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

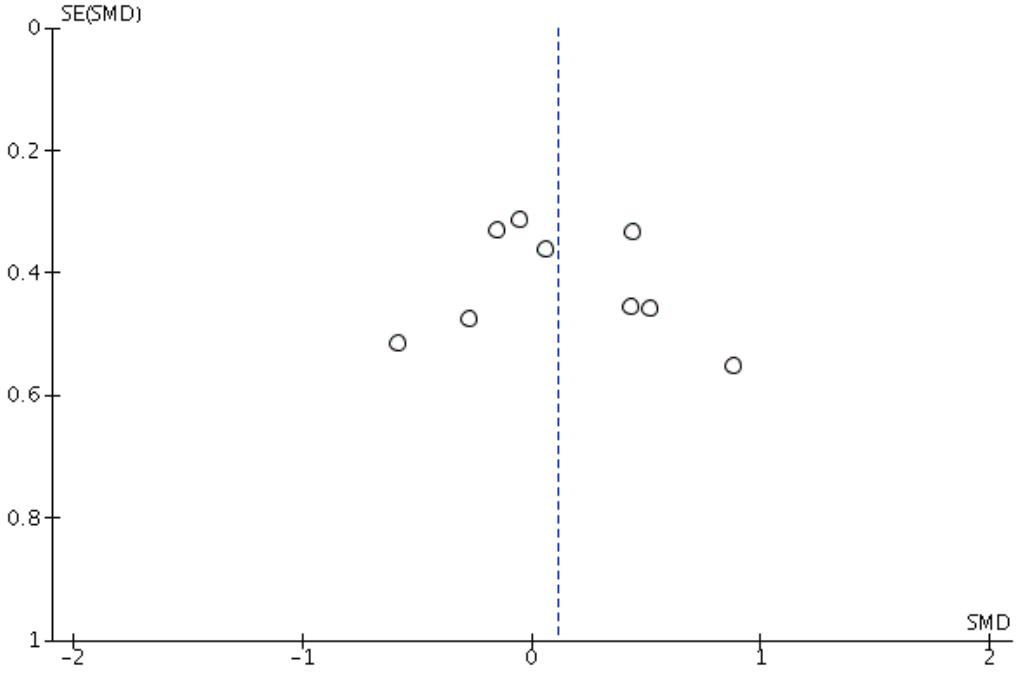
Appendix 3. Search strategy

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. 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	

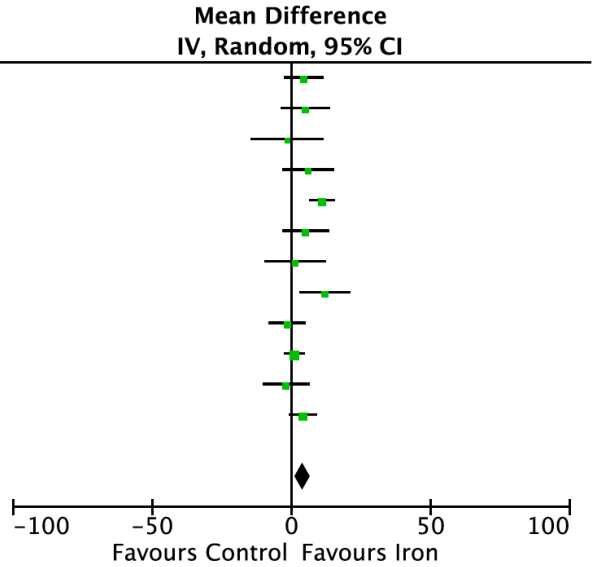
Biologic sex, duration of follow-up and risk of bias subgroup analyses were unevaluable in subgroup analyses as all trials enrolled females and had a follow-up period of less than two months. All trials were of unclear risk of bias. CI = confidence interval; SMD = standardized mean difference; IV = intravenous;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

Appendix 8. Effect of iron supplementation on serum hemoglobin

Study or Subgroup	Iron Therapy Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference 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)

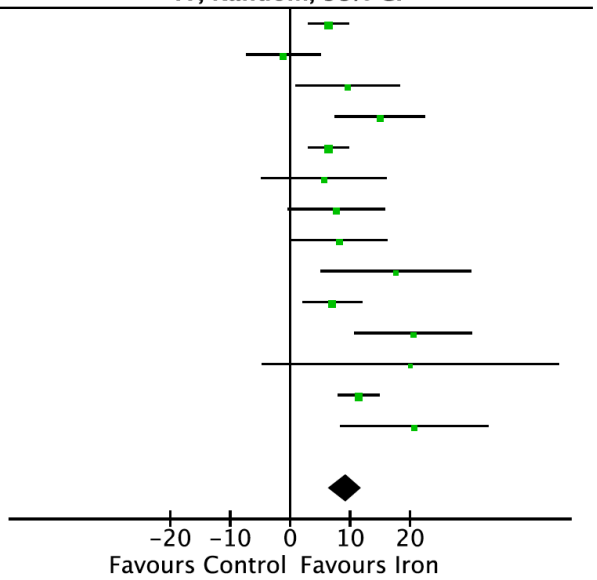


Appendix 9. Effect of iron supplementation on serum ferritin

	Iron Therapy			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	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