro or conferon or eisendragees-ratiopharm or eisensulfat stada or elite iron or femiron or feosol or feospan or fer-gen-sol or fer-in-sol or feratab or fergon or ferodan or fero-gradumet or ferro-gradumet or ferogradumet or ferrex 150 or ferrogamma or ferrograd or haemoprotect or hemobion or hemofer or hamatopan or haematopan or kendural or "Mol-Iron" or plastufer or "Slow-Fe" or vitaferro kapseln).tw,kw. (4979)
51 ferrous fumarate/ (831)
52 (ferrous fumarate or feostat or ferrocap or fersaday or fersamal or ferval or fumar or galfer or ircon or nephro-fer or palafer or rulofer).tw,kw. (510)
53 ferrous gluconate/ (1490)
54 (ferrous gluconate or apo-ferrous gluconate or dextriferron or eisen-sandoz or "FeG Iron" or ferroglucon or ferrogluconaat or ferrum verla or loesferron or losferron or simron or vitaferro brause).tw,kw. (226)
55 or/38-54 (62415)
56 37 and 55 (22461)
57 randomized controlled trial/ or controlled clinical trial/ (1035658)
58 exp "clinical trial (topic)"/ (172053)
59 (randomi#ed or randomly or RCT$1 or placebo*).tw. (1644668)
60 ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).tw. (324774)
61 trial.ti. (344352)
62 or/57-61 (2260510)
63 56 and 62 (3652)
64 exp animal experimentation/ or exp models animal/ or exp animal experiment/ or nonhuman/ or exp vertebrate/ (40209729)
65 exp humans/ or exp human experimentation/ or exp human experiment/ (31154163)
66 64 not 65 (9057215)
67 63 not 66 (3517)
68 editorial.pt. (898431)
69 letter.pt. not (letter.pt. and randomized controlled trial/) (1866594)
70 67 not (68 or 69) (3492)
71 70 use oemez (2075)

72 33 or 71 (3448)
73 remove duplicates from 72 (2408) [TOTAL UNIQUE RECORDS]
74 73 use prmz (1319) [MEDLINE UNIQUE RECORDS]
75 73 use oemez (1089) [EMBASE UNIQUE RECORDS]

Appendix 4. Funnel plot of studies which captured VO_2 max



Appendix 5. Physical capacity tests investigated among the trials reporting measures of physical capacity

Study	Population details	Work capacity tests	Intervention outcomes
Brownlie[19]	Physically active untrained women	VO_2 max RER, HRmax 15km time trial TT RER, TT VO_2max, TT lactates	Significant increases in VO_2 max and decreases in RER in iron treated group compared to placebo; no difference in HRmax Significant reduction in TT in iron group compared to placebo; no differences between TT RER, TT %VO_2 max, TT lactates
Brutsaert[20]	Untrained women	Dynamic knee extension to fatigue	Significant reduction in MVC decline in iron group compared to placebo
Burden[27]	University endurance runners	VO_2 Time to exhaustion RPE	No significant difference in VO_2 max, time to exhaustion or RPE in iron group compared to placebo.
Fogelholm[30]	Female athletes	VO_2 max Lactate levels	No significant difference in VO_2 max and lactate levels between iron and placebo group
Hinton[22]	Recreationally trained individuals	VO_2 max Submaximal test Ventilatory threshold	Significant improvements in gross energetic efficiency and VT among iron groups compared to placebo; no significant difference in VO_2 max between groups
Klingshirn[23]	Female endurance runners	VO_2 max Time to exhaustion Lactate threshold	No significant differences in all measures between iron group and placebo
LaManca[26]	Healthy females	VO_2 max Time to exhaustion RER, HR, lactate	Significant increases in VO_2 max in iron group compared to placebo; no difference in time to exhaustion, RER, HR or lactate.
Newhouse[24]	Young women	VO_2 max Wingate anaerobic test Anaerobic speed test	No significant differences were observed between iron group and placebo

		Ventilatory threshold Muscle enzyme assessments	
Peeling[34]	Well-trained female athletes	VO_2 max Submaximal economy test Time to exhaustion	No significant differences were observed between iron and placebo group
Zhu[25]	Physically active women	VO_2 max 15km time trial TT lactates	No significant differences were observed between iron and placebo group

RER = respiratory exchange ratio; HRmax = maximum heart rate; km = kilometer; TT = time trial; RPE = rated perceived exertion; MVC = maximum ventilator capacity

Appendix 6. Subgroup analysis for fatigue

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Method of iron administration						
Oral	[32,33]	173	161	SMD -0.39 (-0.61, -0.18)	0%	p = 0.82
Intravenous	[28,31]	187	193	SMD -0.36 (-0.56, -0.16)	0%	I^2 = 0%
Duration of study follow-up						
<2 months	[28,33]	215	211	SMD -0.43 (-0.62, -0.23)	0%	p = 0.41
>2 months	[31,32]	145	143	SMD -0.30 (-0.53, -0.07)	0%	I^2 = 0%

Biologic sex, athletic status (athlete vs. non-athlete) and risk of bias subgroup analyses were unevaluable in subgroup analyses as all participants were females, of uncategorized athletic status. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference

Appendix 7. Subgroup analysis for physical capacity

Subgroup	Studies	Participants (iron)	Participants (control)	Effect estimate (95% CI)	I^2 Value	Across Subgroups
Population						
Athlete	[22-24,26,27,30,34]	77	80	SMD 0.22 (-0.10, 0.55)	3%	p = 0.25 I^2 = 24%
Non-athlete	[19,25]	42	36	SMD -0.10 (-0.55, 0.35)	0%	
Method of iron administration						
Oral	[19,22-26,30]	104	100	SMD 0.12 (-0.16, -0.39)	0%	p = 0.15 I^2 = 47%
IV	[27]	7	8	SMD 0.88 (-0.20, 1.95)	NA	
IM	[34]	8	8	SMD -0.59 (-1.59, 0.42)	NA	

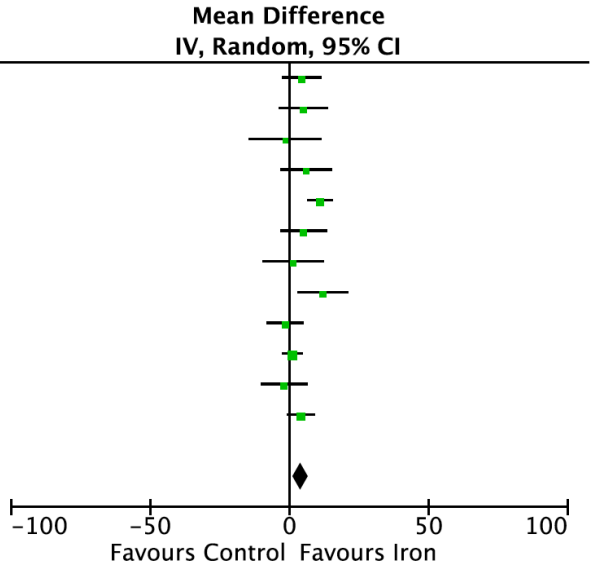
Biologic sex, duration of follow-up and risk of bias subgroup analyses were unevaluable in subgroup analyses as all trials enrolled females and had a follow-up period of less than two months. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference; IV = intravenous; IM = intramuscular

Appendix 8. Effect of iron supplementation on serum hemoglobin

	Iron Therapy			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Brownlie 2002	135.2	9.4	22	130.8	13.1	19	8.7%	4.40 [-2.68, 11.48]
Brutsaert 2003	139	6.3	10	134	12.6	10	6.7%	5.00 [-3.73, 13.73]
Burden 2015	137.2	13.8	7	138.7	11.7	8	3.7%	-1.50 [-14.55, 11.55]
Donangelo 2002	131	7	12	125	14	11	6.3%	6.00 [-3.17, 15.17]
Fogelholm 1992	139	6.7	14	128	5.9	17	12.9%	11.00 [6.51, 15.49]
Hinton 2007	136	10	10	131	9	10	7.1%	5.00 [-3.34, 13.34]
Klingshirn 1992	143.6	12.8	9	142.3	11.1	9	4.8%	1.30 [-9.77, 12.37]
LaManca 1993	141	6.3	10	129	13	10	6.5%	12.00 [3.05, 20.95]
Leonard 2014	130.1	6.1	7	131.6	8.7	13	9.4%	-1.50 [-8.04, 5.04]
Newhouse 1989	135	5	19	134	6	18	14.8%	1.00 [-2.57, 4.57]
Peeling 2007	125	8.5	8	127	8.5	8	7.1%	-2.00 [-10.33, 6.33]
Zhu 1998	136.3	8	20	132.2	7.6	17	11.9%	4.10 [-0.93, 9.13]
Total (95% CI)			**148**			**150**	**100.0%**	**4.01 [1.22, 6.81]**

Heterogeneity: $Tau^2 = 10.46$; $Chi^2 = 21.03$, df = 11 (P = 0.03); $I^2 = 48\%$

Test for overall effect: Z = 2.81 (P = 0.005)

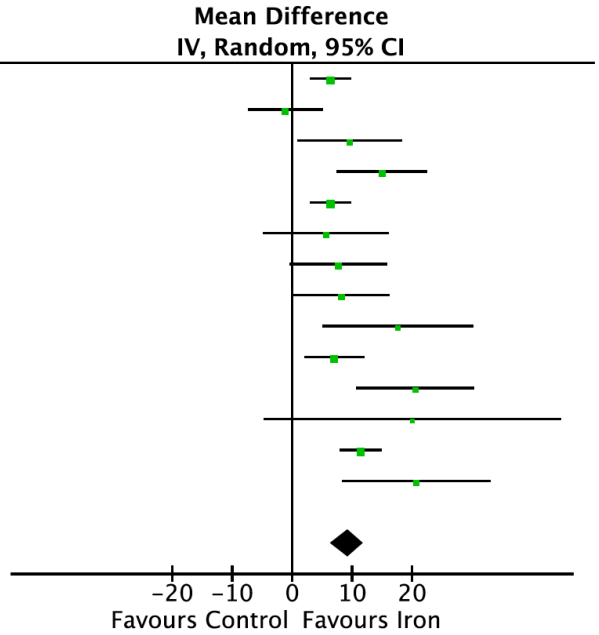


Appendix 9. Effect of iron supplementation on serum ferritin

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Brownlie 2002	14.52	7	22	8.11	3.9	19	12.2%	6.41 [3.00, 9.82]
Brutsaert 2003	15.02	7	10	16.18	7.2	10	8.5%	-1.16 [-7.38, 5.06]
Flink 2006	33	14	24	23.4	15.8	22	6.0%	9.60 [0.94, 18.26]
Fogelholm 1992	26	13.3	14	11	5.9	17	7.1%	15.00 [7.49, 22.51]
Hinton 2000	14.52	7	22	8.11	4	20	12.2%	6.41 [3.00, 9.82]
Hinton 2007	20.82	11.6	10	15.18	12.23	10	4.7%	5.64 [-4.81, 16.09]
Klingshirn 1992	23.44	6.65	9	15.77	10.45	9	6.5%	7.67 [-0.42, 15.76]
LaManca 1993	22.5	10.8	10	14.3	7	10	6.6%	8.20 [0.22, 16.18]
Leonard 2014	32.69	22.1	13	15.1	4	5	3.6%	17.59 [5.08, 30.10]
Moafi 2012	22.39	15.1	36	15.39	1.57	36	10.1%	7.00 [2.04, 11.96]
Newhouse 1989	37.7	19.7	19	17.2	8.9	18	5.2%	20.50 [10.73, 30.27]
Peeling 2007	57	33.9	8	37	11.3	8	1.1%	20.00 [-4.76, 44.76]
Vaucher 2012	11.6	13.7	102	0.2	11	96	12.2%	11.40 [7.95, 14.85]
Zhu 1998	36.9	24	20	16.2	13.5	17	3.7%	20.70 [8.38, 33.02]
Total (95% CI)			**319**			**297**	**100.0%**	**9.23 [6.48, 11.97]**

Heterogeneity: $Tau^2 = 12.99$; $Chi^2 = 30.87$, df = 13 (P = 0.004); $I^2 = 58\%$

Test for overall effect: Z = 6.59 (P < 0.00001)



Appendix 10. Adverse effects in trials of iron supplementation in iron-deficient, non-anemic individuals

Study	Constipation	Diarrhea	Nausea	GI intolerance
Intravenous				
Burden[27]	NR	NR	NR	NR
Favrat[28]	NR	NR	Iron: 8; Control: 2	NR
Krayenbuehl[31]	NR	Iron: 0; Control: 1	Iron: 6; Control: 1	NR
Intramuscular				
Flink[29]	NR	NR	NR	Iron: 14; Control: 2
Oral				
Brownlie[19]	NR	NR	NR	NR
Brutsaert[20]	"Frequency and severity of reported side effects due to supplementation was very low and did not differ significantly between groups"			
Donangelo[21]	NR	NR	NR	NR
Fogelholm[30]	NR	NR	NR	NR
Hinton[22]	NR	NR	NR	NR
Klingshirn[23]	NR	NR	NR	Iron: 1; Control: 0
LaManca[26]	NR	NR	NR	NR
Leonard[18]	Iron: 1; Control: 0	Iron: 2; Control: 2	Iron: 2; Control: 1	NR
Moafi[35]	"When symptoms occurring immediately before or during menses were excluded, there were no significant differences either in frequency or severity of symptoms experienced"			
Newhouse[24]	NR	NR	NR	NR
Peeling[34]	NR	NR	NR	NR
Vaucher[32]	NR	NR	NR	Iron: 12; Control: 10
Verdon[33]	NR	NR	Iron: 0; control: 1	NR
Zhu[25]	NR	NR	NR	NR

NR, not reported; GI, gastrointestinal

Appendix 11. Compliance with the study intervention

	Iron (%)	Control (%)
Intravenous		
Burden[27]	100	100
Favrat[28]	100	100
Krayenbuehl[31]	NR	NR
Intramuscular		
Peeling[34]	NR	NR
Oral		
Brownlie[19]	91	89
Brutsaert[20]	NR	NR
Donangelo[21]	NR	NR
Flink[29]	71	82
Fogelholm[30]	NR	NR
Hinton[22]	98	99
Klingshirn[23]	89	91
LaManca[26]	82	85
Leonard[18*]	89	92
Moafi[35]	89	92
Newhouse[24]	>75	>75
Vaucher[32]	93	94
Verdon[33]	95	98
Zhu[25]	88	87

*weighted averaged between two iron treatment groups; NR = not reported

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp 3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9, Fig 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10, 11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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