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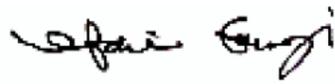
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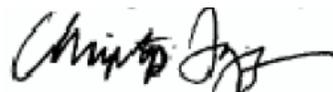
This Dissertation, “The Etiology and Treatment of Anemia”, presented by Christopher T. Andersen, and Submitted to the Faculty of The Harvard T.H. Chan School of Public Health in Partial Fulfillment of the Requirements for the Degree of Doctor of Science in the Department of Epidemiology, has been read and approved by:



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The Etiology and Treatment of Anemia

Christopher T. Andersen

A Dissertation Submitted to the Faculty of
The Harvard T. H. Chan School of Public Health
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Science
in the Department of Epidemiology

Harvard University

Boston, Massachusetts

March, 2021

The Etiology and Treatment of Anemia

Abstract

Anemia is a significant global health challenge. While the subject of anemia has been well-studied, critical questions regarding its causes and treatment remain.

Chapter 1 investigates the optimal schedule, duration, dose, and co-supplementation regimen for iron supplementation as a treatment of anemia among children. A systematic review and meta-analysis of randomized controlled trials was conducted. A total of 123 eligible trials were identified. Frequent (3-7/week) and intermittent (1-2/week) iron regimens were similarly effective at increasing hemoglobin and decreasing anemia, iron deficiency, and iron deficiency anemia (p -heterogeneity >0.05). Varying durations of supplementation showed similar benefits after controlling for baseline anemia status, except for serum ferritin, which showed larger increases with longer duration of iron ($p=0.04$). Moderate- and high-dose supplements were more effective than low-dose supplements at improving hemoglobin ($p=0.03$) and ferritin ($p=0.003$). Co-supplementation of iron with zinc or vitamin A modestly attenuated impacts for anemia ($p=0.048$) and hemoglobin ($p=0.03$), respectively, although benefits remained even in co-supplemented groups.

In Chapter 2, the risks and benefits of iron supplementation among HIV-infected children are examined. A prospective cohort study of 4,229 children were observed for a mean follow-up of

2.9 years. After adjustment for time-varying clinical covariates, time-varying iron supplementation was associated with a 2.87 times higher hazard rate of mortality (95% CI: 1.70, 4.87) and a 1.48 times higher hazard rate of HIV disease stage progression (95% CI: 1.10, 1.98). Iron supplementation was also associated with a lower rate of anemia persistence (HR=0.47; 95% CI: 0.37-0.61). No differences in the association between iron supplementation and clinical outcomes were observed by ART or anemia status.

Chapter 3 quantifies the proportion of anemia cases attributable to selected nutritional, infectious disease, and other risk factors. A population-based cross-sectional study of women, men, and children was conducted. Low serum ferritin contributed to 11% (-1, 22) of anemia cases among women, 9% (0, 17) among men, and 19% (3, 33) among children. The proportion of anemia attributable to low serum folate was estimated at 25% (4, 40) among women and 28 (11, 42) among men. Inflammation and malaria were responsible less than one in ten anemia cases.

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Acknowledgements

My academic training has benefited above all from the mentorship of Dr. Wafaie Fawzi, who served as Chair of my dissertation committee. Dr. Fawzi guided me to research questions of public health importance and cultivated my ability to pursue those questions as an independent researcher. He also supported my interest in working on-site in Ethiopia, as well as promoted my efforts (along with other students at the school) to establish a reciprocal peer-mentorship program between students at Harvard and various African universities.

I am indebted to each of my other committee members for the expertise they shared and the time they dedicated to my training. Dr. Donna Spiegelman provided critical guidance in the use of statistical methods, both those which are standard to the field and those at the cutting-edge of development. Dr. Christopher Duggan's wisdom as a pediatrician helped me develop my understanding of the biology and clinical implications of the topics I studied. Dr. George Seage reinforced in me the fundamental skill of an epidemiologist to assess bias and evaluate a study design. He also invested in me financially by selecting me for support on his T32 Infectious Disease Epidemiology training grant. With his tragic passing in January 2021, we lost both an excellent scientist and a good man.

I had the pleasure to work with many other faculty at Harvard and elsewhere. Dr. Sheila Isanaka permitted me opportunities to learn a new subject area and contribute to work with critical policy implications. Dr. Amare Tadesse was my guide to research implementation and organizing a study team. Dr. Bethany Hedt-Gauthier, Dr. Albert Hofman, and Dean Michelle Williams, and

many others supported efforts to establish the Global Cohort of Doctoral Students. There are too many others to list here, but each have left their mark.

I would also like to thank Ellen Furxhi, Eric DiGiovanni, Caroline Huntington, and Warisha Amin for helping me to navigate the Harvard system, and to David Rogers for career advice and support.

My parents, Marie and Andrew, raised me with two core values: service and learning. Those undoubtedly started me on the path that led to graduate school. My brother and sister, Will and Kirsten, have kept me grounded. Many friends have come along side me through these years as well, and served as both an escape from and a sounding board for my graduate school worries.

Finally, my wife, Farrah, has been a source of encouragement through many long years of studies. I am so glad to have grown alongside her through this process.

Introduction

Anemia is a condition of major public health significance globally, and is associated with considerable health consequences. Among pregnant women who are anemic, there is a 41% increase in the odds of mortality associated with a 10 g/L decrease in hemoglobin.¹ Children born to anemic mothers are at a 31% increased risk of low birth weight, and 172% increased risk of death in the first 4 weeks after birth.² Regarding anemia among children themselves, a 10 g/L decrease in hemoglobin is associated with 32% increased odds of mortality.³ Anemia among children is also associated with impaired development, on the order of 0.5-1.3 standard deviations lower scores of mental development, and 0.7-1.1 standard deviations lower scores of motor development.⁴ Adults with anemia are more likely to experience fatigue and decreased work capacity.⁵

While anemia is an important issue globally, it affects certain populations considerably more than others. Menstruating women and pregnant women are at high risk of anemia. In high-income countries, an estimated 18.2% of women of reproductive age and 23.9% of pregnant women are anemic.⁶ Nearly double that number (35.4% and 41.6%, respectively) are anemic in low- and middle-income countries. Children are also at high risk, affecting 14.5% of children 6-59 months of age in high-income countries and 44.6% of children in low- and middle-income countries.

The underlying causes of anemia at the individual-level are relatively well-understood, including nutritional deficits (e.g. deficiencies in iron, folate, vitamin B12), infections (e.g. malaria,

hookworm, HIV), and hemoglobinopathies (e.g. thalassemias, sickle cell).⁷ Iron-deficiency is estimated to be the primary cause of anemia worldwide, and considerable attention has accordingly been placed on iron supplementation or fortification strategies to combat anemia.⁸⁻¹⁰ However, despite the large body of research on the causes and treatment of anemia, important questions remain.

One area in need of inquiry involves the optimal use of iron supplements to prevent and treat anemia in children. While many randomized trials have shown iron supplements are effective in reducing anemia, there is a broad spectrum of treatment protocols that have been used.¹¹⁻¹³ The World Health Organization has made recommendations on the use of iron supplements among children – including a recommended dose, frequency, and duration of supplementation – based on this evidence.⁹ However, there has not yet been a comprehensive analysis to compare the effectiveness of different doses, schedules, durations, or co-supplementation of iron with other nutrients. Such evidence could serve to inform and enhance these global guidelines. This question of comparative efficacy serves as the topic of Paper 1.

A second key question is whether iron supplementation for the treatment of anemia is safe in all circumstances. Iron is an essential nutrient for both humans and the pathogens which infect them.¹⁴ Some evidence indicates that iron supplementation can have adverse effects when administered to populations with inadequate malaria prevention and treatment measures.¹⁵ Similar hypotheses exist for other infections, including HIV.¹⁶ Paper 2 explores the risks and benefits of iron supplementation amongst a cohort of children infected with HIV.

Finally, the view that iron deficiency is the primary cause of anemia globally has been generally accepted given trial evidence which shows considerable reductions in anemia with iron therapy. However, anemia has a multicausal etiology, and there is a dearth of research that captures individual-level data on a broad array of risk factors to estimate the proportion of anemia due to each cause. Paper 3 undertakes such a study in the context of Ethiopia, with the aim to identify the primary causes of anemia in the population and therefore inform policy making for anemia reduction.

This dissertation has leveraged three different study designs in distinct populations to expand the current body of knowledge on the etiology and treatment of anemia. The results of each of these studies has practical implications for public health decision-making.

In Paper 1, it was shown that intermittent iron supplementation may be considered as an alternative to daily supplementation among children. Additionally, the effect of iron supplementation on hematologic indices was somewhat attenuated when delivered alongside zinc or vitamin A. Guideline-making bodies, such as the World Health Organization, may consider incorporating these findings into their recommendations. If the WHO were to affirm that intermittent iron supplementation has similar efficacy as daily supplementation, there may be contexts where a transition would be made daily to intermittent supplementation in order to ease service delivery challenges and increase adherence to treatment. Furthermore, delivery of iron supplementation alongside zinc or vitamin A may be of use in contexts where deficiency in both

micronutrients is common. However, strategies to address the potential negative interaction between nutrients when co-administered need to be examined.

Paper 2 has presented concerning evidence that iron supplementation increases the risk of mortality and disease progression among HIV-infected children, despite reductions in anemia. Although this evidence comes from a single observational study, the robustness of the results to potential confounding and the severity of the risk imply that the findings should be seriously considered by clinicians and those providing guidance on iron supplementation. Further research to confirm this finding is warranted.

While the first two papers of this dissertation have explored questions related to iron-deficiency anemia, the third demonstrates that iron deficiency is not the most common cause of anemia among adults in Ethiopia. Rather, folate deficiency contributed to a plurality of cases, which implies that interventions to improve intake and absorption of folate could provide substantial benefit in Ethiopia. Among children, iron deficiency was the most common cause. However, population iron interventions must be targeted to children since high levels of iron were detected among men in some regions, thereby precluding the potential for national fortification as a strategy to address iron deficiency in this population. These findings can aid the government of Ethiopia and other partners in developing specific strategies to prevent and treat anemia in the country.

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Chapter 1: Oral iron supplementation and anemia in children according to schedule, duration, dose, and co-supplementation: a systematic review and meta-analysis of 123 randomized trials

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Abstract

Background: The World Health Organization (WHO) recommends daily oral iron supplementation for children aged 6 months to 12 years living in regions with a high burden of anemia. However, the optimal delivery of iron supplementation is not established, and the 2016 WHO guidelines call for further research to identify the optimal schedule, duration, dose, and co-supplementation regimen.

Objective: To estimate the safety and efficacy of pediatric oral iron supplementation according to schedule, duration, dose, and co-supplementation regimen.

Design: Systematic review and meta-analysis of randomized controlled trials.

Data sources: PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials from inception through April, 2017; reference lists of eligible trials; related reviews.

Study selection: Randomized controlled trials providing ≥ 30 days of oral iron supplementation versus placebo or control to children and adolescents aged < 20 years. No language restrictions were applied to the search.

Review methods: Reviewers identified studies, extracted data, and assessed the quality of the evidence, independently and in duplicate. Random effects meta-analysis was used to summarize the potential benefits and harms of iron supplementation. Meta-regression was used to estimate iron effect heterogeneity according to schedule, duration, dose, and co-supplementation regimen.

Results: 123 trials with 190 intervention arms randomized 33,328 children to iron vs. placebo or control. Iron supplementation significantly improved mean hemoglobin (mean difference

[MD]=6.5 g/L [5.7, 7.4]) and ferritin (MD=19.1 ng/mL [16.6, 21.7]), as well as reduced the prevalence of anemia (prevalence ratio [PR]=0.60 [0.54, 0.67]), iron deficiency (PR=0.27 [0.21,0.35]), and iron deficiency anemia (PR=0.19 [0.12, 0.30]). Frequent (3-7/week) and intermittent (1-2/week) iron regimens were similarly effective at increasing hemoglobin and decreasing anemia, iron deficiency, and iron deficiency anemia (p -heterogeneity >0.05), although serum ferritin levels increased more with frequent supplementation ($p=0.002$). Varying durations of supplementation showed similar benefits after controlling for baseline anemia status, except for serum ferritin, which showed larger increases with longer duration of iron ($p=0.04$). Moderate- and high-dose supplements were more effective than low-dose supplements at improving hemoglobin ($p=0.03$) and ferritin ($p=0.003$). Co-supplementation of iron with zinc or vitamin A modestly attenuated impacts for anemia ($p=0.048$) and hemoglobin ($p=0.03$), respectively, although benefits remained even in co-supplemented groups.

Conclusions: These results generally support the WHO recommendations regarding the frequency, duration, and dose of iron supplementation. However, weekly iron supplementation might be considered as an alternative to the recommended daily regimen in some contexts. Iron supplements may deliver greater hematological benefits in the absence of co-supplementation with zinc or vitamin A.

Introduction

Iron deficiency is the most common micronutrient deficiency globally, with children at particular risk.(1,2) Among children aged 6-59 months, approximately half of the 273 million cases of anemia in 2011 were estimated to be due to iron deficiency.(3,4) Iron deficiency is furthermore a key risk factor for cognitive impairment, impaired immune function, and decreased capacity for physical activity.(5-7)

The World Health Organization (WHO) recommends daily oral iron supplementation for all children in regions with a prevalence of anemia $\geq 40\%$. In malaria-endemic settings, iron supplementation is recommended in conjunction with measures to prevent, diagnose, and treat malaria.(8) These recommendations are supported by prior meta-analyses establishing the benefits of iron supplementation in the treatment of anemia along with safety in the presence of malaria control programs.(9-12) However, prior meta-analyses have not investigated the optimal delivery of iron supplementation. The 2016 WHO guidelines therefore highlight the need for additional evidence regarding the optimal schedule, duration, and dose of iron supplementation, as well as the efficacy of iron supplementation in the presence of co-supplemented micronutrients.(8)

We conducted a systematic review and meta-analysis of randomized controlled trials of oral iron supplementation among children and adolescents aged < 20 years and compared the impact of interventions by schedule, duration, dose, and co-interventions. Hematologic, developmental, infectious, and anthropometric outcomes were included to assess both safety and efficacy.

Methods

Search strategy and selection criteria

We adhered to the Cochrane Collaboration's guidelines for this review.(13)

The protocol was pre-registered with the International Prospective Register of Systematic Reviews, number CRD42016039948. Systematic literature searches were performed using PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials from inception through April 1, 2017. References of eligible articles and previous systematic reviews were additionally reviewed. No language restrictions were placed on the search strategy. Search terms are presented in Appendix S1.1.

Studies were eligible for inclusion if they met the following criteria:

1. Oral iron supplementation was randomly assigned.
2. Oral iron supplementation was compared with control or placebo. (Studies comparing multiple doses of iron supplementation and which did not include a group randomized to no iron were excluded due to the lack of a common referent for meta-analysis.)
3. Intervention groups differed by iron alone. (For example, studies randomizing children to iron-folate versus placebo were not eligible unless the placebo also included folate, due to potential independent effects of these other components.)
4. Oral iron supplementation was administered for a minimum period of ≥ 1 month (≥ 30 days).
5. Participants were aged < 20 years.
6. Participants did not have any chronic illness (e.g. HIV) or belong to a special

subpopulation (e.g. athletes).

Data extraction and management

One reviewer screened the titles and abstracts of records identified by the search, and excluded those that indicated clear ineligibility. Two reviewers independently reviewed the full text of all positively screened studies to establish final eligibility, after which data were extracted from eligible studies in duplicate using standardized forms. Appendix S1.2 provides a list of variables extracted from eligible studies. Discrepancies between reviewers were resolved through discussion or through arbitration with a third reviewer. Factorial trials were extracted as two separate experiments (iron versus control, iron+co-intervention versus control+co-intervention).

Assessment of risk of bias

Two authors independently assessed risk of bias using the Cochrane Risk of Bias Tool, and discrepancies were resolved by discussion.(13)

Outcomes

To assess the relative benefits and risks of oral iron supplementation among children and adolescents, we included the following outcomes: hemoglobin (g/L), anemia (defined by study authors), serum ferritin ($\mu\text{g/L}$), iron deficiency (ID, defined by study authors), iron deficiency anemia (IDA, defined by study authors), child development (standardized means of cognitive, motor, and socioemotional domain scores), physical growth (WHO Z scores of height-for-age,

weight-for-height, and weight-for-age(14,15); stunting, wasting, and underweight), and indicators of infection (diarrhea, respiratory infection, malaria, and intestinal helminths). In order to account for repeated use of the same control group in trials comparing multiple iron treatments to a single control (n=17 trials), the variance of the outcome in the control group was adjusted by dividing the control group sample size by the number of comparisons.

Statistical analysis

Meta-analysis was conducted for outcomes reported by four or more trials. We used inverse-variance weighted random-effects meta-analysis to account for underlying differences in the trial populations.(16) Binary outcomes were summarized using risk ratios, prevalence ratios, or rate ratios with 95% confidence intervals. Continuous measures on the same scale were presented using mean differences, and measures reported on different scales using standardized mean differences (SMD). For ferritin, geometric means or medians were included when arithmetic means were not reported. Heterogeneity of effects was measured using the I^2 statistic. We assessed effect modification using univariate meta-regression for pre-specified supplementation variables: schedule (1-2 times/week; 3-7 times/week), duration (1-3 months; 4-6 months; ≥ 7 months), dose (age-adjusted tertiles; see Table S3), and co-interventions (zinc; vitamin A).(17,18) In secondary analyses, we explored effect modification by baseline anemia (all anemic; all non-anemic; mixed population of anemic and non-anemic; missing baseline anemia data), child age (0-5 mo; 6-23 mo; 2-4 y; 5-11 y; 12-19 y), child sex (all female; all male; mixed female and male; missing baseline sex data), WHO region (Africa; Americas; Eastern Mediterranean; Europe; South-East Asia; Western Pacific), and iron formulation (ferrous sulfate; ferrous fumarate; other or unspecified). We conducted multivariate meta-regression to

investigate colinearity between potential effect modifiers. Small study effects were assessed using funnel plots and Egger's test for all outcomes reported by ≥ 10 iron intervention groups. Sensitivity analyses correcting for small study effects were conducted using trim and fill.(19)

Role of the funder

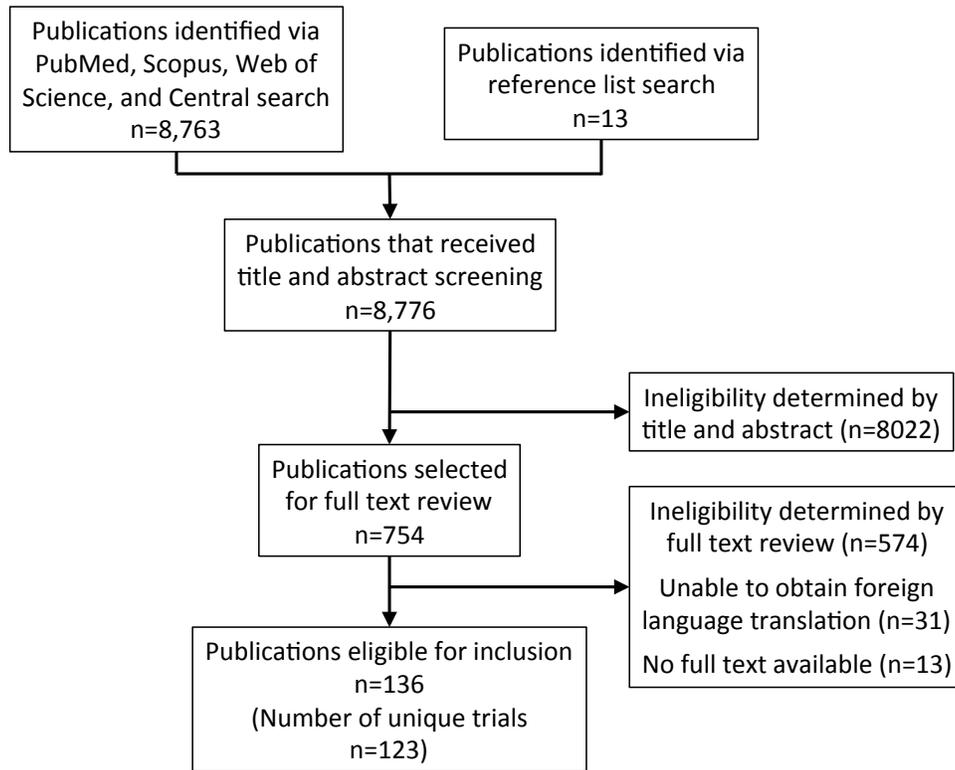
The funder was not involved in the study design, data collection, analysis, interpretation, or manuscript writing. The corresponding author had access to all data in the study and assumes full responsibility for the accuracy of the results.

Results

Literature search

A total of 8,763 unique publications were retrieved from the PubMed, Scopus, Web of Science, and Cochrane Central search. An additional 13 records identified from the reference lists of eligible articles or prior meta-analyses were also screened. Of these, 754 records were selected for full text review, among which 136 met the final eligibility criteria for inclusion (Figure 1.1). Appendix S1.3 provides references to all included publications.

Figure 1.1 Flow diagram for selection process of eligible studies



Trial characteristics

The 136 eligible publications represented 123 unique trials with 190 trial arms randomized to iron supplementation and 166 trial arms randomized to control or placebo, and 33,328 total participants analyzed (Table 1.1). Children under 5 years of age were most frequently studied, with children aged <6 months (n=44 trial arms, 23%), 6-23 months (n=41, 22%), and 2 to <5 years (n=35, 18%) represented roughly equal proportions of this population. School-aged children of 5-11 years (n=50, 26%) and adolescents aged 12-19 years (n=20, 11%) were less frequently studied than children under 5. About half of studies were in anemic only (16%) or mixed anemic and non-anemic (35%) children, and about 1 in 6 (18%) in non-anemic children; the remainder (32%) did not report baseline anemia status. Most studies (n=157, 83%) provided

Table 1.1 Characteristics of studies included in meta-analysis of randomized iron supplementation trials for child health.

Study characteristics	
Eligible publications, <i>n</i>	136
Unique trials, <i>n</i>	123
Trial arms randomized to iron, <i>n</i>	190
Trial arms randomized to placebo or control, <i>n</i>	166
Individuals randomized to iron, <i>n</i>	17,424
Individuals randomized to placebo or control, <i>n</i>	15,904
World Health Organization region, <i>n</i> (%) *	
Africa	37 (19.5)
Americas	36 (19.0)
Eastern Mediterranean	11 (5.8)
Europe	26 (13.7)
South-East Asia	60 (31.6)
Western Pacific	20 (10.5)
Decade of publication, <i>n</i> (%) †	
1970-1979	3 (2.2)
1980-1989	17 (12.5)
1990-1999	26 (19.1)
2000-2009	69 (50.7)
2010-2017	21 (15.4)
Population characteristics	
Age, <i>n</i> (%) *	
0 to 5 months	44 (23.2)
6 to 23 months	41 (21.6)
2 to <5 years	35 (18.4)
5 to <12 years	50 (26.3)
≥12 years	20 (10.5)
Percent female, median (IQR) *	
All female, <i>n</i> (%)	23 (12.1)
All male, <i>n</i> (%)	6 (3.2)
Mixed male and female, <i>n</i> (%)	118 (62.1)
Missing baseline sex data, <i>n</i> (%)	43 (22.6)
Baseline percent anemic, median (IQR) *	
All anemic, <i>n</i> (%)	30 (15.9)
All non-anemic, <i>n</i> (%)	33 (17.5)
Mixed anemic and non-anemic, <i>n</i> (%)	67 (35.3)
Missing baseline anemia data, <i>n</i> (%)	60 (31.6)

Table 1.1 (Continued)

Intervention characteristics	
Frequency, n (%) *	
1 to 2 days per week	30 (15.8)
3 to 7 days per week	157 (82.6)
Missing frequency data	3 (1.6)
Duration, n (%) *	
1 to 3 months	103 (54.2)
4 to 6 months	61 (32.1)
≥7 months	26 (13.7)
Weekly iron dose (mg) by child age category, median (IQR)*	
0 - 5 mo	56.3 (49.7, 70)
6 - 23 mo	70 (55.3, 168)
24-59 mo	114 (70, 216.3)
5 - 11 y	265.1 (120, 403.1)
12 - 19 y	250 (120, 420)
Missing dose information, n	28
Formulation, n (%) *	
Ferrous sulfate	122 (64.2)
Ferrous fumarate	18 (9.5)
Other or unspecified	50 (26.3)
Factorial trials, n (%) ‡	28 (22.8)
Zinc, n (%) §	10 (35.7)
Vitamin A, n (%) §	5 (17.9)
Other, n (%) §	13 (46.4)

* Denominator is the number of unique groups randomized to iron (n=190)

† Denominator is the number of publications (n=136)

‡ Denominator is the number of unique trials (n=123)

§ Denominator is the number of factorial trials (n=28)

Abbreviations: IQR, inter-quartile range

iron supplementation 3 to 7 days per week, although many (n=30, 16%) supplemented only 1 to 2 days per week. About half of studies (54%) provided supplementation for 1-3 months; about one-third (32%) for 4-6 months; and the remainder (14%) for ≥7 months. About one fifth (n=28, 23%) of eligible studies were factorial trials, among which the most frequent co-interventions were zinc (n=10) and vitamin A (n=5). The characteristics of individual studies are available in Table S1.2.

Effects of iron supplementation on hematologic outcomes

In aggregate, oral iron supplementation versus placebo or control demonstrated clear benefits for hematologic indices (Table 1.2). Hemoglobin levels rose by 6.5 g/L (95% CI: 5.7, 7.4) along with serum ferritin increases of 19.1 ng/mL (16.6, 21.7). Iron supplementation reduced the prevalence of overall anemia by 40% (33%, 46%), and even larger impacts were observed for iron deficiency (reduction of 73% [65%, 79%]) and iron deficiency anemia (reduction of 81% [70%, 88%]). Heterogeneity was observed between studies for these hematologic outcomes (I^2 ranging from 80 to 100%), which was further explored using meta-regression (see below).

Assessment of heterogeneity

When comparing trials of frequent (3-7 times/week) versus intermittent (1-2 times/week) supplementation, no significant differences in treatment effects were observed for hemoglobin, anemia, iron deficiency, and iron deficiency anemia outcomes (Table 1.3). However, trials of frequent supplementation achieved larger increases in serum ferritin than trials of intermittent supplementation (21.1 ng/mL [18.3, 24.0] vs. 7.1 ng/mL [3.6, 10.5]; p -interaction=0.002). Similar results were observed after control for baseline anemia (Table S1.3).

Table 1.2. Effect of oral iron supplementation versus placebo or control among children and adolescents aged <20 years.

	n*	Estimate type	Estimate of effect (95% CI)	p-value	I ² (%)
<i>Hematology</i>					
Hemoglobin (g/L)	159	WMD	6.5 (5.7, 7.4)	<0.001	92.2
Serum ferritin (ng/mL)	99	WMD	19.1 (16.6, 21.7)	<0.001	99.5
Anemia	66	RR	0.60 (0.54, 0.67)	<0.001	86.1
Iron deficiency	45	RR	0.27 (0.21, 0.35)	<0.001	90.7
Iron deficiency anemia	26	RR	0.19 (0.12, 0.30)	<0.001	79.9
<i>Anthropometry</i>					
Height-for-age Z score	41	WMD	0.00 (-0.03, 0.03)	0.98	35.5
Weight-for-height Z score	26	WMD	0.01 (-0.05, 0.08)	0.71	71.7
Weight-for-age Z score	40	WMD	0.01 (-0.04, 0.05)	0.79	66.1
Stunting	13	RR	1.07 (0.96, 1.18)	0.22	0
Wasting	6	RR	1.12 (0.85, 1.48)	0.42	0
<i>Infections</i>					
Diarrhea (cumulative incidence)	15	RR	0.97 (0.84, 1.11)	0.63	0
Diarrhea (incidence rate)	8	IRR	1.08 (0.98, 1.19)	0.10	63.4
Respiratory illness (cumulative incidence)	8	RR	1.16 (0.93, 1.45)	0.20	66.2
Respiratory illness (incidence rate)	9	IRR	0.98 (0.92, 1.06)	0.66	0
Malaria (prevalence)	10	PR	1.11 (0.98, 1.25)	0.09	0
Malaria (incidence rate)	7	IRR	0.91 (0.82, 1.01)	0.08	0
Hookworm (prevalence)	4	PR	0.94 (0.85, 1.03)	0.19	0
Ascaris lumbricoides (prevalence)	4	PR	1.04 (0.88, 1.25)	0.68	0
Trichuris trichiura (prevalence)	4	PR	0.97 (0.90, 1.06)	0.52	0
<i>Development</i>					
Bayley Mental Index	9	SMD	0.26 (0.00, 0.51)	0.05	67.8
Bayley Psychomotor Index	9	SMD	0.21 (-0.06, 0.48)	0.13	70.4

* Number of trial arms randomized to iron

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; PR, prevalence ratio; RR, risk ratio; SMD, standardized mean difference; WMD, weighted mean difference

Table 1.3. Comparison of iron supplementation effects across schedule, duration, and dosing schemes.

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Weekly frequency					
Frequent (3-7 times/week)					
n	129	85	54	41	23
Estimate	6.7	21.1	0.61	0.28	0.19
95% CI	(5.7, 7.8)	(18.3, 24.0)	(0.55, 0.68)	(0.22, 0.36)	(0.11, 0.31)
Intermittent (1-2 times/week)					
n	27	13	10	4	3
Estimate	5.4	7.1	0.61	0.24	0.22
95% CI	(3.8, 6.9)	(3.6, 10.5)	(0.41, 0.92)	(0.09, 0.66)	(0.06, 0.77)
P-value for interaction	0.31	0.002	0.84	0.87	0.96
Duration §					
1-3 months					
n	89	39	23	15	6
Estimate	7.8	15.4	0.50	0.32	0.23
95% CI	(6.4, 9.1)	(12.8, 18.0)	(0.43, 0.59)	(0.19, 0.54)	(0.07, 0.81)
4-6 months					
n	52	52	33	26	18
Estimate	5.8	24.4	0.64	0.21	0.13
95% CI	(4.5, 7.1)	(19.3, 29.5)	(0.55, 0.73)	(0.15, 0.30)	(0.09, 0.18)
≥7 months					
n	18	7	8	4	2
Estimate	2.6	11.8	0.84	0.84	0.86
95% CI	(1.4, 3.8)	(5.9, 17.7)	(0.69, 1.03)	(0.67, 1.06)	(0.63, 1.18)
P-value for interaction	0.005	0.03	0.03	0.14	<0.001

Table 1.3. (Continued)

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Dose					
Low (1st tertile for age)					
n	54	36	27	17	13
Estimate	4.9	13.7	0.68	0.35	0.23
95% CI	(3.7, 6.1)	(9.1, 18.3)	(0.56, 0.81)	(0.23, 0.52)	(0.14, 0.39)
Moderate (2nd tertile for age)					
n	30	21	21	15	9
Estimate	8.3	26.7	0.54	0.23	0.08
95% CI	(6.1, 10.5)	(21.1, 32.2)	(0.45, 0.64)	(0.15, 0.35)	(0.05, 0.14)
High (3rd tertile for age)					
n	36	23	8	8	2
Estimate	7.7	18.6	0.65	0.20	0.08
95% CI	(5.1, 10.3)	(12.8, 24.4)	(0.49, 0.87)	(0.11, 0.35)	(0.01, 0.69)
P-value for interaction	0.03	0.003	0.39	0.41	0.05

* Weighted mean difference

† Pooled risk ratio

§ Trials of short duration were more likely to have a higher proportion of anemic patients. No significant associations were observed between duration and hemoglobin, anemia, iron deficiency, and iron deficiency anemia outcomes after controlling for baseline anemia. After control for anemia, an increase in serum ferritin was associated with trials of longer duration (see Table S1.2).

Abbreviations: CI, confidence interval

Increasing duration of supplementation was generally associated with diminished impacts on hemoglobin, anemia, and iron deficiency anemia (Table 1.3); however, trials of short duration were more likely to have a higher proportion of anemic patients. After controlling for baseline anemia, no significant associations were observed between duration of supplementation and hemoglobin, anemia, iron deficiency, or iron deficiency anemia outcomes. Trials of longer duration were associated with greater increases in serum ferritin after adjustment for baseline anemia (Table S1.3).

When we evaluated age-adjusted doses of supplementation (Table S1.1), trials in the lowest dose tertile across all ages utilized lower amounts than recommended by WHO (10-12.5 mg/d for ages 6-23 mo, 30 mg/d for ages 24-59 mo, and 30-60 mg/d ages 5-12 y) (8). Compared with lower age-adjusted doses of supplementation and adjusting for baseline anemia, moderate doses were associated with greater improvements in hemoglobin and ferritin, and nonsignificant trends toward greater reductions in anemia, iron deficiency, and iron deficiency anemia (Table 1.3; Table S1.3). However, the lower dose still produced benefits; and no dose-response was seen: compared with lower dose, the highest doses were not associated with significantly greater effects.

When comparing the effect of iron stratified by the baseline prevalence of anemia, trials conducted among entirely anemic populations demonstrated approximately twofold increases in hemoglobin (global p-interaction<0.001) and reductions in endline anemia (global p-interaction=0.004) relative to non-anemic or mixed populations (Appendix 7). No significant

heterogeneity was observed by child age or sex. Effects of iron on hematologic outcomes were similar across WHO regions, except for a potentially smaller effect on prevalence of iron deficiency anemia in the Americas compared to other regions (global p-interaction=0.01). Comparing types of supplements, ferrous sulfate and ferrous fumarate were associated with greater reductions in iron deficiency anemia (global p-interaction<0.001) and greater increases in serum ferritin (global p-interaction=0.02) compared with other iron formulations.

Co-supplementation

In factorial trials of iron and zinc supplementation (n=10), a borderline interaction was seen for iron effects on the prevalence of anemia, with stronger reductions in anemia among children not receiving zinc (prevalence ratio [PR] = 0.41, [0.33, 0.50]) vs. those receiving zinc (PR=0.64 [0.41, 0.62]) (p-interaction=0.048) (Table 1.4). No significant differences by zinc co-supplementation were seen for hemoglobin, ferritin, iron deficiency, or iron deficiency anemia, although effects generally appeared qualitatively stronger without zinc co-supplementation for each of these outcomes. Iron supplementation improved hemoglobin to a greater extent without vitamin A co-supplementation (12.1 g/L [10.3, 13.9]) than with vitamin A co-supplementation (5.9 g/L [2.1, 9.7]) (p-interaction=0.03), with similar but non-significant directions of interaction for prevalence of anemia and iron deficiency, but not ferritin.

Table 1.4. Comparison of iron supplementation effects within factorial trials.

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Zinc					
Iron+Zinc vs. Zinc					
n	10	9	6	6	5
Estimate	4.2	21.1	0.64	0.18	0.15
95% CI	(1.5, 6.9)	(16.0, 26.1)	(0.48, 0.84)	(0.12, 0.28)	(0.09, 0.24)
Iron vs. Control/Placebo					
n	10	9	6	6	5
Estimate	6.6	28.8	0.41	0.15	0.08
95% CI	(3.6, 9.6)	(22.2, 35.4)	(0.33, 0.50)	(0.09, 0.24)	(0.05, 0.15)
P-value for interaction	0.27	0.19	0.048	0.62	0.13
Vitamin A					
Iron+Vitamin A vs. Vitamin A					
n	4	1	2	1	0
Estimate	5.9	10.6	0.54	0.51	-
95% CI	(2.1, 9.7)	(7.8, 13.4)	(0.11, 2.56)	(0.31, 0.85)	-
Iron vs. Control/Placebo					
n	4	1	2	1	0
Estimate	12.1	8.2	0.27	0.34	-
95% CI	(10.3, 13.9)	(5.0, 11.4)	(0.16, 0.45)	(0.18, 0.66)	-
P-value for interaction	0.03	n/a	0.49	n/a	n/a

* Weighted mean difference

† Pooled risk ratio

Abbreviations: CI, confidence interval

Safety outcomes

No statistically significant changes due to oral iron supplementation were observed for anthropometric, infectious, or developmental indices (Table 1.2). In meta-regression analyses, no significant differential impacts of iron on these outcomes were seen by schedule, duration, or dose, except for an observed increase in the cumulative incidence of respiratory illness associated with iron supplements that were 1-2 per week, low dose, or lasting for ≥ 7 months (Appendix 8). However, these differential effects were due to a single factorial trial of iron and polyunsaturated fatty acids that differed from the other trials in terms of schedule, dose, and duration;(20) excluding this trial, no significant difference remained. Iron increased HAZ by 0.20 (95% CI: 0.07, 0.33) in trial arms composed exclusively of anemic children (but based on only n=2 trial arms); no effect on HAZ was seen among trial arms of non-anemic children (-0.01 [-0.20, 0.17]; n=3) or mixed anemic and non-anemic children (0.00 [-0.03, 0.03]; n=25) (p-interaction=0.013). Improvements in cognitive development were seen in one trial arm among anemic children (Bayley Cognitive SMD=1.39 [0.75, 2.04]; Bayley Motor SMD=1.46 [0.81, 2.11]) compared to no effect seen in 4 trial arms among non-anemic children (Bayley Cognitive SMD=0.29 [-0.12, 0.71]; Bayley Motor SMD=0.03 [-0.31, 0.38]) or 4 trial arms among mixed anemic and non-anemic children (Bayley Cognitive SMD=0.07 [-0.10, 0.23]; Bayley Motor SMD=0.11 [-0.16, 0.37]) (p-interaction=0.020 for cognitive and 0.023 for motor scores).

Risk of bias

The risk of bias within each trial is reported in Appendix 9. Many trials (21-78%) did not report sufficient information to calculate the risk of bias according to one or more of the five criteria

(Appendix 10). 47 of the 123 trials (38%) were judged to be at high risk of bias for at least one of the criteria, with incomplete outcome data being the most frequent reason for a study to be assessed at high risk of bias (n=25, 20%). In sensitivity analyses excluding these 47 studies, the effects of iron supplementation on hematologic outcomes were similar (Appendix 11).

Small study effects

For hematologic outcomes, studies with larger standard errors tended to demonstrate more protective effect sizes (Egger's test p-value < 0.05) (Appendix 12). Attenuated but still statistically significant benefits were obtained during trim-and-fill sensitivity analyses for hemoglobin (4.8 g/L [3.8, 5.8]) and ferritin (4.4 ng/mL [1.7, 7.0]).

Discussion

This systematic review and meta-analysis of 123 randomized controlled trials, including 166 trial arms of oral iron supplementation in children, demonstrates significant benefits on hematologic outcomes including hemoglobin (+6.5 g/L; 95% CI: 5.7, 7.4), ferritin (+19.1 ng/mL; 16.6, 21.7), and prevalence of anemia (40% reduction; 33%, 46%), iron deficiency (73% reduction; 65%, 79%), and iron deficiency anemia (81% reduction; 70%, 88%). Children under age 5 years were most frequently studied (63% of trial arms), including trials throughout this age range, but with meaningful numbers of trials among children age 5-11 years (26%) and adolescents age 12-19 years (11%). In sum, these findings provide strong evidence for the benefits of iron supplementation among children.

Importantly, the number and diversity of identified trials allowed us to assess factors that might modify these benefits. Our results suggest that frequent (3-7 times/week) and intermittent (1-2 times/week) iron supplementation may be equally effective at increasing hemoglobin and decreasing anemia, iron deficiency, and iron deficiency anemia. While the WHO recommends daily oral iron supplementation for all children in regions with an anemia prevalence of 40% or more, success of such programs may be threatened by low adherence from adverse gastrointestinal reactions or high caregiver burden to provide daily supplements.(21) Weekly iron supplementation has been promoted as an alternative to reduce these barriers.(22,23) Furthermore, since mammalian gastrointestinal epithelial cells turn over every 2 to 6 days, weekly supplementation may not be at a great disadvantage relative to daily supplementation with respect to the total amount of absorbed iron.(24,25) Some evidence points to changes in gastrointestinal epithelial cells following a large bolus of iron that results in reduced transport of iron into portal blood.(26) A prior meta-analysis of 21 trials concluded that, compared with daily iron supplements, intermittent supplementation had similar effects on hemoglobin levels but was less effective in reducing anemia.(27) Our findings, based on a much larger number of trials, suggest that frequent and intermittent supplementation are similarly effective in reducing anemia.

Studies of longer duration were more likely to have a lower prevalence of baseline anemia (Table S1.2). As a result, the apparently diminishing impacts of iron with increased duration were entirely explained upon control for baseline anemia (Table S1.3). Some prior studies have shown that impacts of iron on hemoglobin persist for several months after the cessation of supplementation,(28,29) though the period of durability likely depends on the availability of dietary iron, burden of infection, and degree of blood loss experienced by the population. The

findings of this study support the current WHO recommendation of a 3-month course of iron supplementation, though a longer duration may be considered in order to maintain hemoglobin levels.

For all ages, the lowest dose tertile of iron received less than the WHO recommended daily supplement (10-12.5 mg/day ages 6-23 mo, 30 mg/day ages 24-59 mo, 30-60 mg/day ages 5-12 y). Moderate age-adjusted doses appeared to be more effective than lower doses at increasing hemoglobin, but all doses effectively improved outcomes, even doses below current WHO recommendations. Interestingly, more frequent supplementation, longer durations of supplementation, and higher doses each were associated with greater increases in serum ferritin. These novel findings can inform the design of future supplementation programs, suggesting that flexibility is possible depending on specific aims.

Importantly, we found that benefits were generally similar across diverse ages from <5 months to >12 years, in males and in females, and across world regions. This supports initiation of iron supplementation programs in a diverse range of young populations. As might be expected, increases in hemoglobin and reductions in anemia were approximately twice as large in populations in which all children were anemic at baseline, compared with mixed or non-anemic populations. Yet even among children who were non-anemic at baseline, iron supplementation effectively reduced the future risk of anemia by 37%, and of iron deficiency and iron deficiency anemia by about 80%. These findings indicate that iron supplementation is effective for primary prevention among at-risk children.

Factorial trials of iron and zinc supplementation (n=10) found that co-supplementation of iron with zinc did not diminish impacts on hemoglobin, serum ferritin, iron deficiency, or iron deficiency anemia. However, there is some evidence that iron supplementation alone decreases anemia more than when given along with zinc supplementation. Uptake of both iron and zinc is mediated by divalent metal transporter-1 and ferroportin, which may result in absorptive antagonism.(30,31) However, the evidence from this meta-analysis suggests that in contexts where prevalent zinc deficiency is suspected, co-supplementation of iron can still yield population benefits.

The benefit of iron on hemoglobin was attenuated in the presence of vitamin A (n=4 trials). The mechanism of iron and vitamin A interaction remains incompletely understood. Prior research has indicated that vitamin A may increase nonheme iron absorption, which is perhaps achieved through mobilizing iron stores or stimulating the synthesis of transferrin.(32-34) However, one bioavailability study in non-anemic adults found that absorption of a single dose of iron (10 mg) was increased when co-supplemented with a dose of vitamin A of 1500 or 3000 IU, but inhibited when co-supplemented with dose of vitamin A of 6,000 IU.(35) Two of the trials in this meta-analysis gave single vitamin A doses of 200,000 IU at enrollment, while the other two gave daily doses of 5,000 or 10,000 (approximately 400,000 to 600,000 IU total over the study period). Although this evidence was generated by only four trials, the factorial nature of these studies ensures comparability between groups, and the large effect sizes resulted in statistical significance. When planning population interventions, the benefits of vitamin A supplementation should be considered in combination with the risks of diminished iron effects on hemoglobin.

Importantly, we also evaluated the evidence for effects of iron supplementation on non-hematologic outcomes. No effects were seen on child growth or infections, consistent with results of prior meta-analyses based on fewer studies.(9-11) While 40 trials reported data on height-for-age, data on infections were less common and reported as a more diverse set of variables (e.g. incidence, prevalence, mean number of episodes, average duration). Furthermore, numerous studies did not report quantitative data for infectious morbidity or excluded children on the basis of an infection, creating a possibility of reporting and selection bias. A diverse set of measures and reporting scales was similarly used to assess cognitive function; using the most frequently reported measure, the Bayley Index, we found some evidence of benefits of iron supplementation on mental performance ($p=0.05$).

Potential limitations of this research should be considered. Many studies had insufficient information that could be used to determine risk of bias, thereby impeding the exploration of iron effects according to study quality. Furthermore, when data was available on adherence to iron supplements, it was reported in numerous ways, similarly preventing a quantitative summary. As with all meta-analyses, publication bias cannot be ruled out; although trim-and-fill methods suggested that conclusions would not materially change with potentially missing studies. Lastly, results for effect heterogeneity can be confounded due to co-linear effect modifiers. Although we conduct sensitivity analyses controlling for baseline anemia, the lack of individual-level data reduces the degree to which this and other factors can be precisely controlled.

The 2016 WHO guidelines on iron supplementation in infants and children were informed by systematic reviews of randomized controlled iron supplementation trials that found clear benefits of iron supplementation on hematologic outcomes.(9-12) In this large systematic review of iron supplementation trials, our work extends these prior meta-analyses by exploring effect heterogeneity according to schedule, duration, dose, and co-supplementation regimen. We find that the evidence supports the currently recommended dose and duration of iron supplementation, although weekly supplementation might be considered as an alternative to the recommended daily supplementation. Furthermore, decisions to co-supplement iron with zinc or vitamin A should consider the population effect of these other nutrient supplements, as there is some evidence that the impacts of iron on hematologic parameters is attenuated by co-supplementation. Our findings could be considered in clinical decision-making and the development of further guidelines on oral iron supplementation among children and adolescents.

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Supplemental Appendix

Appendix S1.1: Search terms.

Title and abstract searches of the terms below were performed in PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials from inception through April 1, 2017.

Infant OR Infan* OR newborn* OR new-born* OR perinat* OR neonat* OR child OR child OR child* OR children* OR schoolchild* OR schoolchild OR "school child" OR adolescent OR adolescen* OR youth* OR teen* OR pubescen* OR pediatrics OR pediatric* OR paediatric* OR peadiatric* OR school OR school* OR prematur* OR preterm* OR "Pregnant Women" OR Pregnancy OR pregnan* OR gravid OR obstetric OR antenatal OR antepartum OR gestation* OR lactation OR "breast feeding" OR lactation OR "breast feeding"[Title/Abstract] OR breastfe* OR breastfe* OR breast-milk OR breastmilk OR "lactating mother*" OR "lactating woman" OR "lactating women"

AND

iron OR hematinics OR "ferrous" OR "ferric" OR "hematinic" OR "haematinic" OR "haematinics" OR "iron compounds"

AND

“randomized controlled trial” OR “controlled clinical trial” OR “clinical trial” OR randomized
OR randomised OR placebo OR random* OR trial

Appendix S1.2: Variables extracted from eligible studies.

Study characteristics

Endnote Record Number
First author (last name)
Publication year
Corresponding author (full name)
Corresponding author (email address)
Study name
Country
Rural/Urban
Altitude for the study site (meters)
According to study report, is malaria endemic?
According to study report, are helminths endemic?
Is this a subgroup analysis of a priorly extracted study?
Which subgroup is being reported here?
Does this paper reference other publications using the same trial or data? If so, indicate the Endnote record numbers for those studies.
Notes on this paper

Intervention characteristics

Mode of supplementation
Dose of intervention (mg of elemental iron)
Dose of intervention (mg) per kg bodyweight
Mean body weight for the intervention group (kg).
Frequency of intervention
Unit of intervention frequency
Formulation of Iron
Total dose of supplement (used for conversion to elemental iron if elemental unavailable)
Unit for dose
Duration of the intervention (weeks) (there are 4.3 weeks per month)
Is outcome data post intervention endline available?
Year at baseline (if range pick median)
Were all children in the study dewormed as part of the study protocol?
What other compounds were given along with iron (to both intervention & control groups)?
Is this a factorial trial?
Daily dose consumed of other compound given.
Unit for daily dose of other compound given.
Did the control group receive a placebo?
Is the control group being compared to multiple intervention groups?
Population group targeted by intervention

List study inclusion and exclusion criteria
Is compliance reported in the paper?
Indicate the compliance data reported
Other notes on intervention

Population characteristics

Is population characteristic data given for the full sample or for the control group only? (Use the full sample data whenever possible.)
Proportion of female children at baseline (%)
Child age at supplementation start/baseline (mean/median months)
Child age at baseline (SD)
Child age at baseline (min)
Child age at baseline (max)
Child age at outcome assessment (mean/median months)
Child age at outcome assessment (SD)
Age reported if other than age at supplementation start
Mean HAZ at baseline
Mean WHZ at baseline
Mean WAZ at baseline
Mean BAZ at baseline
Proportion of iron deficient children if reported (%)
Cutoff used to define iron deficiency
Proportion of anemic children if reported (%)
Cutoff used to define anemia (g/dl)
Proportion of children with iron deficiency anemia (%)
Cutoff used to define iron deficiency anemia
Proportion of children stunted at baseline (%)
Proportion of children low birth weight at baseline (%)
Cutoff used to define low birth weight (g)
Proportion of children born premature (%)
Cutoff used to define prematurity (wks)
Maternal age at baseline (mean/median years)
Maternal age at baseline (SD)
If pregnant, gestational age at baseline (mean/median weeks)
If pregnant, gestational age (sd)
If any family socioeconomic status measures are reported, please describe
Proportion of mothers who are iron deficient, if reported (%)
Proportion of mothers anemic at baseline, if reported (%)

Outcome characteristics

Specify outcome
Outcome unit
Method of Assessment
Extra information pertaining to outcome
Comparator group

How many weeks after baseline was the outcome measured?
 How many participants were initially recruited for treatment?
 How many participants were initially recruited for control?
 Intervention total group sample size (total analyzed)
 Was the difference-in-differences measure calculated in an adjusted regression model?
 Difference in the differences between baseline & follow-up between intervention & control group (mean)
 Difference in the differences between baseline & follow-up between intervention & control group (SD)
 Difference in the differences between baseline & follow-up between intervention & control group (SE)
 Difference in the differences between baseline & follow-up between intervention & control group (LCI)
 Difference in the differences between baseline & follow-up between intervention & control group (UCI)
 Difference in the differences between baseline & follow-up between intervention & control group (p-value)
 Intervention group baseline sample size
 Intervention group baseline estimate (mean)
 Intervention group baseline estimate (SD)
 Intervention group baseline estimate (SE)
 Intervention group baseline estimate (LCI)
 Intervention group baseline estimate (UCI)
 Intervention group baseline estimate (LIQR)
 Intervention group baseline estimate (UIQR)
 Intervention group follow-up sample size
 Intervention group follow-up estimate (mean)
 Intervention group follow-up estimate (SD)
 Intervention group follow-up estimate (SE)
 Intervention group follow-up estimate (LCI)
 Intervention group follow-up estimate (UCI)
 Intervention group follow-up estimate (LIQR)
 Intervention group follow-up estimate (UIQR)
 Was the intervention group difference measure calculated in an adjusted regression model?
 Intervention group difference sample size
 Intervention group difference in outcome between baseline & follow-up (mean)
 Intervention group difference in outcome between baseline & follow-up (SD)
 Intervention group difference in outcome between baseline & follow-up (SE)
 Intervention group difference in outcome between baseline & follow-up (LCI)
 Intervention group difference in outcome between baseline & follow-up (UCI)
 Intervention group difference in outcome between baseline & follow-up (LIQR)
 Intervention group difference in outcome between baseline & follow-up (UIQR)
 Intervention group difference in outcome between baseline & follow-up (p-value)

Control group baseline sample size
Control group baseline estimate (mean)
Control group baseline estimate (SD)
Control group baseline estimate (SE)
Control group baseline estimate (LCI)
Control group baseline estimate (UCI)
Control group baseline estimate (LIQR)
Control group baseline estimate (UIQR)
Control group follow-up sample size
Control group follow-up estimate (mean)
Control group follow-up estimate (SD)
Control group follow-up estimate (SE)
Control group follow-up estimate (LCI)
Control group follow-up estimate (UCI)
Control group follow-up estimate (LIQR)
Control group follow-up estimate (UIQR)
Was the control group difference measure calculated in an adjusted regression model?
Control group difference sample size
Control group difference in outcome between baseline & follow-up (mean)
Control group difference in outcome between baseline & follow-up (SD)
Control group difference in outcome between baseline & follow-up (SE)
Control group difference in outcome between baseline & follow-up (LCI)
Control group difference in outcome between baseline & follow-up (UCI)
Control group difference in outcome between baseline & follow-up (LIQR)
Control group difference in outcome between baseline & follow-up (UIQR)
Control group difference in outcome between baseline & follow-up (p-value)
Flag indicating whether SD/SE was imputed during data entry
Note other outcomes reported in the study but not extracted

Subgroup data

Was sub-group data on outcome presented by gender?
Was sub-group data on outcome presented by age group?
Was sub-group data on outcome presented by SES group?
Was sub-group data on outcome presented by baseline anemia status?
Was sub-group data on outcome presented by baseline iron status?
Was sub-group data on outcome presented by baseline stunting/birthweight status?
Other subgroup reported? (specify)

Cochrane risk of bias assessment

Method for generating randomization sequence
How were individuals randomly assigned to treatment?
Number of clusters randomized (total)
Risk of bias due to randomization method?
Method for concealing treatment allocation
Risk of bias due to allocation concealment?

Were participants blinded?
Were supplementation providers blinded?
Bias due to lack of participant or supplement provider blinding?
Were outcome assessors blinded?
Bias due to lack of outcome assessor blinding?
Percent drop-out in intervention group
Percent drop-out in control group
Were reasons for withdrawals similar across treatment groups?
Were reasons for withdrawals associated with other important covariates?
If withdrawals were related to the outcome, describe the reason for withdrawal.
Were appropriate methods used to impute missing data?
Was an intention-to-treat analyses conducted (i.e. analyzed as randomized)?
Was a per protocol analysis conducted?
Risk of bias due to incomplete outcome data?
Were data reported for all pre-specified outcomes?
Were data reported for all pre-specified sub-group analyses
Risk of bias due to selective outcome reporting?

Table S1.1. Elemental iron supplementation dose tertile ranges.

	Low	Tertile Middle	High
mg/week			
0 - 5 mo	18.2 - 49.7	51.5 - 70	79.8 - 99.4
6 - 23 mo	20 - 70	87.5 - 87.5	105 - 462
24 - 59 mo	20 - 80	114 - 207.9	216.3 - 420
5 - 11 y	18.2 - 120	143.6 - 320.8	420 - 420
12 - 19 y	50 - 150	250 - 300	350 - 1820
mg/day			
0 - 5 mo	2.6 - 7.1	7.4 - 10	11.4 - 14.2
6 - 23 mo	2.9 - 10	12.5 - 12.5	15 - 66
24 - 59 mo	2.9 - 11.4	16.3 - 29.7	30.9 - 60
5 - 11 y	2.6 - 17.1	20.5 - 45.8	60 - 60
12 - 19 y	7.1 - 21.4	35.7 - 42.9	50 - 260

Note 1: The recommended dose of iron increases as children age. To account for the strong correlation between age and dose, we grouped children into five age categories and then categorized them into low, moderate, or high tertiles based on the total amount of iron they received each week.

Note 2: Tertiles reported here are for studies which reported hematologic outcomes and had sufficient data to calculate dose (n=94 iron treatment groups).

Appendix S1.3. References to included studies.

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Table S1.2. Characteristics of included studies.

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Adish, 1997	Ethiopia	24 mo - 5 yr	30 mg elemental Fe as ferrous sulfate daily for 3 months	Placebo; or Placebo + Vitamin A (factorial trial)	n=399 total participants analyzed; n=196 treatment, n=203 control	Hemoglobin, Diarrhea, Respiratory infection
Aggarwal et al, 2005	India	50 days - 80 days	3 mg/kg elemental Fe as ferric ammonium citrate daily for 8 weeks	Placebo	n=26 total participants analyzed; n=13 treatment, n=13 control	Hemoglobin, Ferritin
Aguayo et al, 2000	Bolivia	6 yr - 12 yr	3 mg/kg elemental Fe as ferrous sulfate 1 time per week for 18 weeks	Placebo	n=64 total participants analyzed; n=33 treatment, n=31 control	Hemoglobin, Anemia, HAZ, WAZ
Akman et al, 2004	Turkey	6 mo - 30 mo	6 mg/kg elemental Fe as ferrous glisine-sulphate daily for 3 months	Control	n=40 total participants analyzed; n=21 treatment, n=19 control	Hemoglobin, Ferritin
Angeles et al, 1993	Indonesia	24 mo - 6 yr	30 mg elemental Fe as ferrous sulfate + Vitamin C daily for 2 months	Placebo + Vitamin C	n=76 total participants analyzed; n=39 treatment, n=37 control	Hemoglobin, Ferritin, HAZ, WHZ, WAZ, Diarrhea, Respiratory infection
Angulo-Barro et al, 2016	China	~1 mo (mean age)	1 mg/kg elemental Fe as iron protein succinylate daily for 7.5 months	Placebo; or Placebo + Maternal iron (factorial trial)	n=1196 total participants analyzed; n=610 treatment, n=586 control	Cognitive
Arcanjo et al, 2011	Brazil	~5 yr (mean age)	50 mg elemental Fe as ferrous sulfate 1 time per week for 14 weeks	Placebo	n=99 total participants analyzed; n=50 treatment, n=49 control	Hemoglobin, Anemia
Arcanjo et al, 2013	Brazil	12 mo - 24 mo	25 mg elemental iron (formulation not specified) 1 time per week for 4 months; 12.5 mg elemental iron (formulation not specified) daily for 4 months	Placebo	n=176 total participants analyzed; n=120 treatment, n=56 control	Hemoglobin, Anemia

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Aukett et al, 1986	United Kingdom	17 mo - 19 mo	24 mg elemental Fe as ferrous sulfate + Vitamin C daily for 2 months	Placebo + Vitamin C	n=97 total participants analyzed; n=48 treatment, n=49 control	Hemoglobin, Ferritin, Anemia, Iron deficiency
Ayoya et al, 2009	Mali	7 yr - 13 yr	60 mg elemental Fe as ferrous sulfate + Praziquantal 5 times per week for 12 weeks	Control + Praziquantal	n=202 total participants analyzed; n=105 treatment, n=97 control	Hemoglobin, Ferritin, Anemia, Iron deficiency anemia, Malaria
Ayoya et al, 2012	Mali	7 yr - 13 yr	60 mg elemental Fe as ferrous sulfate + Praziquantal 5 times per week for 12 weeks	Control + Praziquantal	n=202 total participants analyzed; n=105 treatment, n=97 control	Cognitive
Ballin et al, 1992	Israel	16 yr - 18 yr	105 mg elemental Fe as iron polystyrene sulfonate adsorbate daily for 2 months	Placebo	n=59 total participants analyzed; n=29 treatment, n=30 control	Iron deficiency
Baqui et al, 2003	Bangladesh	~6 mo (mean age)	20 mg elemental Fe as ferrous sulfate 1 time per week for 6 months	Placebo + Riboflavin; or Placebo + Zinc + Riboflavin (factorial trial)	n=249 total participants analyzed; n=125 treatment, n=124 control	Hemoglobin, Ferritin, Diarrhea, Respiratory infection
Barclay et al, 1991	United Kingdom	~1 mo (mean age)	13.8 mg elemental Fe as NaFeEDTA daily for 16 weeks; 7 mg elemental Fe as NaFeEDTA daily for 16 weeks	Control + Mutivitamin	n=55 total participants analyzed; n=36 treatment, n=19 control	Hemoglobin, Ferritin
Baumgartner et al, 2012	South Africa	6 yr - 12 yr	50 mg elemental Fe as ferrous sulfate 4 times per week for 8.5 months	Placebo + Vitamin C; or Placebo + n-3 fatty acids + Vitamin C (factorial trial)	n=294 total participants analyzed; n=145 treatment, n=149 control	Hemoglobin, Ferritin, HAZ, WHZ, WAZ

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Berger et al, 1997	Bolivia	3 yr - 8 yr	3-4 mg/kg elemental Fe as ferrous sulfate 1 time per week for 16 weeks; 3-4 mg/kg elemental Fe as ferrous sulfate 5 times per week for 16 weeks	Placebo	n=173 total participants analyzed; n=116 treatment, n=57 control	Hemoglobin, Anemia
Berger et al, 2000	Togo	6 mo - 3 yr	2-3 mg/kg elemental Fe as iron betainate daily for 3 months	Placebo	n=163 total participants analyzed; n=84 treatment, n=79 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Diarrhea, Respiratory infection, Malaria
Berger et al, 2006	Vietnam	4 mo - 7 mo	10 mg elemental Fe as ferrous sulfate 7 times per week for 6 months	Placebo + Vitamin A; or Placebo + Vitamin A + Zinc (factorial trial)	n=770 total participants analyzed; n=384 treatment, n=386 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia, HAZ, WHZ, WAZ, Stunting, Wasting, Diarrhea, Respiratory infection
Berglund et al, 2010	Sweden	~1 mo (mean age)	1 mg/kg elemental Fe as ferrous succinate daily for 4.5 months; 2 mg/kg elemental Fe as ferrous succinate daily for 4.5 months	Placebo	n=243 total participants analyzed; n=160 treatment, n=83 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Bhatia et al, 1993	India	~4 yr (mean age)	40 mg elemental iron (formulation not specified) daily for 6 months	Placebo	n=156 total participants analyzed; n=84 treatment, n=72 control	Hemoglobin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Black et al, 2004	Bangladesh	~7 mo (mean age)	20 mg elemental Fe as ferrous sulfate 1 time per week for 6 months	Placebo + Riboflavin + Vitamin A; or Placebo + Zinc + Riboflavin + Vitamin A (factorial trial)	n=186 total participants analyzed; n=92 treatment, n=94 control	Cognitive
Bruner et al, 1996	USA	13 yr - 19 yr	260 mg elemental Fe as ferrous sulfate daily for 8 weeks	Placebo	n=73 total participants analyzed; n=37 treatment, n=36 control	Hemoglobin, Ferritin
Burman et al, 1972	United Kingdom	~3 mo (mean age)	10 mg elemental Fe as colloidal ferric hydroxide daily for 21 months	Placebo	n=192 total participants analyzed; n=86 treatment, n=106 control	Hemoglobin
Buzina-Suboticaneć et al, 1998	Croatia	8 yr - 10 yr	100 mg elemental Fe as ferri-glycine sulfate 6 times per week for 10 weeks	Placebo	n=60 total participants analyzed; n=31 treatment, n=29 control	Hemoglobin, Cognitive
Charoenlarp et al, 1980	Thailand	6 yr - 13 yr	40 mg elemental Fe as ferrous sulfate 5 times per week for 5 months	Placebo	n=72 total participants analyzed; n=33 treatment, n=39 control	Hemoglobin, Helminth Infection
Chen et al, 2011	China	3 yr - 7 yr	12 mg elemental Fe as NaFeEDTA + Vitamin A 5 times per week for 26 weeks	Placebo + Vitamin A	n=132 total participants analyzed; n=71 treatment, n=61 control	Hemoglobin, Ferritin
Chen et al, 2013	China	3 yr - 7 yr	1.5 mg/kg elemental Fe as ferrous sulfate 5 times per week for 6 months	Placebo; or Placebo + Vitamin A (factorial trial)	n=387 total participants analyzed; n=188 treatment, n=199 control	Diarrhea, Respiratory infection
Chen et al, 2014	China	3 yr - 6 yr	1-2 mg/kg elemental Fe as ferrous sulfate 5 times per week for 6 months	Control; or Control + Vitamin A (factorial trial)	n=387 total participants analyzed; n=188 treatment, n=199 control	Hemoglobin, Ferritin, Anemia, Iron deficiency

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Cheng et al, 2001	China	7 yr - 12 yr	2.6 mg elemental Fe as daily for 1 month	Placebo	n=108 total participants analyzed; n=53 treatment, n=55 control	Hemoglobin
Choe et al, 1999	Korea	10 yr - 18 yr	6 mg/kg elemental Fe as ferrous sulfate + H. pylori therapy (bismuth subcitrate, amoxicillin, metronidazole) daily for 10 weeks	Placebo + H. pylori therapy (bismuth subcitrate, amoxicillin, metronidazole)	n=11 total participants analyzed; n=6 treatment, n=5 control	Hemoglobin, Ferritin
Chwang et al, 1988	Indonesia	8 yr - 14 yr	2 mg/kg elemental Fe as ferrous sulfate 5 times per week for 12 weeks	Placebo	n=119 total participants analyzed; n=59 treatment, n=60 control	Hemoglobin, HAZ, WAZ
Das et al, 1984	India	6 mo - 7 yr	5 mg elemental Fe as neoferrum infants drop 5 times per week for 12 weeks; 10 mg elemental Fe as neoferrum infants drop 5 times per week for 12 weeks; 20 mg elemental Fe as neoferrum infants drop 2 times per week for 12 weeks; 40 mg elemental Fe as neoferrum infants drop 2 times per week for 12 weeks; 20 mg elemental Fe as neoferrum infants drop 1 time per week for 12 weeks; 40 mg elemental Fe as neoferrum infants drop 1 time per week for 12 weeks	Placebo	n=151 total participants analyzed; n=115 treatment, n=36 control	Hemoglobin
de Silva et al, 2003	Sri Lanka	5 yr - 11 yr	60 mg elemental Fe as ferrous sulfate daily for 8 weeks	Placebo	n=363 total participants analyzed; n=261 treatment, n=102 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Diarrhea, Respiratory infection

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Desai et al, 2003	Kenya	2 mo - 3 yr	3.83 mg/kg elemental Fe as ferrous sulfate daily for 12 weeks	Placebo; or Placebo + Antimalarial (sulfadoxine-pyrimethamine at 4 and 8 weeks) (factorial trial)	n=491 total participants analyzed; n=256 treatment, n=235 control	Hemoglobin, Anemia, Malaria
Devaki et al, 2008	India	15 yr - 19 yr	100 mg elemental Fe as iron(iii) hydroxide polymaltose complex 6 times per week for 8 months	Placebo	n=60 total participants analyzed; n=30 treatment, n=30 control	Hemoglobin, Ferritin
Dewey et al, 2002	Sweden	~6 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 5 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 5 months	Placebo	n=172 total participants analyzed; n=136 treatment, n=36 control	HAZ, WAZ, Diarrhea
Dijkhuizen et al, 2001	Indonesia	~4 mo (mean age)	10 mg elemental Fe as ferrous sulfate 5 times per week for 6 months	Placebo; or Placebo + Zinc (factorial trial)	n=360 total participants analyzed; n=172 treatment, n=188 control	Hemoglobin, Ferritin, Anemia, Iron deficiency anemia, HAZ, WHZ, WAZ, Stunting, Wasting
Dijkhuizen et al, 2008	Indonesia, Thailand, Vietnam	4 mo - 6 mo	10 mg elemental Fe as ferrous sulfate + Zinc to half the population 5-7 times per week for 6 months	Placebo	n=2451 total participants analyzed; n=1212 treatment, n=1239 control	HAZ, WHZ, WAZ, Stunting, Wasting, Underweight

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Domellöf et al, 2001	Sweden	~6 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 5 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 1 mg/kg elemental Fe as ferrous sulfate daily for 5 months	Placebo	n=171 total participants analyzed; n=136 treatment, n=35 control	Hemoglobin, Ferritin
Dossa et al, 2001	Benin	3 yr - 6 yr	60 mg elemental Fe as ferrous sulfate daily for 3 months	Placebo; or Placebo + Albendazole (factorial trial)	n=140 total participants analyzed; n=70 treatment, n=70 control	Hemoglobin, HAZ, WHZ
4 Dossa et al, 2001	Benin	18 mo - 30 mo	66 mg elemental Fe as ferrous fumarate daily for 6 weeks	Placebo	n=74 total participants analyzed; n=35 treatment, n=39 control	Hemoglobin
Eftekhari et al, 2006	Iran	14 yr - 19 yr	60 mg elemental Fe as ferrous sulfate 5 times per week for 12 weeks	Placebo; or Placebo + Iodine (factorial trial)	n=94 total participants analyzed; n=47 treatment, n=47 control	Hemoglobin, Ferritin
Elwood et al, 1970	United Kingdom	14 yr - 15 yr	10 mg elemental Fe as ferrous fumarate daily for 36 months; 30 mg elemental Fe as ferrous fumarate daily for 36 months	Placebo + Riboflavin	n=601 total participants analyzed; n=386 treatment, n=215 control	Hemoglobin
Engstrom et al, 2008	Brazil	5 mo - 7 mo	12.5 mg elemental Fe as ferrous sulfate daily for 24 weeks; 25 mg elemental Fe as ferrous sulfate 1 time per week for 24 weeks	Placebo	n=391 total participants analyzed; n=297 treatment, n=94 control	Hemoglobin, Anemia

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Ermis et al, 2002	Turkey	~5 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate every other day for 4 months; 2 mg/kg elemental Fe as ferrous sulfate every other day for 4 months; 2 mg/kg elemental Fe as ferrous sulfate every other day for 4 months	Placebo	n=107 total participants analyzed; n=84 treatment, n=23 control	Hemoglobin, Ferritin, Iron deficiency, Iron deficiency anemia
Fahmida et al, 2007	Indonesia	3 mo - 6 mo	10 mg elemental Fe as ferrous sulfate + Zinc + Vitamin A daily for 6 months	Placebo + Zinc + Vitamin A	n=377 total participants analyzed; n=186 treatment, n=191 control	Hemoglobin, Ferritin, Anemia, HAZ, WHZ, WAZ, Stunting, Wasting, Underweight
Fallahi et al, 2007	Iran	~11 yr (mean age)	20 mg elemental Fe as ferrous sulfate + Zinc 6 times per week for 4 months	Placebo + Zinc	n=54 total participants analyzed; n=26 treatment, n=28 control	Hemoglobin, Ferritin
Franz et al, 2000	Germany	~0 mo (mean age)	2-4 mg/kg elemental Fe as ferrous sulfate daily for 8 weeks	Control	n=133 total participants analyzed; n=68 treatment, n=65 control	Ferritin, Iron deficiency
Friel et al, 2003	Canada	~1 mo (mean age)	7.5 mg elemental Fe as ferrous sulfate daily for 5 months	Placebo	n=49 total participants analyzed; n=28 treatment, n=21 control	Hemoglobin, Ferritin, Iron deficiency, Iron deficiency anemia
Fujiu et al, 2004	Japan	~1 mo (mean age)	4 mg/kg elemental iron (formulation not specified) + Recombinant human erythropoietin daily for 8 weeks	Control + Recombinant human erythropoietin	n=24 total participants analyzed; n=12 treatment, n=12 control	Hemoglobin, Ferritin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Gebresellacie, 1996	Ethiopia	5 yr - 15 yr	60 mg elemental Fe as ferrous sulfate daily for 12 weeks	Placebo	n=480 total participants analyzed; n=239 treatment, n=241 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Malaria
Geltman et al, 2001	USA	~6 mo (mean age)	10 mg elemental iron (formulation not specified) + Multivitamin (A, B, C, D, E) daily for 3 months	Placebo + Multivitamin (A, B, C, D, E)	n=240 total participants analyzed; n=117 treatment, n=123 control	Anemia, Iron deficiency
Geltman et al, 2004	USA	~6 mo (mean age)	10 mg elemental Fe as + Multivitamin daily for 3 months	Placebo + Multivitamin	n=284 total participants analyzed; n=138 treatment, n=146 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Gokcay et al, 2012	Turkey	~6 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate + Vitamin D daily for 6 months	Control + Vitamin D	n=105 total participants analyzed; n=51 treatment, n=54 control	Hemoglobin, Anemia
Gopaldas et al, 1985	India	8 yr - 16 yr	26.2 mg elemental Fe as ferrous sulfate daily for 2 months; 37.4 mg elemental Fe as ferrous sulfate daily for 2 months	Placebo	n=210 total participants analyzed; n=140 treatment, n=70 control	Hemoglobin, Anemia
Gopaldas et al, 1985	India	8 yr - 16 yr	30 mg elemental Fe as ferrous sulfate daily for 2 months; 40 mg elemental Fe as ferrous sulfate daily for 2 months	Placebo	n=48 total participants analyzed; n=32 treatment, n=16 control	Cognitive
Greisen et al, 1986	Zambia	5 yr - 16 yr	66 mg elemental Fe as ferrous fumarate 5 times per week for 6 weeks	Placebo	n=430 total participants analyzed; n=218 treatment, n=212 control	Hemoglobin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Hacihamdioglu et al, 2013	Turkey	~4 mo (mean age)	10 mg elemental Fe as ferrous sulfate daily for 2 months	Control	n=53 total participants analyzed; n=27 treatment, n=26 control	Hemoglobin, Ferritin, Iron deficiency
Harvey et al, 1989	Papua New Guinea	8 yr - 13 yr	130 mg elemental Fe as ferrous sulfate 5 times per week for 16 weeks	Placebo	n=298 total participants analyzed; n=156 treatment, n=142 control	Hemoglobin, Malaria
Hathirat et al, 1992	Thailand	9 yr - 12 yr	93.75 mg elemental Fe as ferrous sulfate 5 times per week for 16 weeks	Placebo	n=1772 total participants analyzed; n=885 treatment, n=887 control	Hemoglobin, Ferritin
Hess et al, 2002	Ivory Coast	5 yr - 15 yr	60 mg elemental Fe as ferrous sulfate 4 times per week for 16 weeks	Placebo	n=166 total participants analyzed; n=85 treatment, n=81 control	Hemoglobin, Ferritin, Anemia, Iron deficiency
Hettiarachchi et al, 2008	Sri Lanka	12 yr - 17 yr	50 mg elemental Fe as ferrous fumarate 5 times per week for 24 weeks	Placebo; or Placebo + Zinc (factorial trial)	n=774 total participants analyzed; n=392 treatment, n=382 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, HAZ, WAZ
Hieu et al, 2012	Vietnam	6 yr - 10 yr	1-2 mg/kg elemental Fe as ferrous fumarate 1 time per week for 21-23 weeks	Control	n=221 total participants analyzed; n=95 treatment, n=126 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Hop et al, 2005	Vietnam	6 mo - 12 mo	10 mg elemental Fe as ferrous fumarate 7 times per week for 6 months	Placebo	n=152 total participants analyzed; n=75 treatment, n=77 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, HAZ, WHZ, WAZ
Idjradinata et al, 1993	Indonesia	12 mo - 18 mo	3 mg/kg elemental Fe as ferrous sulfate daily for 4 months	Placebo	n=119 total participants analyzed; n=60 treatment, n=59 control	Hemoglobin, Ferritin
Idjratinata et al, 1994	Indonesia	12 mo - 18 mo	3 mg/kg elemental Fe as ferrous sulfate daily for 16 weeks	Placebo	n=44 total participants analyzed; n=22 treatment, n=22 control	HAZ, WAZ

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Irigoyen et al, 1991	USA	~6 mo (mean age)	3 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 6 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Placebo	n=225 total participants analyzed; n=150 treatment, n=75 control	Hemoglobin, Ferritin, Diarrhea
Kapur et al, 2003	India	9 mo - 3 yr	20 mg elemental Fe as ferium, m/s emcure pharm india 1 time per week for 8 weeks	Placebo; or Placebo + Nutrition education (factorial trial)	n=232 total participants analyzed; n=116 treatment, n=116 control	Hemoglobin, Ferritin
Kashyap et al, 1987	India	8 yr - 15 yr	60 mg elemental Fe as ferrous sulfate daily for 4 months	Placebo	n=166 total participants analyzed; n=83 treatment, n=83 control	Hemoglobin, Anemia
Kashyap et al, 1987	India	8 yr - 15 yr	60 mg elemental Fe as ferrous sulfate daily for 4 months	Placebo	n=130 total participants analyzed; n=65 treatment, n=65 control	Cognitive
Kianfar et al, 2000	Iran	~16 yr (mean age)	50 mg elemental Fe as ferrous sulfate daily for 3 months; 50 mg elemental Fe as ferrous sulfate 2 times per week for 3 months; 50 mg elemental Fe as ferrous sulfate 1 time per week for 3 months	Control	n=268 total participants analyzed; n=194 treatment, n=74 control	Hemoglobin, Ferritin
Kordas et al, 2005	Mexico	~7 yr (mean age)	30 mg elemental Fe as ferrous fumarate daily for 6 months	Placebo	n=527 total participants analyzed; n=271 treatment, n=256 control	Cognitive

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Lambert et al, 2002	New Zealand	12 yr - 18 yr	105 mg elemental Fe as ferrous sulfate daily for 8 weeks	Placebo	n=116 total participants analyzed; n=57 treatment, n=59 control	Hemoglobin, Ferritin
Latham et al, 1990	Kenya	~8 yr (mean age)	80 mg elemental Fe as ferrous sulfate daily for 15 weeks	Placebo	n=55 total participants analyzed; n=29 treatment, n=26 control	Hemoglobin, Malaria, Helminth Infection
Lawless et al, 1994	Kenya	6 yr - 12 yr	47 mg elemental Fe as ferrous sulfate daily for 14 weeks	Placebo	n=86 total participants analyzed; n=44 treatment, n=42 control	Hemoglobin, Ferritin, HAZ, WHZ, WAZ, Diarrhea, Malaria
Lee et al, 1997	Korea	12 yr - 16 yr	60 mg elemental Fe as ferrous sulfate daily for 9 weeks	Placebo	n=15 total participants analyzed; n=9 treatment, n=6 control	Hemoglobin, Ferritin
Leenstra et al, 2009	Kenya	12 yr - 19 yr	120 mg elemental Fe as ferrous sulfate + Vitamin A 1 time per week for 3 months	Placebo + Vitamin A	n=249 total participants analyzed; n=124 treatment, n=125 control	Hemoglobin, Ferritin, Anemia, Malaria
Lind et al, 2003	Indonesia	~6 mo (mean age)	10 mg elemental Fe as ferrous sulfate daily for 6 months	Placebo + Vitamin C; or Placebo + Vitamin C + Zinc (factorial trial)	n=549 total participants analyzed; n=272 treatment, n=277 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Lind et al, 2004	Indonesia	~6 mo (mean age)	10 mg elemental Fe as ferrous sulfate daily for 6 months	Placebo + Vitamin C; or Placebo + Zinc + Vitamin C (factorial trial)	n=656 total participants analyzed; n=324 treatment, n=332 control	HAZ, WHZ, WAZ, Stunting, Wasting, Underweight
Loría et al, 1979	Mexico	~2 mo (mean age)	1-2 mg/kg elemental Fe as ferrous sulfate daily for 16 months	Placebo; or Placebo + Prenatal iron (factorial trial)	n=92 total participants analyzed; n=48 treatment, n=44 control	Hemoglobin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Lozoff et al, 2016	China	~2 mo (mean age)	1 mg/kg elemental Fe as iron proteinsuccinylate oral solution daily for 7.5 months	Placebo; or Placebo + Maternal iron (factorial trial)	n=1276 total participants analyzed; n=648 treatment, n=628 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia, HAZ, WHZ, WAZ
López de Romaña et al, 2005	Peru	6 mo - 12 mo	10 mg elemental Fe as ferrous fumarate 7 times per week for 6 months	Placebo	n=146 total participants analyzed; n=74 treatment, n=72 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, HAZ, WHZ, WAZ, Diarrhea, Respiratory infection
Majumdar et al, 2003	India	6 mo - 24 mo	2 mg/kg elemental iron (formulation not specified) daily for 4 months	Placebo	n=100 total participants analyzed; n=50 treatment, n=50 control	Hemoglobin, Ferritin
Malan et al, 2015	South Africa	6 yr - 12 yr	50 mg elemental Fe as ferrous sulfate 4 times per week for 8.5 months	Placebo + Vitamin C; or Placebo + n-3 fatty acids + Vitamin C (factorial trial)	n=296 total participants analyzed; n=146 treatment, n=150 control	Diarrhea, Respiratory infection
Massaga et al, 2003	Tanzania	3 mo - 4 mo	7.5 mg elemental Fe as ferric ammonium citrate daily for 6 months	Placebo; or Placebo + Amodaquine (antimalarial) (factorial trial)	n=291 total participants analyzed; n=146 treatment, n=145 control	Anemia, Malaria
Mebrahtu et al, 2004	Tanzania	4 mo - 6 yr	10 mg elemental Fe as ferrous sulfate + Placebo & Anthelmintic (Mebendazole) to half daily for 12 months	Placebo + Placebo & Anthelmintic (Mebendazole) to half	n=538 total participants analyzed; n=273 treatment, n=265 control	Malaria
Mejía et al, 1988	Guatemala	12 mo - 9 yr	3 mg/kg elemental Fe as ferrous sulfate daily for 2 months	Placebo; or Placebo + Vitamin A (factorial trial)	n=99 total participants analyzed; n=54 treatment, n=45 control	Hemoglobin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Menendez et al, 1997	Tanzania	~2 mo (mean age)	12 mg elemental Fe as ferrous glycine sulphate daily for 16 weeks	Placebo; or Placebo + Malaria prophylaxis (Deltaprim) (factorial trial)	n=832 total participants analyzed; n=417 treatment, n=415 control	Malaria
Menendez et al, 2004	Tanzania	~2 mo (mean age)	12 mg elemental Fe as ferrous glycine sulphate daily for 4 months	Placebo; or Placebo + Malaria prophylaxis (Deltaprim) (factorial trial)	n=601 total participants analyzed; n=307 treatment, n=294 control	Ferritin, Iron deficiency
Metallinos-Katsaras et al, 2004	Greece	3 yr - 5 yr	15 mg elemental Fe as ferrous fumarate + Multivitamin (Vitamins A, B, C, D, E) 5 times per week for 2 months	Placebo + Multivitamin (Vitamins A, B, C, D, E)	n=48 total participants analyzed; n=31 treatment, n=17 control	Hemoglobin, Ferritin
Mitra et al, 1997	Bangladesh	2 mo - 4 yr	15 mg elemental Fe as ferrous gluconate + Multivitamin (A, C, D) daily for 15 months	Placebo + Multivitamin (A, C, D)	n=289 total participants analyzed; n=141 treatment, n=148 control	Diarrhea, Respiratory infection
Mozaffari-Koshravi et al, 2010	Iran	14 yr - 17 yr	150 mg elemental Fe as ferrous sulfate 1 time per week for 16 weeks	Control	n=193 total participants analyzed; n=96 treatment, n=97 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Mwanri et al, 2000	Tanzania	9 yr - 12 yr	40 mg elemental Fe as ferrous sulfate 3 times per week for 12 weeks	Placebo; or Placebo + Vitamin A (factorial trial)	n=136 total participants analyzed; n=68 treatment, n=68 control	Hemoglobin, Anemia
Nagpal et al, 2004	India	4 mo - 6 mo	2 mg/kg elemental Fe as ferric ammonium citrate daily for 8 weeks	Placebo	n=43 total participants analyzed; n=19 treatment, n=24 control	Hemoglobin, Ferritin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Nair et al, 2017	India	4 mo - 6 mo	2 mg/kg elemental Fe as colloidal iron daily for 7 months	Control	n=44 total participants analyzed; n=22 treatment, n=22 control	Hemoglobin, Anemia
Nchito et al, 2009	Zambia	~10 yr (mean age)	60 mg elemental Fe as ferrous dextran 5 times per week for 10 months	Placebo; or Placebo + Multivitamin (factorial trial)	n=215 total participants analyzed; n=118 treatment, n=97 control	Helminth Infection
Northrop-Clewes et al, 1996	Pakistan	~14 mo (mean age)	15 mg elemental Fe as ferrous sulfate daily for 12 weeks	Placebo	n=191 total participants analyzed; n=95 treatment, n=96 control	Hemoglobin, Ferritin
Olsen et al, 2000	Kenya	4 yr - 16 yr	60 mg elemental Fe as ferrous dextran 2 times per week for 12 months	Placebo	n=200 total participants analyzed; n=108 treatment, n=92 control	Helminth Infection
Olsen et al, 2006	Kenya	~9 yr (mean age)	60 mg elemental Fe as ferrous dextran 2 times per week for 12 months	Placebo	n=200 total participants analyzed; n=108 treatment, n=92 control	Hemoglobin, Ferritin
Palupi et al, 1997	Indonesia	24 mo - 6 yr	30 mg elemental Fe as ferrous sulfate 1 time per week for 9 weeks	Placebo	n=194 total participants analyzed; n=96 treatment, n=98 control	Hemoglobin, Anemia, HAZ, WHZ, WAZ, Helminth Infection
Paracha et al, 1993	Pakistan	8 yr - 11 yr	76 mg elemental Fe as ferrous gluconate + Multivitamin daily for 11 weeks	Control + Multivitamin	n=119 total participants analyzed; n=61 treatment, n=58 control	Hemoglobin, Ferritin
Perrone et al, 1999	Italy	4 yr - 12 yr	12 mg elemental Fe as iron polymaltosate + Zinc daily for 12 months	Control + Zinc	n=20 total participants analyzed; n=10 treatment, n=10 control	Ferritin, HAZ, WAZ

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Prasetyani et al, 2017	Indonesia	5 yr - 19 yr	100 mg elemental Fe as iron(iii)-hydroxide polymaltose complex 6 times per week for 8 weeks	Placebo	n=578 total participants analyzed; n=290 treatment, n=288 control	Hemoglobin, Ferritin, Malaria
Reeves et al, 1985	USA	11 mo - 14 mo	3 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Placebo	n=179 total participants analyzed; n=77 treatment, n=102 control	Diarrhea
Rezaeian et al, 2014	Iran	14 yr - 19 yr	50 mg elemental Fe as ferrous sulfate 2 times per week for 16 weeks	Placebo	n=200 total participants analyzed; n=100 treatment, n=100 control	Hemoglobin
Richard et al, 2006	Peru	6 mo - 16 yr	15 mg elemental Fe as ferrous sulfate daily for 7 months	Placebo; or Placebo + Zinc (factorial trial)	n=758 total participants analyzed; n=378 treatment, n=380 control	Hemoglobin, HAZ, WAZ, Diarrhea, Respiratory infection, Malaria
Rosado et al, 1997	Mexico	18 mo - 3 yr	20 mg elemental Fe as ferrous sulfate 5 times per week for 12 months	Placebo; or Placebo + Zinc (factorial trial)	n=217 total participants analyzed; n=108 treatment, n=109 control	Hemoglobin, Ferritin, Iron deficiency, HAZ, WHZ, WAZ, Diarrhea, Respiratory infection
Rosado et al, 2006	Mexico	6 yr - 8 yr	30 mg elemental Fe as ferrous fumarate 5 times per week for 6 months	Placebo; or Placebo + Zinc (factorial trial)	n=517 total participants analyzed; n=265 treatment, n=252 control	Hemoglobin, Ferritin
Roschnik et al, 2004	Philippines	7 yr - 13 yr	108 mg elemental Fe as ferrous sulfate 1 time per week for 10 weeks	Control	n=1510 total participants analyzed; n=708 treatment, n=802 control	Hemoglobin, Anemia
Sarker et al, 2008	Bangladesh	24 mo - 6 yr	3 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Placebo; or Placebo + H-pylori treatment (factorial trial)	n=197 total participants analyzed; n=99 treatment, n=98 control	Hemoglobin, Ferritin

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Seshadri et al, 1989	India	8 yr - 16 yr	30 mg elemental Fe as ferrous sulfate daily for 2 months; 40 mg elemental Fe as ferrous sulfate daily for 2 months	Placebo	n=113 total participants analyzed; n=97 treatment, n=16 control	Hemoglobin, Anemia
Smith et al, 1989	Gambia	6 mo - 6 yr	3-6 mg/kg elemental Fe as ferrous sulfate daily for 12 weeks	Placebo	n=186 total participants analyzed; n=97 treatment, n=89 control	Malaria
Smuts et al, 2005	South Africa	6 mo - 12 mo	10 mg elemental iron (formulation not specified) daily for 6 months	Placebo	n=99 total participants analyzed; n=49 treatment, n=50 control	Hemoglobin, Ferritin, HAZ, WHZ, WAZ, Diarrhea, Respiratory infection
Smuts et al, 2014	South Africa	6 yr - 12 yr	50 mg elemental Fe as ferrous sulfate 4 times per week for 8.5 months	Placebo + Vitamin C; or Placebo + n-3 fatty acids + Vitamin C (factorial trial)	n=86 total participants analyzed; n=43 treatment, n=43 control	Cognitive
Soemantri et al, 1989	Indonesia	8 yr - 12 yr	2 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Placebo	n=130 total participants analyzed; n=71 treatment, n=59 control	Hemoglobin, Cognitive
Soewondo et al, 1989	Indonesia	~5 yr (mean age)	50 mg elemental Fe as ferrous sulfate daily for 8 weeks	Placebo	n=176 total participants analyzed; n=77 treatment, n=99 control	Hemoglobin, Ferritin
Stoltzfus et al, 2001	Tanzania (Zanzibar)	12 mo - 4 yr	10 mg elemental Fe as ferrous sulfate daily for 12 months	Placebo	n=538 total participants analyzed; n=277 treatment, n=261 control	Hemoglobin, Ferritin, Helminth Infection, Cognitive
Stoltzfus et al, 2004	Tanzania	6 mo - 29 mo	10 mg elemental Fe as ferrous sulfate daily for 12 months	Placebo; or Placebo + Mebendazole (factorial trial)	n=145 total participants analyzed; n=74 treatment, n=71 control	Anemia, Stunting

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Sungthong et al, 2002	Thailand	6 yr - 14 yr	60 mg elemental Fe as ferrous sulfate daily for 16 weeks; 60 mg elemental Fe as ferrous sulfate 1 time per week for 16 weeks	Placebo	n=396 total participants analyzed; n=274 treatment, n=122 control	Hemoglobin, Ferritin, Iron deficiency anemia, HAZ, WAZ
Sungthong et al, 2004	Thailand	6 yr - 14 yr	60 mg elemental Fe as ferrous sulfate 5 times per week for 16 weeks; 60 mg elemental Fe as ferrous sulfate 1 time per week for 16 weeks	Placebo	n=391 total participants analyzed; n=269 treatment, n=122 control	Cognitive
Thibault et al, 1993	France	6 mo - 4 yr	38.6 mg elemental Fe as hydroxyproline iron daily for 2 months	Placebo	n=70 total participants analyzed; n=32 treatment, n=38 control	Hemoglobin, Ferritin
Untoro et al, 2005	Indonesia	6 mo - 12 mo	10 mg elemental Fe as ferrous sulfate 7 times per week for 23 weeks	Placebo	n=134 total participants analyzed; n=69 treatment, n=65 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, HAZ, WHZ, WAZ, Diarrhea, Respiratory infection
van den Hombergh et al, 1996	Tanzania	0 mo - 30 mo	40 mg elemental Fe as ferrous sulfate + Malaria treatment + folic acid daily for 12 weeks	Placebo + Malaria treatment + folic acid	n=95 total participants analyzed; n=48 treatment, n=47 control	Hemoglobin, WAZ, Malaria
Verhoef et al, 2002	Kenya	2 mo - 3 yr	3 mg/kg elemental Fe as ferrous fumarate 2 times per week for 12 weeks	Placebo; or Placebo + Antimalarial (sulfadoxine-pyrimethamine) (factorial trial)	n=328 total participants analyzed; n=164 treatment, n=164 control	Hemoglobin, Iron deficiency, Malaria

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Wang et al, 2012	China	~4 mo (mean age)	1 mg/kg elemental Fe for 2 months	Control	n=60 total participants analyzed; n=26 treatment, n=34 control	Hemoglobin, Ferritin
Wasantwisut et al, 2006	Thailand	4 mo - 6 mo	10 mg elemental Fe as ferrous sulfate daily for 6 months	Placebo + Vitamin C, A; or Placebo + Zinc + Vitamins C, A (factorial trial)	n=609 total participants analyzed; n=305 treatment, n=304 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia, HAZ, WHZ, WAZ
Wieringa et al, 2007	Indonesia, Thailand, Vietnam	4 mo - 6 mo	10 mg elemental Fe as ferrous sulfate 5-7 times per week for 6 months	Placebo; or Placebo + Zinc (factorial trial)	n=2049 total participants analyzed; n=1017 treatment, n=1032 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Yalçın et al, 2000	Turkey	~6 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Control	n=16 total participants analyzed; n=7 treatment, n=9 control	Hemoglobin, Ferritin, Anemia, Iron deficiency, Iron deficiency anemia
Yip et al, 1985	USA	11 mo - 13 mo	30 mg/kg elemental Fe as ferrous sulfate daily for 3 months	Placebo	n=291 total participants analyzed; n=146 treatment, n=145 control	Hemoglobin, Ferritin
Yurdakök et al, 2004	Turkey	~4 mo (mean age)	1 mg/kg elemental Fe as ferrous sulfate daily for 3 months; 7 mg/kg elemental Fe as ferrous sulfate 1 time per week for 3 months	Control	n=53 total participants analyzed; n=37 treatment, n=16 control	Hemoglobin, Ferritin
Zavaleta et al, 2000	Peru	12 yr - 19 yr	60 mg elemental Fe as ferrous sulfate 2 times per week for 17 weeks	Placebo	n=198 total participants analyzed; n=101 treatment, n=97 control	Anemia
Zhao et al, 2004	China	3 yr - 6 yr	4 mg elemental Fe as NaFeEDTA for 18 months	Placebo	n=213 total participants analyzed; n=120 treatment, n=93 control	Hemoglobin, Anemia

Table S1.2. (Continued)

Author & Year	Country	Age	Intervention	Comparison group	Sample size	Outcomes
Ziegler et al, 2009	USA	4 mo - 4 mo	7.5 mg elemental Fe as ferrous sulfate daily for 5 months	Control	n=98 total participants analyzed; n=42 treatment, n=56 control	Hemoglobin, Ferritin
Ziegler et al, 2009	USA	~1 mo (mean age)	7 mg elemental Fe as ferrous sulfate + Multivitamins (A, C, D) daily for 4.5 months	Placebo + Multivitamins (A, C, D)	n=63 total participants analyzed; n=31 treatment, n=32 control	Hemoglobin, Ferritin, Iron deficiency, Iron deficiency anemia
Zlotkin et al, 2003	Ghana	8 mo - 20 mo	40 mg elemental Fe as ferrous fumarate daily for 6 months; 12.5 mg elemental Fe as ferrous sulfate daily for 6 months	Placebo	n=241 total participants analyzed; n=161 treatment, n=80 control	Hemoglobin, Ferritin, Anemia
Zlotkin et al, 2013	Ghana	6 mo - 4 yr	12.5 mg elemental Fe as microencapsulated ferrous fumarate + Ascorbic acid + vitamin a + zinc daily for 5 months	Placebo + Ascorbic acid + vitamin a + zinc	n=1815 total participants analyzed; n=900 treatment, n=915 control	Hemoglobin, Anemia, Iron deficiency, Malaria

Table S1.3. Modification of iron effects after control for baseline anemia.

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Weekly frequency					
Frequent (3-7 times/week)	ref	ref	ref	ref	ref
n	94	59	47	28	n/e
Intermittent (1-2 times/week)					
n	18	11	9	4	
Estimate	-1.3	-14.8	1.01	1.20	
95% CI	(-4.6, 2.1)	(-26.2, -3.5)	(0.63, 1.61)	(0.48, 3.02)	
p-interaction	0.45	0.01	0.97	0.69	
Duration					
1-3 months					
n	69	38	23	12	5
Estimate	ref	ref	ref	ref	ref
4-6 months					
n	38	29	29	20	12
Estimate	-1.6	9.8	1.32	0.69	1.02
95% CI	(-4.2, 1.1)	(1.1, 18.4)	(0.96, 1.81)	(0.35, 1.38)	(0.22, 4.73)
≥7 months					
n	8	4	6	0	0
Estimate	-3.2	37.9	1.71	n/e	n/e
95% CI	(-8.0, 1.6)	(-1.3, 77.1)	(1.06, 2.75)		
Global p-interaction	0.27	0.02	0.05	0.29	0.98
Dose					
Low (1st tertile for age)					
n	32	22	22	10	8
Estimate	ref	ref	ref	ref	ref
Moderate (2nd tertile for age)					
n	26	21	21	14	8
Estimate	3.3	14.3	0.80	0.61	0.49
95% CI	(-0.2, 6.7)	(5.5, 23.1)	(0.54, 1.18)	(0.25, 1.48)	(0.20, 1.20)
High (3rd tertile for age)					
n	30	18	7	5	1
Estimate	1.7	6.0	0.97	0.71	0.45
95% CI	(-1.7, 5.1)	(-3.4, 15.3)	(0.56, 1.66)	(0.24, 2.06)	(0.01, 20.63)
Global p-interaction	0.17	0.007	0.49	0.52	0.11

* Difference in the weighted mean effect of iron in the index category compared to the weighted mean effect of iron in the reference category, holding baseline anemia constant.

† Ratio of the prevalence ratio for iron in the index category relative to the prevalence ratio for iron in the reference category, holding baseline anemia constant.

Abbreviations: CI, confidence interval; ref, reference category; n/e, not estimable

Table S1.4. Effect modification of iron by baseline anemia, child age, child sex, WHO region, and iron formulation.

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Baseline anemia					
All anemic					
n	28	12	9	4	1
Estimate	12.2	16.2	0.35	0.13	0.15
95% CI	(8.5, 15.9)	(9.5, 22.8)	(0.26, 0.47)	(0.06, 0.31)	(0.03, 0.89)
Mixed anemic and non-anemic					
n	54	30	39	23	12
Estimate	6.5	22.7	0.63	0.25	0.11
95% CI	(5.4, 7.5)	(17.4, 27.9)	(0.56, 0.72)	(0.18, 0.35)	(0.07, 0.16)
All non-anemic					
n	28	20	10	5	4
Estimate	5.1	18.7	0.63	0.20	0.22
95% CI	(3.5, 6.7)	(13.5, 23.9)	(0.41, 0.97)	(0.11, 0.35)	(0.07, 0.67)
Missing baseline anemia data					
n	49	37	8	13	9
Estimate	3.5	16.9	0.80	0.59	0.46
95% CI	(2.5, 4.5)	(12.7, 21.1)	(0.66, 0.96)	(0.45, 0.77)	(0.28, 0.77)
Global p-interaction	<0.001	0.48	0.004	0.08	0.02
Child age					
0 to 5 months					
n	38	32	18	19	17
Estimate	5.3	24.2	0.57	0.22	0.17
95% CI	(3.9, 6.8)	(18.7, 29.6)	(0.48, 0.70)	(0.15, 0.34)	(0.09, 0.30)
6 to 23 months					
n	29	21	20	13	4
Estimate	5.3	15.7	0.79	0.34	0.28
95% CI	(3.4, 7.2)	(9.5, 21.9)	(0.67, 0.93)	(0.23, 0.49)	(0.08, 0.99)
2 to <5 years					
n	25	10	6	4	0
Estimate	5.7	19.5	0.76	0.31	n/e
95% CI	(3.8, 7.7)	(11.3, 27.7)	(0.53, 1.09)	(0.14, 0.67)	
5 to <12 years					
n	29	13	13	5	4
Estimate	8.5	17.9	0.48	0.21	0.20
95% CI	(5.7, 11.4)	(10.9, 24.9)	(0.38, 0.60)	(0.06, 0.73)	(0.07, 0.53)
≥12 years					
n	16	15	5	3	1
Estimate	8.1	11.9	0.49	0.30	0.09
95% CI	(5.8, 10.4)	(9.9, 13.9)	(0.31, 0.79)	(0.11, 0.80)	(0.01, 1.64)
Global p-interaction	0.15	0.10	0.05	0.85	0.70
Child sex					
All female					
n	17	12	5	2	1
Estimate	8.2	10.3	0.39	0.74	0.09
95% CI	(5.6, 10.8)	(8.5, 12.1)	(0.28, 0.55)	(0.46, 1.19)	(0.01, 1.64)
All male					
n	6	0	2	0	0
Estimate	12.1	n/e	0.54	n/e	n/e
95% CI	(10.1, 14.2)		(0.46, 0.64)		
Mixed female and male					
n	97	67	55	35	24

Table S1.4. (Continued)

	Hemoglobin (g/L) *	Ferritin (ng/mL) *	Anemia †	Iron deficiency †	Iron deficiency anemia †
Estimate	5.6	20.3	0.63	0.29	0.19
95% CI	(4.7, 6.5)	(16.7, 23.9)	(0.56, 0.70)	(0.22, 0.37)	(0.11, 0.31)
Missing baseline sex data					
n	39	20	4	8	1
Estimate	7.6	21.5	0.61	0.17	0.11
95% CI	(4.9, 10.3)	(12.3, 30.6)	(0.43, 0.86)	(0.09, 0.31)	(0.01, 1.99)
Global p-interaction	0.06	0.16	0.44	0.17	0.87
WHO region					
Africa					
n	24	9	17	8	1
Estimate	6.4	22.1	0.70	0.23	0.15
95% CI	(4.1, 8.8)	(16.7, 27.4)	(0.59, 0.84)	(0.12, 0.45)	(0.03, 0.89)
Americas					
n	29	13	13	7	2
Estimate	4.6	19.3	0.63	0.73	0.66
95% CI	(2.6, 6.7)	(13.1, 25.5)	(0.43, 0.91)	(0.50, 1.08)	(0.20, 2.14)
Eastern Mediterranean					
n	12	11	1	1	1
Estimate	8.6	8.9	0.14	0.79	0.09
95% CI	(6.1, 11.2)	(5.8, 12.0)	(0.03, 0.62)	(0.48, 1.29)	(0.01, 1.64)
Europe					
n	21	19	5	10	6
Estimate	4.4	17.4	0.31	0.28	0.17
95% CI	(2.8, 6.0)	(11.8, 23.1)	(0.13, 0.70)	(0.20, 0.40)	(0.06, 0.45)
South-East Asia					
n	55	33	20	11	10
Estimate	8.3	24.6	0.57	0.21	0.13
95% CI	(6.6, 10.0)	(18.5, 30.7)	(0.48, 0.67)	(0.18, 0.25)	(0.09, 0.18)
Western Pacific					
n	18	14	10	8	5
Estimate	5.4	14.1	0.58	0.34	0.45
95% CI	(3.6, 7.3)	(8.3, 19.9)	(0.44, 0.76)	(0.22, 0.52)	(0.24, 0.86)
Global p-interaction	0.05	0.07	0.23	0.25	0.01
Iron formulation					
Ferrous sulfate					
n	110	66	46	27	20
Estimate	7.2	21.8	0.56	0.21	0.12
95% CI	(6.1, 8.3)	(18.5, 25.1)	(0.49, 0.63)	(0.14, 0.29)	(0.09, 0.16)
Ferrous fumarate					
n	13	9	7	8	1
Estimate	5.0	20.8	0.66	0.25	0.40
95% CI	(2.1, 8.0)	(13.0, 28.5)	(0.47, 0.91)	(0.15, 0.43)	(0.08, 2.01)
Other or unspecified					
n	36	24	13	10	5
Estimate	4.6	11.4	0.77	0.53	0.80
95% CI	(3.1, 6.2)	(5.8, 17.1)	(0.60, 0.98)	(0.39, 0.70)	(0.57, 1.14)
Global p-interaction	0.09	0.02	0.20	0.07	<0.001

* Weighted mean difference

† Pooled risk ratio

Abbreviations: CI, confidence interval; n/e, not estimable

Table S1.5. Effect heterogeneity p-values for anthropometric, infectious, and development outcomes. *†

	Frequency	Duration	Dose for age tertile	Baseline percent anemic	Modifiers Age category	Baseline percent female	Factorial trial	Formulation
<i>Anthropometry</i>								
Height-for-age Z score	0.262	0.574	0.835	0.013	0.256	n/e	0.054	0.293
Weight-for-height Z score	0.970	0.665	0.291	n/e	0.311	n/e	0.559	0.843
Weight-for-age Z score	0.520	0.519	0.861	0.709	0.250	n/e	0.355	0.815
Stunting	n/e	0.452	0.393	n/e	0.378	0.378	0.700	n/e
Wasting	n/e	n/e	0.522	n/e	n/e	n/e	0.336	n/e
<i>Infections</i>								
Diarrhea (cumulative incidence)	0.136	0.282	0.274	0.358	0.884	n/e	0.642	0.904
Diarrhea (incidence rate)	0.962	0.471	0.435	n/e	0.446	n/e	0.760	0.760
Respiratory illness (cumulative incidence)	0.006	0.018	0.032	0.125	n/e	n/e	0.129	0.683
Respiratory illness (incidence rate)	0.902	0.866	0.750	n/e	0.887	n/e	0.421	0.421
Malaria (prevalence)	n/e	n/e	0.571	0.910	n/e	n/e	0.238	0.601
Malaria (incidence rate)	n/e	0.469	0.965	0.871	n/e	n/e	0.463	0.467
Hookworm (prevalence)	0.968	0.812	0.880	n/e	n/e	n/e	n/e	0.823
Ascaris lumbricoides (prevalence)	0.390	0.456	0.647	n/e	n/e	n/e	n/e	0.932
Trichuris trichiura (prevalence)	0.501	0.501	0.431	n/e	n/e	n/e	n/e	0.584
<i>Development</i>								
Bayley Mental Index	0.651	0.892	0.888	0.020	n/e	n/e	0.280	0.454
Bayley Psychomotor Index	0.807	0.432	0.738	0.023	n/e	n/e	0.672	0.634

* Categories used for modifiers: Frequency (1-2 times per week vs. 3-7 times per week); Dose for age tertile (1st vs. 2nd vs. 3rd); Baseline percent anemic (<20% anemic vs. 20-39% anemic vs. ≥40% anemic); Age category (0-5 mo vs. 6-23 mo vs. 24-59 mo vs. 5-11 y vs. 12-19 y); Baseline percent female (All participants female vs. no participants female vs. some participants female); Factorial trial (Yes vs. no); Formulation (Ferrous sulfate vs. ferrous fumarate vs. other)

† P-values calculated from meta-regression Wald tests.

Abbreviations: n/e, not estimable.

Table S1.6. Risk of bias within included studies.

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)
Adish 1997	Unclear	Unclear	Low	Low	Low
Aggarwal 2005	Low	Unclear	Low	Low	High
Aguayo 2000	Low	Unclear	Low	Low	Low
Akman 2004	Low	Unclear	High	Low	Low
Angeles 1993	Unclear	Unclear	Unclear	Unclear	Low
Angulo-Barro 2016	Low	Low	Low	Low	Unclear
Arcanjo 2011	High	Unclear	Low	Low	Unclear
Arcanjo 2013	High	Unclear	Low	Low	Unclear
Aukett 1986	Unclear	Low	Low	Low	Low
Ayoya 2009	Unclear	Unclear	High	Low	High
Ayoya 2012	Unclear	Unclear	High	Low	High
Ballin 1992	High	High	Low	Low	Unclear
Baqui 2003	Unclear	Unclear	Unclear	Unclear	Low
Barclay 1991	Unclear	Unclear	High	High	Unclear
Baumgartner 2012	Low	Unclear	Low	Low	Low
Berger 1997	Unclear	Unclear	Low	Unclear	Low
Berger 2000	Unclear	Unclear	Low	Low	Unclear
Berger 2006	Low	Low	Low	Low	High
Berglund 2010	Low	Unclear	Low	Low	High
Bhatia 1993	High	Unclear	Unclear	Unclear	Unclear
Black 2004	Unclear	Unclear	Low	Low	Low
Bruner 1996	Low	Unclear	Low	Low	High
Burman 1972	High	Unclear	High	High	High
Buzina-Suboticaneec 1998	High	Unclear	Low	Low	Unclear
Charoenlarp 1980	Unclear	Unclear	Unclear	Unclear	Unclear
Chen 2011	Unclear	High	Unclear	Low	Unclear
Chen 2013	Low	Unclear	Low	Low	Low
Chen 2014	Low	Unclear	High	High	Low
Cheng 2001	Unclear	Unclear	Unclear	Unclear	Unclear
Choe 1999	Low	Unclear	Low	Low	Unclear
Chwang 1988	Unclear	Unclear	Low	Low	Low
Das 1984	High	Unclear	Unclear	Unclear	Low
de Silva 2003	Unclear	Unclear	Low	Low	Unclear
Desai 2003	Low	Low	Low	Low	Low
Devaki 2008	Unclear	Unclear	Unclear	Unclear	Low
Dewey 2002	Unclear	Unclear	Low	Low	Low
Dijkhuizen 2001	Low	Low	Low	Low	High
Dijkhuizen 2008	Low	Low	Low	Low	Unclear
Domellöf 2001	Unclear	Unclear	Low	Low	Unclear
Dossa 2001	Low	Unclear	Low	Low	Low
Dossa 2001	Unclear	Unclear	Low	Low	Unclear
Eftekhari 2006	Unclear	Unclear	Low	Low	High
Elwood 1970	Unclear	Unclear	Unclear	Unclear	Unclear
Engstrom 2008	Unclear	Unclear	High	High	Unclear
Ermis 2002	Unclear	Unclear	Unclear	Unclear	Unclear
Fahmida 2007	Unclear	Unclear	Low	Low	Low
Fallahi 2007	Unclear	Unclear	Low	Unclear	Low

Table S1.6. (Continued)

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)
Franz 2000	Unclear	Unclear	Unclear	Unclear	High
Friel 2003	Unclear	Unclear	Low	Low	High
Fujiu 2004	Unclear	Unclear	Unclear	Unclear	Low
Gebresellasi 1996	Low	Low	Low	Low	Low
Geltman 2001	Unclear	Low	Low	Low	Unclear
Geltman 2004	Low	Unclear	Low	Low	Unclear
Gokcay 2012	Unclear	Unclear	High	Unclear	High
Gopaldas 1985	Unclear	Unclear	Low	Low	Low
Gopaldas 1985	Unclear	Unclear	Low	Low	Low
Greisen 1986	Unclear	Unclear	Unclear	Unclear	Unclear
Hacihamdioglu 2013	Unclear	Unclear	High	Unclear	Unclear
Harvey 1989	Unclear	Unclear	Low	Low	Low
Hathirat 1992	Unclear	Unclear	Low	Unclear	Unclear
Hess 2002	Unclear	Unclear	Low	Low	Low
Hettiarachchi 2008	Unclear	Unclear	Low	Low	Low
Hieu 2012	Low	Low	Low	Low	Unclear
Hop 2005	Low	Low	Low	Low	Unclear
Idjradinata 1993	Low	Unclear	Low	Low	Low
Idjradinata 1994	Low	Unclear	Unclear	Unclear	Unclear
Irigoyen 1991	Unclear	Unclear	Low	Low	High
Kapur 2003	Low	Unclear	Low	Unclear	Unclear
Kashyap 1987	Unclear	Unclear	Low	Unclear	Unclear
Kashyap 1987	Unclear	Unclear	Low	Unclear	Unclear
Kianfar 2000	Unclear	Unclear	High	Unclear	Unclear
Kordas 2005	Low	Low	Low	Low	High
Lambert 2002	Unclear	Unclear	Low	Low	Unclear
Latham 1990	Unclear	Unclear	Low	Unclear	Low
Lawless 1994	Low	Unclear	Low	Low	Low
Lee 1997	Unclear	Unclear	Low	Unclear	Unclear
Leenstra 2009	Low	Unclear	Low	Low	Low
Lind 2003	Unclear	Low	Low	Low	Low
Lind 2004	Low	Low	Low	Low	Low
López de Romaña 2005	Low	Low	Low	Low	Low
Loría 1979	Unclear	Unclear	Unclear	Unclear	Unclear
Lozoff 2016	Low	Unclear	Low	Low	Low
Majumdar 2003	Unclear	Unclear	Low	Unclear	High
Malan 2015	Low	Unclear	Low	Low	High
Massaga 2003	Low	Low	Low	Low	High
Mebrahtu 2004	Unclear	Low	Low	Low	Unclear
Mejía 1988	Low	Low	Unclear	Unclear	High
Menendez 1997	Low	Low	Low	Low	High
Menendez 2004	Low	Low	Low	Low	High
Metallinos-Katsaras 2004	Unclear	Unclear	Low	Low	Unclear
Mitra 1997	Unclear	Unclear	Low	Low	Low
Mozaffari-Koshravi 2010	High	Unclear	High	Unclear	Low
Mwanri 2000	Low	Unclear	Low	Low	Low
Nagpal 2004	Low	Unclear	Low	Low	High
Nair 2017	High	High	Unclear	Unclear	Low

Table S1.6. (Continued)

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)
Nchito 2009	Unclear	Unclear	Low	Low	High
Northrop-Clewes 1996	Unclear	Unclear	Low	Low	Unclear
Olsen 2000	Unclear	Unclear	Low	Low	Low
Olsen 2006	Low	Low	Unclear	Unclear	Unclear
Palupi 1997	Unclear	Unclear	Low	Low	Unclear
Paracha 1993	Unclear	Unclear	Low	Unclear	Unclear
Perrone 1999	Unclear	Unclear	High	Unclear	Unclear
Prasetyani 2017	Low	Unclear	Low	Low	Low
Reeves 1985	High	Unclear	Unclear	Unclear	High
Rezaeian 2014	High	Unclear	Low	Low	Low
Richard 2006	Unclear	Unclear	Low	Low	Low
Rosado 1997	Unclear	Unclear	Low	Low	Low
Rosado 2006	Low	Unclear	Low	Low	Unclear
Roschnik 2004	Low	Unclear	High	Unclear	Unclear
Sarker 2008	Low	Low	Low	Low	Unclear
Seshadri 1989	Unclear	Unclear	Low	Low	Low
Smith 1989	Unclear	Unclear	Low	Low	Unclear
Smuts 2005	Unclear	Low	Low	Low	High
Smuts 2014	Low	Unclear	Low	Low	Unclear
Soemantri 1989	Unclear	Unclear	Low	Unclear	Unclear
Soewondo 1989	Unclear	Unclear	Low	Low	Unclear
Stoltzfus 2001	Low	Low	Low	Low	Unclear
Stoltzfus 2004	Unclear	Unclear	Low	Low	High
Sunthong 2002	Low	Low	Low	Low	Low
Sunthong 2004	Low	Low	Low	Low	Low
Thibault 1993	Unclear	Unclear	Low	Low	Unclear
Untoro 2005	Unclear	Unclear	Low	Low	Low
van den Hombergh 1996	Unclear	Unclear	Unclear	High	Low
Verhoef 2002	Low	Low	Low	Low	Low
Wang 2012	Unclear	Low	Low	Unclear	High
Wasantwisut 2006	Low	Low	Low	Low	Low
Wieringa 2007	Low	Low	Low	Low	High
Yalçin 2000	Unclear	Unclear	High	High	High
Yip 1985	High	Unclear	Unclear	Unclear	Unclear
Yurdakök 2004	Unclear	Unclear	High	High	Low
Zavaleta 2000	Unclear	Unclear	Low	Low	Low
Zhao 2004	Unclear	Unclear	Low	Low	High
Ziegler 2009	Unclear	Unclear	High	High	Unclear
Ziegler 2009	Unclear	Unclear	Low	Low	Unclear
Zlotkin 2003	Low	Low	Low	Low	Low
Zlotkin 2013	Low	Unclear	Low	Low	Low

Figure S1.1. Cochrane risk of bias assessment of included studies (n=123).

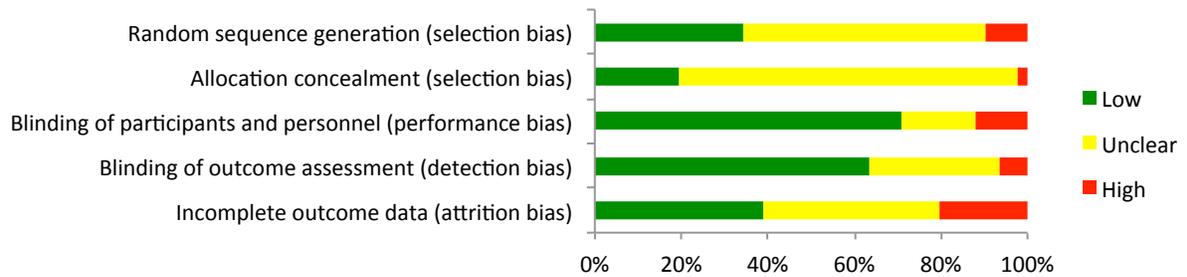


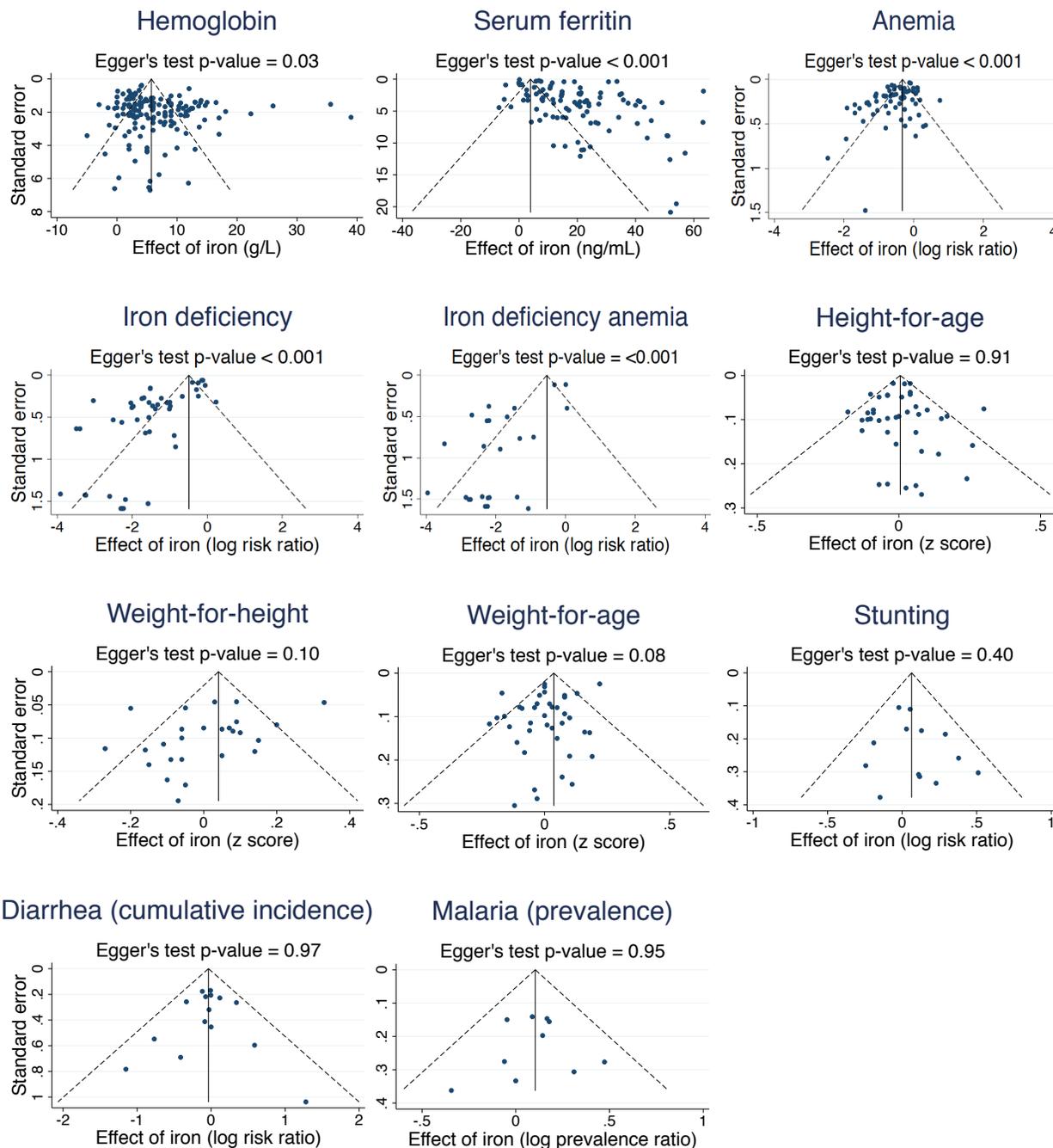
Table S1.7. Effect of oral iron supplementation versus placebo among children and adolescents aged <20 years among trials judged to not be at “high” risk of bias.

	n*	Estimate type	Estimate of effect (95% CI)	p-value	I ² (%)
Hemoglobin (g/L)	98	WMD	6.9 (5.7, 8.1)	<0.001	94.2
Serum ferritin (ng/mL)	61	WMD	21.9 (18.1, 25.8)	<0.001	99.6
Anemia	39	RR	0.57 (0.50, 0.65)	<0.001	87.6
Iron deficiency	31	RR	0.29 (0.22, 0.39)	<0.001	91.7
Iron deficiency anemia	16	RR	0.26 (0.15, 0.44)	<0.001	79.5

* Number of trial arms randomized to iron

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; PR, prevalence ratio; RR, risk ratio; SMD, standardized mean difference; WMD, weighted mean difference

Figure S1.2. Assessment of small study bias for outcomes reported by ≥ 10 groups randomized to iron.



Chapter 2: Iron supplementation and pediatric HIV disease progression: a cohort study

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Abstract

Background: Anemia is common among HIV-infected children and iron supplementation is prescribed routinely for the prevention and management of anemia among children. Limited evidence suggests iron supplementation may have adverse effects among HIV-infected populations. We examined the effect of iron supplement use on mortality, disease progression, and hematological outcomes among HIV-infected children in Dar es Salaam, Tanzania.

Methods: A prospective cohort study was conducted among HIV-infected children (aged 0-14 years) receiving antiretroviral treatment or supportive care between October 2004 and September 2014. Clinical data were recorded on morbidity and vital status, hematological status, and prescriptions at each clinical visit. Cox proportional hazards models adjusted for time-varying covariates were used to estimate the effect of time-varying iron supplementation on the hazard rate of mortality, HIV disease stage progression, tuberculosis incidence, and anemia and microcytosis persistence.

Results: 4,229 children were observed during 149,260 clinic visits for a mean follow-up of 2.9 years. After adjustment for time-varying clinical covariates, time-varying iron supplementation was associated with a 2.87 times higher hazard rate of mortality (95% CI: 1.70, 4.87) and a 1.48 times higher hazard rate of HIV disease stage progression (95% CI: 1.10, 1.98). Iron supplementation was also associated with a lower rate of anemia persistence (HR=0.47; 95% CI: 0.37-0.61). No differences in the association between iron supplementation and clinical outcomes were observed by ART or anemia status.

Conclusions: Iron supplementation may increase the risk of HIV disease progression and mortality among HIV-infected children, while reducing the risk of anemia.

Introduction

Iron deficiency and human immunodeficiency virus (HIV) infection are each an important contributor to morbidity and mortality among children in low- and middle-income countries. Iron deficiency is thought to be responsible for approximately half of the 273 million cases of anemia among children 6-59 months in the year 2011, making it the most common cause of disability in this age group.¹⁻³ To address this challenge, the World Health Organization (WHO) recommends daily oral iron supplementation for all children 6 months to 12 years of age in regions with a prevalence of anemia >40% and adequate malaria control.⁴ With respect to HIV, 1.7 million children <15 years were estimated to be living with an HIV infection in the year 2018.⁵ Although immediate initiation of anti-retroviral therapy (ART) is now recommended for all children diagnosed with HIV, only slightly more than half of children receive treatment.^{6,7} Thirty-four percent of HIV-infected children are estimated to be iron deficient, and iron supplementation is sometimes given in conjunction with ART.⁸

However, the safety of iron supplementation among HIV-infected children is called into question by evidence from molecular biology which indicates that iron facilitates HIV replication and the virulence of opportunistic pathogens. Reverse transcription of HIV RNA, transcription of HIV genes, and assembly of HIV capsid proteins are iron-dependent or iron-regulated processes.⁹ *In vitro* studies have suggested that these processes are sensitive to changes in iron availability, with viral replication increasing with the addition of cellular iron and decreasing with iron chelation.¹⁰⁻¹³ Furthermore, *M. tuberculosis* – the most common cause of death among AIDS patients – competes for iron stores within host macrophages, and downregulation of macrophage iron uptake is thought to be part of innate immune defense.¹⁴

A large, long-term observational cohort study of HIV-infected adults initiating antiretroviral therapy in Tanzania (n=40,657) between the years 2004 and 2012 found a starkly increased rate of mortality among those who were prescribed iron-folic acid supplements. The risk of mortality associated with iron supplement use was higher among patients with no anemia (hazard ratio [HR]=3.8) than those with severe anemia (HR=1.6).¹⁵ These findings raise the question of whether similar risks are apparent for children, as the symptoms and treatment of HIV infection among children differ substantially from those of adults.¹⁶ Additionally, iron supplements are more widely recommended for children than adults.⁴

Two prior studies of iron supplementation among HIV-infected children were done in small populations over short periods of follow-up and do not examine mortality as an outcome.^{17,18} The present study aims to address these gaps. We present a longitudinal cohort study of HIV-infected Tanzanian children who received supportive care or antiretroviral therapy between 2004-2012. We aim to estimate the effect of iron supplement use on disease progression and hematological outcomes, as well as potential effect modification by antiretroviral therapy.

Methods

Study population

A prospective cohort study was prepared using medical record data from HIV-infected children aged 0-14 years in Dar es Salaam, Tanzania. Data on patient visits were available from October 2004 to September 2014. Study participants were enrolled in supportive care and antiretroviral treatment services at Management and Development for Health (MDH), an HIV care provider

supported by the U.S. President's Emergency Plan for AIDS Relief. Patients received treatment free of charge.

Clinical care and data collection

Patients were scheduled for monthly visits with a physician where they received regular evaluations, were given counseling on nutrition and ART adherence, and obtained ART refills. When patients missed a visit or had abnormal laboratory results, they were contacted in person or by phone to encourage them to return to the clinic. A team of community health workers and volunteers worked with the treatment clinics to trace and ascertain the vital status of individuals lost to follow-up.

Blood specimens were scheduled to be drawn every four months by trained phlebotomists. Hemoglobin and mean corpuscular volume (MCV; using ACT5 Diff hematology analyzer; Beckman Coulter, Brea, CA) as well as CD4 cell counts or percent (using FACS Calibur; Becton Dickinson, San Jose, CA) were assessed. Children were eligible for inclusion in the study from the time they had a blood specimen with complete hemoglobin, MCV, and CD4 data. Iron supplements (iron alone, iron folic-acid, or iron-B12 complex) were prescribed by the physician on the basis of hemoglobin levels or severe clinical pallor. Iron supplement prescriptions were typically for a course of one to two months.

The presence of active pulmonary tuberculosis was assessed via chest X-ray and sputum smear for acid-fast bacilli at first visit and subsequently if suggestive symptoms appeared. Clinical stage of HIV disease progression (stage I-IV) was assessed using WHO criteria.¹⁹ Viral load

testing was not routinely performed in this clinical setting. Antiretroviral care was provided per the Tanzania National Guidelines for the Management of HIV and AIDS.²⁰⁻²³ Antiretroviral therapy consisted of three drugs: two nucleoside reverse transcriptase inhibitors (zidovudine, lamivudine, abacavir, stavudine) plus one non-nucleoside reverse transcriptase inhibitor (nevirapine, efavirenz). Stavudine replaced zidovudine in cases of anemia, and efavirenz replaced nevirapine in the case of rifampin-based tuberculosis treatment.

Nurses and physicians collected clinical data on standard case report forms using unique patient identifiers. Data reviewers at each clinic ensured completeness of records and conducted quality assurance checks before entering data into a secure computerized database.

Variable definitions

Exposure to iron supplements was defined as a patient having been prescribed iron alone, iron folic-acid, or iron-B12 complex. For the main analysis, the iron exposure period was considered to continue for 3 months from the date of prescription. Sensitivity analyses were performed by alternatively defining the exposure period to be 6 months, until the end of follow-up, and until the next visit date.

Time to mortality was defined as the number of days from the time of exposure to the date of death as confirmed by clinical records. Other time-to-event outcomes included diagnosis of incident tuberculosis, HIV disease stage increase, anemia persistence, and microcytosis persistence. Incident tuberculosis was defined as the absence of tuberculosis at or prior to the start of follow-up followed by a tuberculosis diagnosis during follow-up. HIV disease stage

increase was defined as an increase of at least one stage from the one recorded at the start of follow-up. Categories of anemia (none, mild, moderate, and severe) were defined using age-specific hemoglobin cutoffs as per the WHO.²⁴ As no WHO guidance exists for defining anemia in children 0-5 months of age, the definitions for children aged 6-59 months were applied. Microcytosis ($MCV < -2$ standard deviations), a marker of iron deficiency, was defined using age-specific cutoffs according to WHO.²⁵ As a majority of children (82.3%) were anemic at baseline, we examined the effect of iron supplementation on the probability of reduction in anemia severity or microcytosis, defined as a change in status to a less severe category than the one observed at the start of follow-up (conversely, persistence was defined as a lack of reduction in severity).

Covariates were assessed at every clinical visit and carried forward in the case of missing data for a given visit. Covariates included age (0-5 months, 6-23 months, 24-59 months, 5-11 years, 12-14 years), sex, Tanzania HIV treatment guideline under effect (1st edition [April 2002 – March 2005], 2nd edition [April 2005 – January 2009], 3rd edition [February 2009 – March 2012], 4th edition [April 2012 – April 2015]), body mass index for age z score (BMIZ; $BMIZ > 1$, $1 \geq BMIZ \geq -1$, $-1 > BMIZ \geq -2$, $BMIZ < -2$), district of Dar es Salaam (Ilala, Kinondoni, Temeke), health facility type (hospital, health center, dispensary), immune deficiency (as defined by CD4 count or percent per WHO criteria¹⁹; none, mild, advanced, severe), HIV disease stage (I, II, III, IV), and ART status (on ART, not on ART).

Statistical methods

The data was structured in Andersen-Gill format, with each row representing exposure, outcome, and covariate data for a given patient on a given date of observation.²⁶ Covariate values were carried forward until the next date when a new measurement was available. Missing indicators were used when covariate values were not available.²⁷ Outlier values of hemoglobin and mean corpuscular volume were defined according to Tukey's criteria and dropped from analysis.²⁸ Cox proportional hazards regression models with time-varying exposure and covariate data were used to estimate the association between iron supplementation and time to event outcomes. Interaction terms were tested for time-varying ART status and anemia severity. Marginal structural models, which account for potential time-varying exposure and covariate feedback, were used as a sensitivity analysis.²⁹ The degree of unmeasured confounding needed to explain the observed hazard ratios was calculated.³⁰ Two-sided statistical tests were used, with statistical significance defined as $p < 0.05$. Statistical analyses were performed using Stata software (version 13, College Station, Texas).

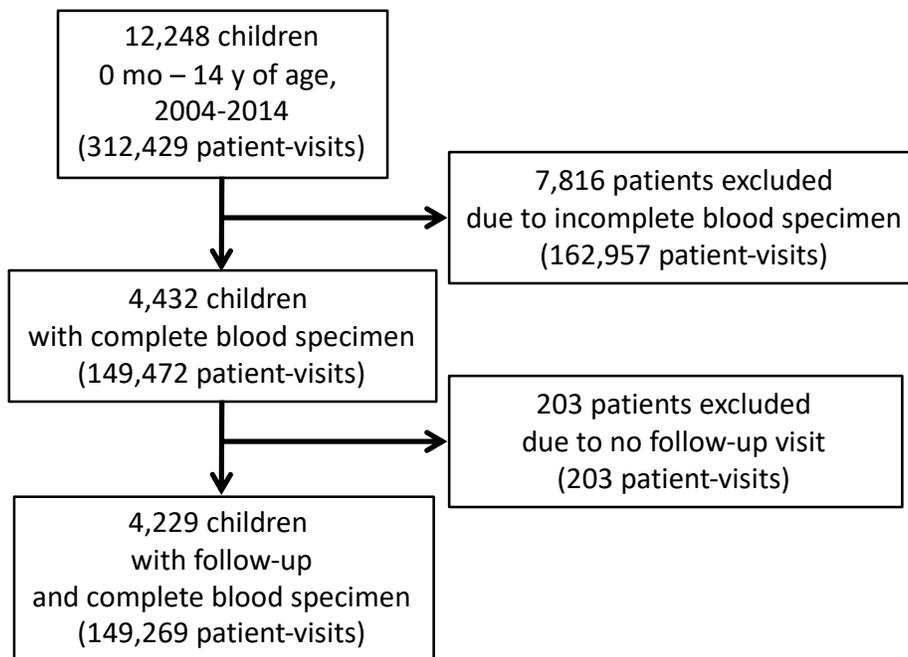
Ethical approval

Ethical approval for this study was granted by the Institutional Review Boards of the Harvard T.H. Chan School of Public Health and the Tanzanian National Institute of Medical Research at the Muhimbili University of Health and Allied Sciences.

Results

There were 12,248 children 0 to 14 years of age who visited a study health center between 2004 and 2014, for a total of 312,429 patient-visits (**Figure 2.1**). Among these children, 7,816 were excluded due to a lack of blood specimens that contained CD4, hemoglobin, or MCV values. After these exclusions, there were an additional 203 patients excluded because they did not have any follow-up visits after their first complete blood specimen. The final sample size is 4,229 children observed over 149,269 patient-visits and a mean follow-up time of 2.90 years.

Figure 2.1. Flowchart of exclusions from clinical database



The plurality of children (41.5%) began follow-up between the ages of five and 11 years, with very few enrolled in the first six months of life (7.4%; **Table 2.1**). Approximately half of the study population was female (50.9%). Anemia was observed in 82.9% of children and microcytosis in 51.5% of children at the start of follow-up. However, only 64.1% of follow-up time was among children with anemia and 39.1% among children with microcytosis. Substantial improvements were seen for immune status, with nearly half of children (44.9%) experiencing some degree of immunodeficiency at the start of follow-up, but only 5.5% of follow-up time spent with immunodeficiency. The plurality of children were observed to have WHO HIV disease stage 3 at both baseline and throughout follow-up. Nearly eight in ten children (78.5%) were on ART during follow-up. The most common drug regimen was lamivudine and nevirapine along with either stavudine or zidovudine.

Iron supplementation was prescribed to 827 (19.6%) of children (**Table 2.2**). Over half of children who received a prescription were prescribed iron only once. Mortality occurred for 247 children at a median time of 0.41 years after the start of follow-up. Incident tuberculosis occurred for approximately one in seven children after a median time of 1.17 years. WHO stage increase was the most commonly occurring adverse outcome, affecting four in ten children. Over half of children experienced a reduction in anemia severity from baseline after a median time of 0.45 years. Transitioning from microcytosis to normocytosis was observed among only 16.3 percent of children.

Table 2.1. Clinical characteristics among study population

	Baseline		Follow-up time in
	n	%	category
			%
Age			
0-5 months	314	7.4	0.8
6-23 months	769	18.2	6.8
24-59 months	835	19.7	21.1
5-11 years	1757	41.5	52.9
12-14 years	554	13.1	18.3
Sex			
Female	2151	50.9	50.3
Male	2078	49.1	49.7
Tanzania HIV Treatment Guideline			
1st Edition (April 2002 - March 2005)	75	1.8	0.2
2nd Edition (April 2005 - January 2009)	2993	70.8	35.1
3rd Edition (February 2009 - March 2012)	1160	27.4	50.5
4th Edition (April 2012 - April 2015)	1	0.0	14.1
BMIZ category^a			
BMIZ>1	221	13.5	17.1
BMIZ≤1 & BMIZ≥-1	703	42.8	54.2
BMIZ≤-1 & BMIZ≥-2	303	18.5	15.8
BMIZ<-2	414	25.2	12.9
District of Dar es Salaam^a			
Ilala	1935	46.9	47.8
Kinondoni	1078	26.1	25.4
Temeke	1114	27.0	26.8
Health facility type^a			
Hospital	3655	87.9	87.9
Health center	288	6.9	7.6
Dispensary	215	5.2	4.5
Anemia status			
No anemia	722	17.1	35.9
Mild anemia	693	16.4	21.0
Moderate anemia	2393	56.6	40.0
Severe anemia	421	10.0	3.1
Mean corpuscular volume			
Normocytosis	2050	48.5	60.8
Microcytosis	2179	51.5	39.1
Immune status			
No immune deficiency	2331	55.1	94.5
Mild deficiency	353	8.3	2.1
Advanced deficiency	479	11.3	1.7
Severe deficiency	1066	25.2	1.7

Table 2.1. (Continued)

	Baseline		Follow-up time in
	n	%	category %
WHO HIV disease stage ^a			
Stage 1	693	17.2	11.6
Stage 2	1195	29.7	28.4
Stage 3	1893	47	55.4
Stage 4	246	6.1	4.6
ART start relative to inclusion in study			
Started ART prior to study inclusion	253	6.0	n/a
Started ART in first month	1548	36.6	n/a
Started ART after first month	1520	35.9	n/a
Did not start ART	908	21.5	n/a
Antiretroviral drugs prescribed to children on ART ^b			
Nucleoside reverse transcriptase inhibitors			
Lamivudine	2635	79.3	82.1
Stavudine	1196	36.0	42.8
Zidovudine	1435	43.2	39.3
Non-nucleoside reverse transcriptase inhibitors			
Efavirenz	508	15.3	11.5
Nevirapine	2110	63.5	70.2
Other or unspecified drug	623	18.8	15.4

^a No data was available on the following variables for some children: BMIZ, n=2588 missing (61.2%); district of Dar es Salaam, n=102 missing (2.4%); health facility type, n=71 missing (1.7%); WHO HIV disease stage, n=202 missing (2.8%)

^b Percent values do not sum to 100 because children were prescribed multiple drugs.

Abbreviations: ART, antiretroviral therapy; BMIZ, body mass index for age z score; HIV, human immunodeficiency virus; WHO, World Health Organization

Table 2.2. Iron supplementation and outcomes among pediatric HIV-infected patients in Dar es Salaam, Tanzania (n=4229)

	Prescribed any during follow-up		Number received during follow-up	
	n	%	median	1st, 3rd quartile
Iron supplement	827	19.6	1	(1, 2)
	At least one event		Time to first event (years)	
	N	%	median	1st, 3rd quartile
Mortality	247	5.8	0.41	(0.14, 1.67)
Incident pulmonary tuberculosis	615	14.5	1.17	(0.52, 2.46)
WHO Stage increase	1671	39.5	0.57	(0.15, 1.77)
Reduction in anemia severity	2246	53.1	0.45	(0.20, 0.94)
Resolution of microcytosis	691	16.3	0.96	(0.45, 1.86)

Abbreviations: WHO, World Health Organization

Iron supplementation was associated with a higher rate of mortality in both unadjusted and adjusted models (**Table 2.3**). In a model examining the association between time-varying iron supplementation adjusted for time-varying covariates, the rate of mortality was nearly three times higher in children who were prescribed iron supplements (HR=2.87; 95% CI: 1.70, 4.87). An association of lesser magnitude was observed for iron supplementation and WHO HIV disease stage progression (HR=1.48; 95% CI: 1.10, 1.98). No association was seen between iron supplementation and incident tuberculosis. Iron supplementation was also significantly associated with reductions in anemia (HR=0.47; 95% CI: 0.37, 0.61) but not for microcytosis (HR=0.63; 95% CI: 0.35, 1.15). The hazard rate of mortality among children not on ART (HR=3.04; 95% CI: 1.14, 8.10) did not differ from that among children on ART (HR=2.83; 95% CI: 1.55, 5.15; p for heterogeneity=0.90; **Table 2.4**). Similar findings by ART status were seen for all other analyzed outcomes. Furthermore, no significant differences in the association of iron supplements with clinical outcomes was observed by time-varying anemia status.

Table 2.3. Iron supplementation effects on health among HIV-infected children (n=4229).^a

	Mortality	HIV stage progression	Tuberculosis incidence ^b	Anemia persistence	Microcytosis persistence
Supplementation in first month					
Bivariate	3.64 (2.56, 5.18)	1.01 (0.81, 1.27)	0.71 (0.47, 1.09)	0.82 (0.68, 0.99)	1.37 (0.92, 2.04)
Baseline covariate adjustment ^c	2.12 (1.45, 3.11)	1.26 (1.00, 1.59)	0.81 (0.52, 1.25)	0.93 (0.76, 1.12)	1.04 (0.69, 1.57)
Time-varying supplementation					
Bivariate	5.43 (3.26, 9.04)	1.51 (1.14, 2.01)	0.92 (0.36, 2.34)	0.68 (0.53, 0.88)	0.72 (0.42, 1.24)
Baseline covariate adjustment ^c	3.00 (1.79, 5.02)	1.86 (1.40, 2.48)	0.98 (0.39, 2.52)	0.85 (0.65, 1.10)	0.64 (0.36, 1.15)
Time-varying covariate adjustment ^d	2.87 (1.70, 4.87)	1.48 (1.10, 1.98)	0.93 (0.36, 2.40)	0.47 (0.37, 0.61)	0.63 (0.35, 1.15)

^a Values are hazard ratio (95% confidence interval)

^b Children with tuberculosis at or prior to the start of study follow-up were excluded, resulting in a sample size of 3420.

^c Controlling for baseline age category, sex, HIV treatment guidelines, BMIZ, facility level, district of Dar es Salaam, anemia, mean corpuscular volume, immune status, WHO stage, and ART use

^d Controlling for time-varying age category, sex, HIV treatment guidelines, BMIZ, facility level, district of Dar es Salaam, anemia, mean corpuscular volume, immune status, WHO stage, and ART use

Table 2.4. Iron supplementation effects on health among HIV-infected children (n=4229), stratified by time-varying ART use and anemia.^{a,b}

	Mortality	HIV stage progression	Tuberculosis incidence	Anemia persistence	Microcytosis persistence
ART use					
Not on ART	3.04 (1.14, 8.10)	1.49 (0.95, 2.32)	1.21 (0.43, 3.42)	0.38 (0.21, 0.67)	1.01 (0.15, 6.58)
On ART	2.83 (1.55, 5.15)	1.49 (1.03, 2.17)	0.65 (0.39, 1.08)	0.46 (0.35, 0.62)	0.67 (0.34, 1.30)
P-value for heterogeneity	0.90	0.99	0.29	0.52	0.69
Anemia					
No anemia	4.43 (1.39, 14.11)	0.73 (0.27, 1.96)	0.19 (0.02, 2.33)	0.57 (0.38, 0.86)	0.67 (0.20, 2.21)
Mild anemia	2.66 (0.28, 24.87)	1.97 (1.01, 3.85)	0.78 (0.24, 2.53)	0.48 (0.32, 0.72)	0.40 (0.12, 1.40)
Moderate anemia	2.09 (0.92, 4.75)	1.79 (1.21, 2.64)	0.70 (0.37, 1.30)	0.28 (0.15, 0.52)	0.99 (0.35, 2.78)
Severe anemia	4.65 (1.89, 11.47)	1.15 (0.54, 2.45)	1.01 (0.46, 2.22)	n/e	0.57 (0.09, 3.70)
P-value for heterogeneity	0.62	0.19	n/e	n/e	n/e

^a Values are hazard ratio (95% confidence interval)

^b Controlling for time-varying age category, sex, HIV treatment guidelines, BMIZ, facility level, district of Dar es Salaam, anemia, mean corpuscular volume, immune status, WHO stage, and ART use

Abbreviations: ART, anti-retroviral therapy; HIV, human immunodeficiency virus; n/e, not estimable

To assess whether the association between iron supplementation and mortality was sensitive to the assumed 3-month period of iron effect, additional models were run specifying alternative effect periods. Models assuming immediate effects (i.e. effects within the next recorded clinical visit), 6-month periods, and enduring effects (i.e. from the time of supplementation to the end of follow-up) all found statistically significant and qualitatively similar associations of iron supplementation with higher rates of mortality (**Table S2.1**). A marginal structural model, which accounts for time-varying treatment and covariate feedback, found similar estimates of elevated mortality associated with iron supplementation (HR=2.40; 95% CI: 1.35, 3.16).

We assessed the possibility whether unmeasured confounding may explain the observed increased hazard ratios for mortality. A 50-percentage point disparity in prevalence of an unmeasured confounder between the iron supplemented and non-supplemented children, along with a hazard ratio between the confounder and mortality of three or more, would be needed to render the observed hazard ratio between iron supplementation and mortality as statistically insignificant (**Table S2.2**).

Discussion

In this cohort study of pediatric HIV-infected patients in Tanzania, we found that iron supplementation was associated with a significant increase in the hazard rate of mortality and HIV disease stage progression. The effects observed for iron did not differ by ART use or anemia status. These associations persisted even after control for a broad set of clinical morbidity and demographic indicators. The observed associations were also robust to alternative model specifications and the possibility of substantial unmeasured confounding. Prior studies, with

smaller sample sizes and shorter periods of follow-up, have not investigated the relationship between iron supplementation and mortality among HIV-infected children.

Prior studies among adults have found mixed results. Two trials and one observational cohort suggest that HIV viral loads are not impacted by iron supplementation. However, their small size, limited use of antiretroviral therapy among participants, and unique study populations prevent a strong generalized conclusion of no evidence for harm. The earliest of these studies randomized ART-naïve adults (n=32) in Kenya to twice-weekly parenteral iron (60 mg), and did not find a difference in the change in viral load over the four month intervention period.³¹ Another study randomized HIV and Hepatitis C co-infected female injection drug users in the USA (n=138) to micronutrients with iron (18 mg daily) versus micronutrients alone for 12 months. CD4 counts and viral loads did not differ at the end of the study.³² The third study, among a cohort of anemic pregnant women in Zambia (n=59), examined the influence of iron supplementation at two weeks postpartum on breastmilk viral load. At six weeks postpartum there was no statistically significant difference in milk viral loads between those who used iron supplements and those who did not.³³ However, a large study of adult patients (n=40,657) receiving HIV care and treatment (conducted at the same facilities as the children analysed in this study) found an increased risk of mortality associated with iron supplements.¹⁵

Two small studies of iron supplementation among HIV-infected children provide some evidence among pediatric populations. One cohort study followed HIV-infected Indian children aged 2-12 years for twelve months (n=194). The analysis compared children who were prescribed iron supplements with those who were not, and found that hemoglobin, CD4 percent, and WHO

clinical stage were similar after one year.¹⁷ However, the analysis did not control for any potentially confounding variables, making questionable any claims of causality with respect to iron supplementation. The strongest evidence comes from a trial in Malawi that randomized 209 HIV-infected children aged 6-59 mo with moderate anemia to three months of iron (3mg/kg daily) plus multivitamins (vitamins A, C and D) versus multivitamins alone. Approximately one third of enrolled children were receiving antiretroviral therapy at baseline. At the conclusion of the iron therapy period, iron-supplemented children had a decreased prevalence of anemia and increased CD4 percent, but the incidence rate of malaria more than doubled.¹⁸ The present study followed a larger cohort of children for a longer period of time and assessed mortality as an outcome.

It is unclear from this study the mechanism by which iron supplements would induce higher rates of mortality and WHO HIV disease stage progression. No association was found between iron supplementation and incident tuberculosis, which suggests that this may not be the primary pathway. An alternative explanation is that impacts are mediated by changes in viral load, which make the immune system susceptible to a broad range of opportunistic infections, including not just tuberculosis but also *Pneumocystis jiroveci* pneumonia, esophageal candidiasis, and recurrent bacterial infections.³⁴ While viral load data was not available for this study, the increase in HIV stage associated with iron supplementation would support the viral load hypothesis. A final hypothesis is malaria infection, as iron supplementation is associated with an increased risk of clinical malaria in areas without effective malaria prevention and control.³⁵ Data on malaria infections were not available for study participants, but malaria is endemic at a low prevalence in Dar es Salaam (among children aged 6-59 months, 1.2% in 2007 and 3.6% in

2011), and therefore may be a driver of increased mortality associated with iron supplementation.^{36,37}

Improvements in anemia were observed, with children receiving iron supplements over twice as likely to improve their anemia status. However, the hematological benefits accrued to some children were not enough to result in improved mortality outcomes in the iron supplemented group. It is important to note there is not sufficient data available for this cohort to ascertain the etiology of anemia for participants. It is likely that anemia of inflammation is an important contributor, but this cannot be confirmed. Whether the effects of iron may differ depending on anemia etiology is an important consideration for future research.

As with all observational research, there is a risk of residual confounding by unmeasured determinants of iron supplementation and clinical outcomes. In this study, we have controlled for a broad set of time-varying clinical indicators. These are the key variables that study clinicians used for decision-making regarding the prescription of iron supplements, which suggests that important unmeasured confounding is unlikely. Even in the case that an important confounder was omitted from this analysis, sensitivity analyses show that only confounders with a very strong association with mortality ($HR \geq 3$) and a large difference in prevalence between iron supplemented and non-supplemented children (prevalence difference $> 50\%$) would be sufficient to explain the observed associations. Another potential source of bias is inaccurate measurement of iron supplementation. This could occur if children were prescribed iron supplements but failed

to consume them, in which case the observed association would be closer to the null than the true association.

Clinicians, particularly in contexts where anemia is presumptively treated with iron supplementation, may use the findings of this study to consider the relative risks and benefits of prescribing iron to HIV-infected children. The findings are also relevant to public health policies, such as the WHO's current recommendation that iron supplements be provided to all children in areas with a high burden of anemia. Currently, a caveat is made in this guidance for malaria-endemic regions, and it may be prudent to consider a caveat for HIV-infected children as well. Evidence from one observational study – even if strongly designed – is rarely a sufficient basis for changes in clinical or public health policy. Additional observational studies of iron supplementation in HIV-infected populations could include those receiving different ART regimens and contexts with differing burdens of comorbidities and risk factors. Consideration should also be given to randomized trials, although the requirement of equipoise may require close scrutiny given the risks observed in this study.

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Supplemental Appendix

Table S2.1. Iron supplementation effects on mortality among HIV-infected children (n=4229): Sensitivity analyses of varying iron exposure periods^{a,b}

	Iron exposure period from the time of prescription			
	Until next clinic visit	For 3 months	For 6 months	Until end of follow-up
Time-varying supplementation				
Bivariate	9.49 (4.71, 19.10)	5.43 (3.26, 9.04)	5.06 (3.16, 8.12)	4.27 (2.96, 6.16)
Baseline covariate adjustment ^b	5.40 (2.70, 10.81)	3.00 (1.79, 5.02)	2.89 (1.78, 4.69)	2.64 (1.79, 3.89)
Time-varying covariate adjustment ^c	4.95 (2.46, 9.96)	2.87 (1.70, 4.87)	2.71 (1.65, 4.45)	2.32 (1.55, 3.46)
Effect modification by time-varying ART use ^c				
Not on ART	4.23 (1.35, 13.26)	3.04 (1.14, 8.10)	2.39 (0.85, 6.68)	2.07 (0.82, 5.21)
On ART	5.62 (2.34, 13.49)	2.83 (1.55, 5.15)	2.82 (1.61, 4.93)	2.42 (1.56, 3.78)
P-value for heterogeneity	0.69	0.90	0.78	0.76
Effect modification by time-varying anemia ^c				
No anemia	10.87 (2.36, 50.13)	4.43 (1.39, 14.11)	3.41 (1.00, 11.62)	3.03 (1.22, 7.48)
Mild anemia	n/e	2.66 (0.28, 24.87)	3.02 (0.72, 12.59)	1.87 (0.59, 5.90)
Moderate anemia	4.42 (1.73, 11.31)	2.09 (0.92, 4.75)	1.97 (0.98, 3.96)	2.04 (1.16, 3.57)
Severe anemia	4.73 (1.30, 17.18)	4.65 (1.89, 11.47)	4.36 (1.71, 11.08)	3.37 (1.49, 7.66)
P-value for heterogeneity	0.37	0.62	0.52	0.09

^a Values are hazard ratio (95% confidence interval)

^b Controlling for time-varying age, sex, HIV treatment guidelines, BMIZ, facility level, district of Dar es Salaam, anemia, mean corpuscular volume, immune status, WHO stage, and ART use

Abbreviations: ART, antiretroviral therapy; n/e, not estimable

Table S2.2. Different assumptions about unmeasured confounding to assess possibility of bias that may affect the association between iron supplements and mortality^a

Prevalence of unmeasured confounder		Effect (HR) of unmeasured confounder on outcome				
No iron supplementation	Iron supplementation	1.5	2	3	5	10
0.1	0.15	1.02	1.05	1.08	1.14	1.24
0.1	0.2	1.05	1.09	1.17	1.29	1.47
0.1	0.3	1.10	1.18	1.33	1.57	1.95
0.1	0.6	1.24	1.45	1.83	2.43	3.37
0.25	0.3	1.02	1.04	1.07	1.10	1.14
0.25	0.35	1.04	1.08	1.13	1.20	1.28
0.25	0.45	1.09	1.16	1.27	1.40	1.55
0.25	0.75	1.22	1.40	1.67	2.00	2.38
0.5	0.55	1.02	1.03	1.05	1.07	1.08
0.5	0.6	1.04	1.07	1.10	1.13	1.16
0.5	0.7	1.08	1.13	1.20	1.27	1.33
0.5	1	1.20	1.33	1.50	1.67	1.82

^a Bias factors shaded in green indicate that the unmeasured confounder would not be sufficient to negate a statistically significant positive association between iron supplements and mortality, in yellow indicate that the association between iron and mortality would no longer be statistically significant, and in red indicate that the association would be null or inverted.

Chapter 3: Anemia etiology in Ethiopia: assessment of nutritional, infectious disease, and other risk factors in a population-based cross-sectional survey of women, men, and children

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Abstract

Background: While the causes of anemia at an individual level (such as certain infections, nutritional deficiencies, and genetic disorders) are well defined, there is limited understanding of the relative burden of anemia attributable to each cause within populations. The study's objective was to estimate the prevalence of anemia and the proportion of anemia cases attributable to nutritional, infectious disease, and other risk factors among women, men, and children in Ethiopia.

Methods: A population-based cross-sectional study using multi-stage sampling was conducted in six regions of Ethiopia in both the wet and dry seasons. Data was obtained from 2,520 women of reproductive age (15-49 years), 1,044 adult men (15-49 years), and 1,528 children (6-59 months). Participants provided venous blood samples for assessment of hemoglobin concentration, ferritin, folate, vitamin B12, C-reactive protein, and malaria infection. Stool samples were collected to ascertain helminth infection status. Sociodemographic questionnaires and a 24-hour diet recall were administered. Population-weighted prevalences of anemia and risk factors were calculated. Multivariable-adjusted associations of risk factors with anemia and partial population attributable risk percentages (pPAR%) were estimated using generalized linear models.

Findings: Anemia prevalence was 17% (95% CI: 13, 21) among women, 8% (6, 11) among men, and 22% (19, 26) among children. Low serum ferritin contributed to 11% (-1, 22) of anemia cases among women, 9% (0, 17) among men, and 19% (3, 33) among children. The proportion of anemia attributable to low serum folate was estimated at 25% (4, 40) among women and 28 (11, 42) among men. Dietary iron intake was adequate for nearly all participants, while inadequacy was common for folate and vitamin B12. Inflammation and malaria were responsible

less than one in ten anemia cases. Unimproved water and sanitation were significant contributors to anemia among adults.

Interpretation: Folate deficiency, iron deficiency, and inflammation appear to be important contributors to anemia in Ethiopia. Folic acid food fortification, targeted iron interventions, and strategies to reduce infections may be considered as potential public health interventions to reduce anemia in Ethiopia.

Introduction

Anemia remains a major public health challenge in low- and middle-income countries (LMIC). In 2016, an estimated 44.6% of children aged <5 years and 35.1% of non-pregnant women aged 15-49 years globally were anemic.⁴ Anemia is associated with increased mortality among children and pregnant women, impaired cognitive development among children, and reduced productivity among adults.⁵⁻⁸ As a result, it was estimated that in 2010 anemia was responsible for 8.8% of global years lived with disability, more than major depression, chronic respiratory disease, or injuries.³

The underlying causes of anemia at the individual-level are relatively well-understood, including nutritional deficits (e.g. iron, folate, vitamin B12), infections (e.g. malaria, hookworm, HIV), and hemoglobinopathies (e.g. thalassemias, sickle cell).⁹ However, it is important for public health policy-makers to understand the relative contribution of each of these causes at the population level when planning programs and allocating resources. Modeling studies have attempted to identify the proportion of anemia cases in the population attributable to each cause by assembling data from multiple sources on risk factor prevalence and the strength of the association between the risk factor and anemia.³ However, it is rare for population-representative participant-level data on multiple risk factors to be collected in a single study and used to directly estimate the proportion of anemia cases due to each cause. It is well known that with multifactorial diseases such as anemia, attributable risk estimates are biased when only one risk factor at a time is considered.¹⁰

In Ethiopia, several studies have collected countrywide data on anemia prevalence or some risk factors, but there is no study which estimates the proportion of anemia attributable to each cause among the full set of known risk factors. The 2015 Ethiopian National Micronutrient Survey collected blood specimens to assess the prevalence of anemia and serum micronutrient levels but did not assess diet and some infectious causes of anemia.¹¹ The 2013 Ethiopian National Food Consumption Survey administered a 24-hour dietary recall questionnaire to participants to estimate iron and other nutrient intake, but hemoglobin and non-dietary risk factors for anemia were not assessed.¹² The 2016 Demographic Health Survey assessed anemia prevalence and malaria, but not micronutrient status.¹³ As a result, gaps in data availability in each of these surveys has not allowed for a complete and valid assessment of the contribution of diet, micronutrient status, infections and other risk factors jointly to anemia prevalence in Ethiopia. Furthermore, none of these surveys attempted to estimate the proportion of anemia cases attributable to each risk factor.

This population-based study assessed the prevalence of anemia along with risk factors including dietary intake, nutritional and infectious disease blood biomarkers, malaria and helminth infections, and socioeconomic factors among women of reproductive age, adult men, and children 6-59 months in six regions of Ethiopia. The relative contribution of risk factors to the burden of anemia was estimated in order to inform decision-making on anemia control strategies.

Methods

Study population and sampling

The Anemia Etiology in Ethiopia (AnemEE) study is a population-based cross-sectional study conducted among women, men, and children. Detailed methods of the survey have been published elsewhere.¹⁴ Administratively, Ethiopia is divided into 10 regions and 2 city administrations. Sampling was stratified across six of these (Addis Ababa, Afar, Amhara, Oromia, Southern Nations Nationalities and Peoples [SNNP], and Tigray). Within each region a multi-stage sampling design was employed using administrative divisions. Random selection was performed for two zones (second-level administrative divisions) within each region, three woredas (third-level) within each selected zone, and two kebeles (fourth-level) within each selected woreda. Households were randomly selected within each kebele and were eligible for inclusion if they included a woman of reproductive age (15-49 years). A target sample size of seventeen women of reproductive age, seven men (15-49 years), and ten children (6-59 months) were selected for data collection in each kebele. The total sample size was selected to have regionally representative anemia estimates for children, adult women, and adult men in the six selected regions. The assumptions used to calculate sample size were: anemia prevalence equal to the 2016 Demographic and Health Survey, 10% precision overall, 90% participation at the household, 90% participation at the individual-level, and a design effect of 2.0. The survey was undertaken twice in the same kebele – once from January to March 2019 and again from June to August 2019 and included different participants from each kebele by round to assess seasonal differences.

Data collection

Data were collected via a standardized questionnaire, 24-hour diet recall and blood and stool collection. Questionnaires were administered by trained enumerators following standard operating procedures and using tablet-based software (Survey CTO, Doherty Inc, Cambridge MA). Data quality was ensured through random spot-checks performed by field supervisors and by central-level weekly monitoring of data submissions. Blood and stool samples were collected by experienced phlebotomists and fieldworkers.

A household questionnaire was administered to collect information on sociodemographic characteristics (e.g. education, income, assets), household infrastructure (e.g. electricity, water, sanitation), health behaviors (e.g. smoking), and morbidity. Diet was assessed using a 24-hour recall tailored to the dietary characteristics of Ethiopia. A multiple-pass method was used to maximize recall accuracy of items consumed, their quantity, and their preparation method.¹⁵ A second 24-hour recall was administered to a subset of participants to assess diet variability.¹⁶ Venous blood was collected; 2 mL were used to assess hemoglobin level and malaria infection and 5 mL were used to measure serum concentrations of ferritin (an indicator of iron deficiency), C-reactive protein (CRP; an indicator of inflammation), folate, and vitamin B12. Participants who refused a blood draw were requested to provide finger-prick capillary blood to assess hemoglobin status. Stool samples were collected to assess the presence of intestinal helminths. Since household eligibility was based on the presence of a woman of reproductive age, a larger number of women of reproductive age were contacted than was needed to achieve in order to achieve the target sample size for men and children. For the women who fell above the target

sample size (approximately half of women in each kebele), questionnaire-based data and a venous blood sample for hemoglobin and malaria assessment were collected.

Defining anemia and risk factors

Hemoglobin values were adjusted for altitude and then categorized as anemic according to World Health Organization (WHO) cutoffs; cutoffs used in this study are provided in **Table S3.1**.^{17,18} Serum ferritin concentrations were adjusted for inflammation (measured by CRP).¹⁹ Serum folate and serum vitamin B12 were categorized as low according to WHO criteria and standard clinical guidance, respectively, and serum ferritin was categorized as low or high according to WHO criteria.²⁰⁻²² Presence of malaria infection was defined as a positive result from a rapid diagnostic test. Microscopic evaluation of Kato–Katz slides of stool specimens were examined for the presence of intestinal helminths and categorized as helminth-infected if they contained ova for roundworm (*Ascaris lumbricoides*), hookworm (*Ancylostoma duodenale*), whipworm (*Trichuris trichiura*), or tapeworm (*Hymenolepis nana* or *Taenia* species).

Dietary intake was assessed using the food items and quantities reported in the 24-hour recall. Nutrient intake was calculated by matching the consumed food items and their quantity to a dataset based on the Ethiopian Food Composition Table (EFCT).²³ For nutrient values not listed in the EFCT, food composition tables from Uganda, Tanzania, and the United States were used.²⁴⁻²⁶ The value for iron content in injera – a commonly consumed food item and important source of iron – was taken from a study that accounted for soil contamination that occurs during teff grain processing.²⁷ Cutoffs for inadequate nutrient intake of iron, folate, and vitamin B12

were defined as consumption less than the age- and sex-specific Estimated Average Requirement of nutrient intake and assuming a low bioavailability of iron absorption (5%).²⁸

Heavy menstruation is a risk factor for anemia among women of reproductive age.²⁹ Women were asked three questions based on a previously validated questionnaire: how heavy they perceived their period to be, how much pain they experienced, and the duration of their period.³⁰ These questions were combined into a single variable using principal components analysis and the highest ten percent of this new variable were classified as having heavy menstruation. Symptoms of diarrhea, cough, and fever during the previous two weeks were self-reported for adults or reported by the caregiver for children. Use of an unimproved source of water and unimproved sanitation was defined according to criteria by the WHO.³¹ An asset index was calculated by conducting principal components analysis using a list of items owned by households and then dividing participants into quintiles. Data collection occurring between June and August was defined as the wet season for all regions except Afar, where the climate was hot and dry during this period.

Statistical analysis

The prevalence of overall anemia by region and season for women, men, and children was estimated. The prevalence of mild, moderate, and severe anemia was also estimated by region. Standard errors were adjusted for correlated outcomes (within kebeles, woredas, and zones) and for stratification by region and season. Estimates of anemia prevalence aggregated across all six regions were weighted according to their relative age and sex-specific population sizes.³² The

prevalence of risk factors in the weighted sample was calculated among both anemic and non-anemic participants and compared using Pearson's design-based F statistic. Possible bias due to missing dietary and specimen collection among participants was accounted for using inverse probability of censoring weights (see **Appendix 3.1** for details).³³ Censoring weights were estimated by logistic regression models using socioeconomic data drawn from the household questionnaire (completion of primary education, improved water source, improved sanitation, asset quintile, region, and season).

Estimates of usual diet were calculated using the IMAPP software for intake distribution based on the Iowa State University methodology for prevalence measures, and based on mixed regression models for risk ratios and partial population attributable risk percentages (pPAR%).³⁴ Estimates of usual dietary intake were obtained by estimating within- and between-person variation in intake by participant type and calculated using data from repeat 24-hour recalls taken for a subset of the sample (n=309).³⁵ Additional information on the methods and results of usual dietary intake estimation are presented in **Appendix 3.2**. Confidence intervals for these nutrient analyses using predicted values of usual diet were calculated using bootstrapping (see **Appendix 3.3** for details).³⁶ The prevalence of inadequate intake of iron was nearly zero, and the prevalence of inadequate vitamin B12 was nearly one hundred percent, so binary cutoffs for dietary intake were not used in the model for these nutrients. Instead, quartiles of nutrient intake adjusted for total energy intake using the residual method were used.³⁷ For iron and vitamin B12 intake, risk ratios and pPAR% were calculated comparing the lowest three quartiles of consumption to the highest quartile, whereas for folate a binary indicator defined by the Estimated Average Requirement was used.

Risk ratios for the association of risk factors with anemia were calculated using generalized linear models with a log link, Poisson distribution, and robust standard error.³⁸ Clustering due to the complex survey sampling design was accounted for using Stata's "svyset" command applying standard methods.^{39,40} A proximal, medial, and distal risk factor model was estimated for each participant type (women, men, and children). The proximal model – which contains risk factors that most immediately precede anemia (serum biomarkers and infections) – includes only participants with complete blood and stool data. The medial models include intermediate risk factors (such as usual diet and morbidity symptoms) and the distal models include socioeconomic risk factors (such as sanitation and assets). Medial and distal models include participants with hemoglobin data. Partial population attributable risk percentages were estimated from multivariate models.^{41,42} Risk ratios and pPAR% were weighted to represent the population distribution aggregated across the six sampled states.³² Given the possibility that altitude adjustment of hemoglobin may introduce bias in risk ratio and PAR% estimates if altitude is correlated with risk factors, sensitivity analyses were conducted using the outcome of anemia without altitude adjustment.

Ethics

The AnemEE protocol was approved by the Harvard T. H. Chan School of Public Health Institutional Review Board (Ref. No. IRB18–0236) and the Addis Continental Institute of Public Health Institutional Review Board (Ref. No. ACIPH/IRB/005/2018). A support letter was also obtained from the Ethiopian Federal Ministry of Health (Ref. No.1/49/44/671). All adult participants were asked to provide written informed consent for study participation. Individuals who consented for study participation were also asked to consent to a blood draw and stool

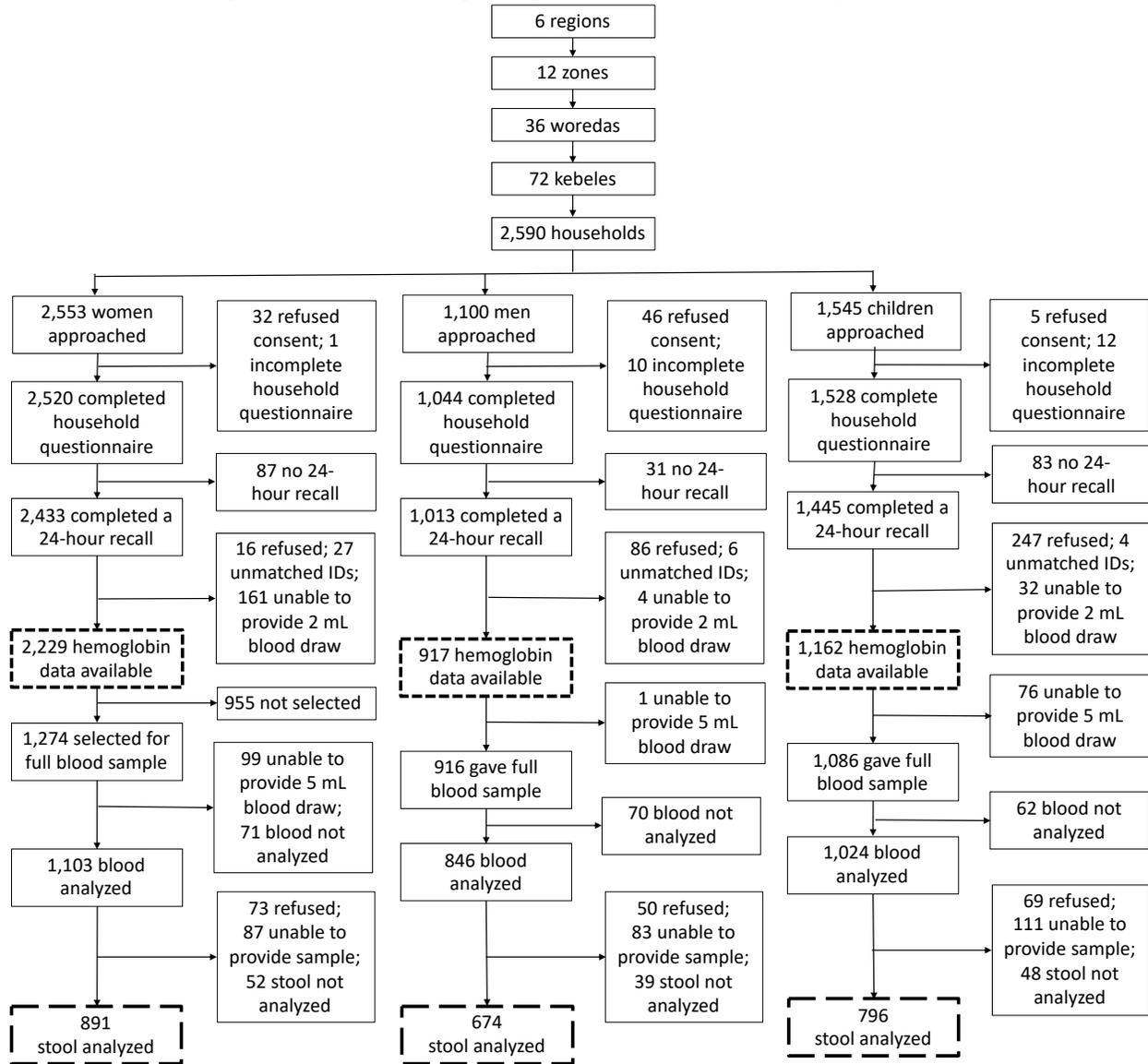
specimen collection. Mothers or guardians provided informed written consent for the children selected to participate in the study.

Results

There were 2,520 women, 1,044 men, and 1,528 children who consented for study participation and completed a household questionnaire (**Figure 3.1**). Among these, 2,229 women (88%), 917 men (88%), and 1,162 children (76%) had hemoglobin concentration data. Among women, 1,274 were randomly selected for serum micronutrient and inflammatory biomarker specimen collection while all 917 men and 1,162 children were eligible for biomarker assessment. Stool samples were obtained for 891 women, 674 men, and 796 children.

The prevalence of anemia among women was 16.6% (95% CI: 12.8, 21.4), among men was 8.1% (5.8, 11.3), and among children was 21.9% (18.8, 25.5; **Table 3.1**). No statistically significant differences in anemia prevalence were observed by season (**Table S3.2**). Significant differences were observed between regions among women, with Afar showing the highest prevalence. The majority of anemia cases were mild (prevalence of mild anemia was 10.3% among women, 5.4% among men, and 13.6% among children; **Table S3.3**). Moderate anemia (5.7% among women, 2.2% among men, and 7.2% among children) was more common than severe anemia (0.5% among women and men, and 1.0% among children). The prevalence of iron

Figure 3.1. Flowchart of participant data collection and study inclusion.



Key: Sample size for medial and distal models Sample size for proximal models

Table 3.1. Prevalence of anemia in Ethiopia among women, men and children by region and time of data collection.*

Participant	Region	Overall			Dry season			Wet season		
		<i>n</i> <i>anemic</i>	<i>n</i> <i>total</i>	<i>weighted %</i> <i>(95% CI)</i>	<i>n</i> <i>anemic</i>	<i>n</i> <i>total</i>	<i>weighted %</i> <i>(95% CI)</i>	<i>n</i> <i>anemic</i>	<i>n</i> <i>total</i>	<i>weighted %</i> <i>(95% CI)</i>
Women	All regions†	425	2229	16.6 (12.8, 21.4)	224	1136	14.3 (11.3, 18.0)	201	1093	18.9 (12.3, 27.9)
	Addis	39	378	10.4 (8.8, 12.3)	22	175	12.6 (10.8, 14.6)	17	203	8.4 (6.1, 11.4)
	Afar	153	371	41.7 (36.3, 47.3)	90	183	49.7 (45.2, 54.2)	63	188	33.6 (24.0, 44.8)
	Amhara	63	357	17.8 (8.7, 32.9)	32	193	16.8 (11.3, 24.3)	31	164	18.8 (4.8, 51.4)
	Oromia	62	368	17.7 (11.5, 26.2)	23	191	12.3 (6.6, 21.7)	39	177	23.1 (13.5, 36.6)
	SNNP	50	404	12.3 (10.5, 14.4)	22	200	10.8 (7.5, 15.3)	28	204	13.8 (13.0, 14.7)
	Tigray	58	351	16.9 (14.3, 19.9)	35	194	17.9 (14.3, 22.3)	23	157	15.9 (12.4, 20.1)
Men	All regions†	87	917	8.1 (5.8, 11.3)	46	467	8.0 (5.6, 11.2)	41	450	8.3 (5.5, 12.5)
	Addis	3	125	2.2 (0.9, 5.5)	1	52	1.8 (0.2, 14.8)	2	73	2.7 (0.6, 11.3)
	Afar	25	187	12.8 (7.5, 20.9)	16	94	17.0 (9.3, 29.1)	9	93	9.5 (3.5, 23.0)
	Amhara	14	148	9.3 (7.9, 11.0)	9	81	10.9 (5.7, 19.8)	5	67	7.4 (2.7, 18.9)
	Oromia	8	151	5.6 (1.6, 17.8)	4	81	5.3 (2.1, 12.7)	4	70	5.9 (1.6, 19.1)
	SNNP	18	162	10.9 (8.7, 13.6)	7	82	8.5 (3.5, 18.9)	11	80	13.5 (6.8, 25.1)
	Tigray	19	144	13.6 (6.1, 27.7)	9	77	12.3 (5.7, 24.8)	10	67	15.4 (6.2, 33.3)
Children	All regions†	267	1162	21.9 (18.8, 25.5)	124	566	20.3 (14.8, 27.2)	143	596	23.4 (20.6, 26.4)
	Addis	29	135	20.9 (12.5, 32.7)	7	42	17.2 (5.4, 43.2)	22	93	23.4 (14.4, 35.8)
	Afar	67	216	31.3 (17.6, 49.4)	36	120	30.2 (13.8, 54.0)	31	96	32.7 (13.0, 61.2)
	Amhara	47	202	23.4 (20.7, 26.4)	19	103	18.2 (14.4, 22.7)	28	99	28.1 (25.2, 31.1)
	Oromia	48	190	24.5 (18.0, 32.4)	26	102	25.3 (13.3, 42.9)	22	88	23.7 (22.6, 24.9)
	SNNP	32	219	14.6 (9.5, 21.8)	11	96	11.5 (9.5, 13.8)	21	123	17.3 (8.5, 31.9)
	Tigray	44	200	22.1 (13.9, 33.6)	25	103	24.2 (9.4, 49.7)	19	97	20.2 (16.2, 24.9)

* Hemoglobin values adjusted for altitude using WHO-recommended regression method. Anemia defined as <12 g/dl for women, <13 g/dl for men, and <11 g/dl for children.

†Average weighted by regional population size.

deficiency anemia (defined according to the WHO definition of concurrent anemia and low serum ferritin) was 1.3% in men, 3.7% in females, and 9.2% in children (**Table S3.4**).

Analysis of serum samples demonstrated that folate deficiency was the most common micronutrient deficiency evaluated among women (41.0% [33.2, 49.3]) and men (38.7% [28.6, 49.9]), and was also common among children (20.9% [16.3, 26.4]; **Table 3.2**). The prevalence of low serum ferritin, an indicator of iron deficiency, was 24.9% among children (18.7, 32.3) and 13.7% among women (9.2, 19.9), but not as high among men (4.5% [3.3, 6.0]). Low serum vitamin B12 was prevalent in about one in four adults and one in five children. High levels of inflammation were seen in 7-9% of women, men, and children. The prevalence of risk factors varied by population and region (**Table S3.5**).

In terms of diet, the mean daily iron intake was 55 mg/d among women, 63 mg/d among men, and 30 mg/d among children. Mean daily folate intake was 305 µg/d among women, 363 µg/d among men, and 174 µg/d among children (**Table S3.6**). Usual iron intake less than the Estimated Average Requirement was found in less than 1% of participants, but 99% of participants had an estimated intake below the tolerable upper level (**Table S3.7**). Inadequate amounts of folate were consumed by 42% of women, 30% of men, and 26% of children.

Inadequate dietary vitamin B12 consumption was observed in 100% of women, 98% of men, and 43% of children. Malaria prevalence was uncommon, appearing in less than 2% of the population. Helminth infections were also rare, appearing in 5% or less of the population. Self-reported diarrhea, cough, and fever in the two weeks prior to survey was not rare.

Table 3.2. Anemia risk factors among women, men, and children in six regions of Ethiopia.

Participant	Risk factor	All		Anemic		Non-Anemic		p-value	
		%	95% CI	%	95% CI	%	95% CI		
Women	Low serum ferritin	13.7	(9.2, 19.9)	22.0	(9.8, 42.3)	12.1	(7.9, 18.2)	0.13	
	Low serum folate	41.0	(33.2, 49.3)	57.4	(41.8, 71.7)	38.0	(31.3, 45.2)	0.003	
	Low serum vitamin B12	25.5	(14.4, 41.1)	15.5	(6.3, 33.4)	27.4	(15.5, 43.6)	0.08	
	High C-reactive protein	6.6	(5.5, 8.0)	14.6	(7.1, 27.7)	5.2	(3.9, 6.9)	0.03	
	Helminth infection	4.4	(1.7, 10.8)	1.0	(0.2, 4.7)	5.1	(1.9, 12.7)	0.03	
	Malaria	1.5	(0.3, 6.3)	2.8	(0.5, 13.8)	1.2	(0.3, 4.7)	0.007	
	Inadequate dietary iron	0.5	(0.2, 1.5)	0.3	(0.0, 2.5)	0.5	(0.2, 1.5)	0.52	
	Inadequate dietary folate	42.2	(39.0, 45.4)	43.2	(32.9, 54.1)	41.9	(39.0, 45.0)	0.80	
	Inadequate dietary vitamin B12	100.0	(99.8, 100.0)	100.0	--	100.0	(99.8, 100.0)	0.67	
	Heavy menstruation	23.6	(21.6, 25.7)	20.9	(17.1, 25.4)	24.1	(21.3, 27.1)	0.30	
	Diarrhea	7.4	(6.1, 9.0)	8.1	(5.7, 11.4)	7.3	(5.6, 9.4)	0.66	
	Cough	16.3	(14.4, 18.4)	20.5	(13.1, 30.7)	15.5	(13.8, 17.4)	0.22	
	Fever	27.4	(25.9, 29.0)	31.9	(25.6, 39.0)	26.6	(24.4, 28.9)	0.17	
	Woman has not completed primary education	69.2	(63.9, 74.1)	74.1	(69.7, 78.1)	68.3	(62.5, 73.5)	0.02	
	Unimproved water source	16.1	(11.4, 22.1)	26.1	(18.0, 36.3)	14.0	(9.3, 20.6)	0.02	
	Unimproved sanitation	72.4	(69.5, 75.2)	77.4	(72.2, 81.9)	71.5	(68.0, 74.7)	0.06	
	Men	Low serum ferritin	4.5	(3.3, 6.0)	12.7	(6.1, 24.4)	3.7	(2.1, 6.5)	0.04
		Low serum folate	38.7	(28.6, 49.9)	52.1	(39.5, 64.4)	37.4	(27.2, 48.9)	0.007
Low serum vitamin B12		23.2	(8.0, 51.1)	13.4	(5.1, 30.6)	24.1	(8.3, 52.8)	0.07	
High C-reactive protein		8.2	(6.8, 9.9)	14.4	(5.9, 31.0)	7.6	(6.0, 9.6)	0.15	
Helminth infection		4.4	(2.3, 8.0)	7.4	(3.0, 17.3)	4.1	(2.2, 7.3)	0.04	
Malaria		1.0	(0.2, 4.9)	7.1	(1.4, 29.8)	0.4	(0.1, 2.2)	<0.001	
Inadequate dietary iron		0.0	--	0.0	--	0.0	--	n/e	
Inadequate dietary folate		29.8	(24.2, 36.1)	28.8	(19.1, 40.9)	29.9	(23.9, 36.7)	0.84	
Inadequate dietary vitamin B12		98.0	(97.1, 98.6)	98.7	(95.2, 99.7)	97.9	(97.0, 98.6)	0.42	
Diarrhea		5.8	(4.5, 7.3)	9.5	(3.4, 24.0)	5.4	(4.3, 6.8)	0.23	
Cough		14.7	(12.3, 17.4)	16.0	(7.5, 30.8)	14.5	(12.0, 17.5)	0.78	
Fever		19.3	(13.9, 26.3)	26.0	(19.4, 33.9)	18.8	(13.0, 26.3)	0.13	
Woman has not completed primary education		69.3	(63.3, 74.8)	67.7	(54.5, 78.6)	69.5	(63.5, 74.9)	0.71	
Unimproved water source		17.3	(9.1, 30.5)	20.4	(8.4, 41.7)	17.0	(9.1, 29.7)	0.34	
Unimproved sanitation	71.9	(64.2, 78.6)	84.1	(76.4, 89.6)	70.9	(62.9, 77.7)	<0.001		

Table 3.2. (Continued)

Participant	Risk factor	%	All	Anemic	Non-Anemic	p-value		
			95% CI	95% CI	95% CI			
Children	Low serum ferritin	24.9	(18.7, 32.3)	40.5	(23.4, 60.3)	20.9	(15.3, 27.9)	0.02
	Low serum folate	20.9	(16.3, 26.4)	30.7	(23.1, 39.5)	18.4	(14.1, 23.5)	<0.001
	Low serum vitamin B12	19.6	(12.9, 28.6)	18.9	(11.5, 29.4)	19.8	(12.8, 29.2)	0.78
	High C-reactive protein	8.7	(5.8, 12.8)	14.3	(10.4, 19.3)	7.3	(4.4, 11.8)	<0.001
	Helminth infection	5.1	(2.0, 12.4)	1.3	(0.3, 6.2)	6.1	(2.3, 15.2)	0.06
	Malaria	1.4	(0.2, 8.6)	0.2	(0.1, 0.5)	1.7	(0.2, 11.1)	0.01
	Inadequate dietary iron	0.6	(0.5, 0.8)	0.4	(0.0, 3.3)	0.7	(0.5, 1.0)	0.63
	Inadequate dietary folate	25.5	(22.6, 28.6)	26.6	(23.6, 30.0)	25.2	(21.2, 29.6)	0.63
	Inadequate dietary vitamin B12	42.6	(40.4, 44.7)	38.5	(32.8, 44.6)	43.7	(40.1, 47.4)	0.23
	Diarrhea	13.8	(11.1, 17.1)	25.5	(19.2, 32.9)	10.6	(8.0, 13.8)	<0.001
	Cough	24.0	(20.5, 27.9)	32.6	(24.7, 41.5)	21.6	(16.7, 27.4)	0.06
	Fever	22.6	(20.2, 25.3)	33.3	(26.2, 41.2)	19.6	(15.9, 24.0)	0.009
	Woman has not completed primary education	69.9	(63.6, 75.6)	75.3	(66.6, 82.3)	68.4	(61.8, 74.3)	0.06
	Unimproved water source	15.4	(9.1, 24.9)	17.1	(10.7, 26.2)	14.9	(8.0, 26.2)	0.64
	Unimproved sanitation	71.7	(67.0, 76.0)	75.0	(67.0, 81.6)	70.8	(64.4, 76.4)	0.41

The proximal model examined the association and contribution to anemia of serum biomarkers and infections. After adjustment for other risk factors in the proximal model for anemia among women, positive associations were observed between anemia and low serum ferritin (relative risk [RR] = 2.05 [0.95, 4.41]), low serum folate (RR=1.74 [1.08, 2.82]), high CRP (RR=2.81 [1.58, 4.98]), and malaria (RR=2.49 [1.65, 3.77]; **Table 3.3**). Among men, a similar set of risk factors were found in the proximal risk factor model (low serum ferritin RR=3.33 [1.17, 9.47]), low serum folate RR=2.18 [1.35, 3.51], high CRP RR=2.43 [0.76, 7.79], malaria RR=14.11 [4.81, 41.46]; **Table 3.4**). For children, low serum ferritin (RR=1.91 [1.17, 3.10]), low serum folate (RR=1.24 [0.84, 1.84]), and high C-reactive protein (RR=1.58 [1.14, 2.17]) were associated with increased risk of anemia (**Table 3.5**).

After adjustment for other proximal risk factors and potential confounders, the proportion of anemia attributable to low serum ferritin among women was 11% (-1, 22), among men was 9% (0, 17), and among children was 19% (3, 33). Low serum folate was estimated to contribute to 25% (4, 40) of anemia cases among women and 28% (11, 42) among men. High CRP contributed to 9% (2, 16) of anemia among women, 8% (-6, 21) among men, and 5% (2, 9) among children. While malaria had strong relative risks for anemia among women and men, the low prevalence of malaria at the population-level resulted in only 3% (2, 4) of anemia cases attributed among women and 8% (3, 13) attributed among men. Low serum vitamin B12 was found to have a protective association with anemia among women (-13% [-23, -3]) and men (-29% [-45, -14]). Risk factors in the proximal model that were significantly associated with increased anemia were together responsible for 34% (13, 49) of anemia cases among women, 41% (24, 59) among men, and 24% (8, 36) among children (**Table S3.8**).

Table 3.3. Proportion of anemia cases attributable to risk factors among women aged 15-49 years in six regions of Ethiopia.

	Risk ratio		Partial population attributable percent	
	<i>RR</i>	<i>95% CI</i>	%	<i>95% CI</i>
Proximal factors model				
Low serum ferritin	2.05	(0.95, 4.41)	11	(-1, 22)
Low serum folate	1.74	(1.08, 2.82)	25	(4, 40)
Low serum vitamin B12	0.55	(0.31, 0.99)	-13	(-23, -3)
High C-reactive protein	2.81	(1.58, 4.98)	9	(2, 16)
Malaria	2.49	(1.65, 3.77)	3	(2, 4)
Helminth infection	0.30	(0.07, 1.32)	-2	(-4, 0)
Medial factors model				
Lower three quartiles of dietary iron intake	0.87	(0.70, 1.31)	-10	(-28, 19)
Insufficient dietary folate intake	1.11	(0.85, 1.39)	4	(-8, 14)
Lower three quartiles of dietary vitamin B12 intake	1.29	(0.91, 1.97)	18	(-7, 44)
Heavy menstruation	0.92	(0.41, 1.12)	-2	(-16, 3)
Diarrhea	1.01	(0.49, 1.54)	0	(-4, 3)
Cough	1.27	(1.07, 2.33)	4	(1, 13)
Fever	1.17	(0.83, 1.54)	4	(-5, 14)
Distal factors model				
Not completed primary education	1.14	(0.84, 1.54)	9	(-11, 25)
Unimproved water source	1.67	(1.08, 2.57)	10	(1, 19)
Unimproved sanitation	1.14	(0.87, 1.51)	10	(-9, 25)
Lower four quintiles of household asset index	1.25	(0.57, 2.71)	18	(-55, 57)
Region				
Addis	ref			
Amhara	1.25	(0.53, 2.92)	6	(-15, 23)
Afar	2.38	(1.33, 4.25)	3	(2, 4)
Oromia	1.16	(0.58, 2.32)	6	(-20, 26)
SNNP	0.92	(0.56, 1.53)	-1	(-9, 6)
Tigray	1.34	(0.87, 2.08)	2	(0, 4)
Wet season	1.35	(0.87, 2.08)	15	(-7, 32)

Note 1: Sample size for proximal model is 891, and for medial and distal models is 2,229.

Note 2: Multivariate estimates for the proximal factors model adjusted for all variables listed under proximal factors plus all variables listed under distal factors and age. Multivariate estimates for the medial factors model adjusted for all variables listed under medial factors plus all variables listed under distal factors and age.

Multivariate estimates for the distal factors model adjusted for all variables listed under distal factors and age.

Table 3.4. Proportion of anemia cases attributable to risk factors among men aged 15-49 years in six regions of Ethiopia.

	Risk ratio		Partial population attributable percent	
	<i>RR</i>	<i>95% CI</i>	%	<i>95% CI</i>
Proximal factors model				
Low serum ferritin	3.33	(1.17, 9.47)	9	(0, 17)
Low serum folate	2.18	(1.35, 3.51)	28	(11, 42)
Low serum vitamin B12	0.32	(0.16, 0.62)	-29	(-45, -14)
High C-reactive protein	2.43	(0.76, 7.79)	8	(-6, 21)
Malaria	14.11	(4.81, 41.46)	8	(3, 13)
Helminth infection	1.00	(0.41, 2.43)	0	(-6, 6)
Medial factors model				
Lower three quartiles of dietary iron intake	2.03	(0.72, 5.69)	40	(-25, 73)
Insufficient dietary folate intake	0.92	(0.29, 1.49)	-2	(-29, 11)
Lower three quartiles of dietary vitamin B12 intake	2.03	(0.76, 5.75)	45	(-23, 78)
Diarrhea	1.70	(0.13, 3.17)	4	(-7, 7)
Cough	0.78	(0.24, 1.59)	-4	(-23, 9)
Fever	1.55	(0.59, 3.20)	9	(-8, 23)
Distal factors model				
Not completed primary education	0.81	(0.38, 1.75)	-16	(-86, 28)
Unimproved water source	1.25	(0.75, 2.07)	4	(-5, 13)
Unimproved sanitation	1.99	(1.05, 3.74)	42	(6, 64)
Lower four quintiles of household asset index	1.66	(0.83, 3.32)	37	(-11, 65)
Region				
Addis	ref			
Amhara	2.51	(1.00, 6.33)	18	(3, 30)
Afar	3.10	(0.97, 9.85)	2	(0, 4)
Oromia	1.40	(0.37, 5.22)	7	(-24, 31)
SNNP	3.11	(1.31, 7.40)	19	(7, 29)
Tigray	3.71	(1.26, 10.90)	8	(0, 15)
Wet season	1.07	(0.57, 2.01)	4	(-29, 28)

Note 1: Sample size for proximal model is 674, and for medial and distal models is 917.

Note 2: Multivariate estimates for the proximal factors model adjusted for all variables listed under proximal factors plus all variables listed under distal factors and age. Multivariate estimates for the medial factors model adjusted for all variables listed under medial factors plus all variables listed under distal factors and age.

Multivariate estimates for the distal factors model adjusted for all variables listed under distal factors and age.

Table 3.5. Proportion of anemia cases attributable to risk factors among children aged 6-59 months in six regions of Ethiopia.

	Risk ratio		Partial population attributable percent	
	<i>RR</i>	<i>95% CI</i>	<i>%</i>	<i>95% CI</i>
Proximal factors model				
Low serum ferritin	1.91	(1.17, 3.10)	19	(3, 33)
Low serum folate	1.24	(0.84, 1.84)	6	(-4, 15)
Low serum vitamin B12	0.85	(0.59, 1.22)	-3	(-10, 3)
High C-reactive protein	1.58	(1.14, 2.17)	5	(2, 9)
Malaria	0.10	(0.02, 0.56)	-1	(-2, -1)
Helminth infection	0.31	(0.05, 1.85)	-3	(-5, -1)
Medial factors model				
Lower three quartiles of dietary iron intake	1.09	(0.63, 1.57)	6	(-40, 29)
Insufficient dietary folate intake	0.98	(0.72, 1.48)	0	(-8, 11)
Lower three quartiles of dietary vitamin B12 intake	1.00	(0.65, 1.66)	0	(-36, 33)
Diarrhea	1.75	(1.48, 3.19)	11	(7, 25)
Cough	1.11	(0.78, 1.70)	3	(-8, 17)
Fever	1.20	(0.78, 1.82)	6	(-8, 18)
Distal factors model				
Not completed primary education	1.30	(0.89, 1.90)	17	(-6, 36)
Unimproved water source	1.10	(0.58, 2.07)	2	(-8, 11)
Unimproved sanitation	1.16	(0.77, 1.75)	10	(-18, 32)
Lower four quintiles of household asset index	0.87	(0.40, 1.92)	-13	(-100, 39)
Region				
Addis	ref			
Amhara	1.12	(0.56, 2.21)	3	(-13, 16)
Afar	1.43	(0.59, 3.50)	1	(-1, 3)
Oromia	1.19	(0.63, 2.25)	7	(-16, 26)
SNNP	0.71	(0.31, 1.63)	-6	(-19, 6)
Tigray	1.00	(0.51, 2.00)	0	(-4, 4)
Wet season	1.18	(0.87, 1.59)	8	(-6, 21)

Note 1: Sample size for proximal model is 796, and for medial and distal models is 1,162.

Note 2: Multivariate estimates for the proximal factors model adjusted for all variables listed under proximal factors plus all variables listed under distal factors and age. Multivariate estimates for the medial factors model adjusted for all variables listed under medial factors plus all variables listed under distal factors and age.

Multivariate estimates for the distal factors model adjusted for all variables listed under distal factors and age.

The medial model examined the contribution of dietary and morbidity risk factors. After adjustment for other medial risk factors and potential confounders, dietary intake of iron, folate and vitamin B12 were not associated with the risk of anemia for any participant group. Anemia was associated with cough among women (RR=1.27 [1.07, 2.33], pPAR%=4 [1, 13]) and with diarrhea among children (RR=1.75 [1.48, 3.19], pPAR%=11 [7, 25]). Heavy menstruation was not found to be an anemia risk factor among women.

The distal risk factor model examined the relation of socioeconomic, geographic, and seasonal variables with anemia. Water and sanitation were found to be important factors for adults. Use of an unimproved water source among women (RR=1.67 [1.08, 2.57], pPAR%=10% [1, 19]), and use of unimproved sanitation among men (RR=1.99 [1.05, 3.74], pPAR%=42 [6, 64]) were associated with increased anemia. Among adults, some regions (Afar for women, SNNP and Tigray for men) were found to be associated with higher risk of anemia compared to Addis Ababa (the reference group), though these associations control only for other distal risk factors and not medial or proximal risk factors. For proximal, medial, and distal risk factors among women, men, and children, similar results were obtained in sensitivity analyses that did not adjust anemia for altitude (**Table S3.9**).

Discussion

This population-based cross-sectional survey is among the few studies to estimate the proportion of anemia attributable to risk factors using individual-level data. The prevalence of anemia in Ethiopia was highest among children and women, with notable variability by region. The study

found that more than a quarter of anemia cases among men and women across the six study regions were attributable to low serum folate. Low serum ferritin, an indicator of iron deficiency, was associated with about one in ten cases among adults and one in five cases of anemia among children. Based on 24-hour recall data, nearly all men, women and children met the recommended dietary intake levels for iron, while inadequate intake of folate and vitamin B12 was relatively common. Inflammation was also a contributor to anemia among women and children, although malaria and soil-transmitted helminths infections did not contribute to a large share of anemia cases.

The prevalence of anemia in Ethiopia varied between women, men, and children, and appeared to be sensitive to differences in region for women. Two prior surveys estimated the prevalence of anemia in Ethiopia, the 2015 Ethiopian Micronutrient Survey (EMNS) and 2016 Demographic and Health Survey (DHS), and presented results that in some cases differed considerably from each other and from the present study.^{11,13} While the present study found estimates of anemia that were similar to those of the EMNS, the DHS found a notably higher prevalence of anemia among men and children. The DHS used capillary blood, while the EMNS and the present study used venous blood for nearly all participants. Research has indicated that hemoglobin concentrations may substantially differ by blood draw collection method, however current evidence is inconclusive as to whether this results in systematic over- or under-estimates of anemia.⁴³ The differing results between surveys may also be due to changes in the distribution of risk factors over time, which in turn supports the argument for public health interventions to reduce the prevalence of risk factors. Prospective and repeated monitoring of anemia prevalence,

with standardized methods of assessment, is warranted to observe whether changes in anemia prevalence can be attributed, at least in part, to intervention programs.

Iron deficiency can result from blood loss, inadequate dietary iron intake, or insufficient absorption. Fourteen percent of women, 5% of men, and 25% of children had low serum ferritin (after adjustment for inflammation), an indicator of iron deficiency. This is consistent with prior studies in Ethiopia.^{44,45} Helminth infection prevalence was low and not associated with anemia, which suggests that this is not a substantial cause of blood loss. The prevalence of inadequate dietary iron intake using the WHO recommended daily intake was virtually zero for men, women and children. Yet low serum ferritin explained 9-19% of anemia (depending on the participant group), which suggests that efforts to improve dietary iron absorption could be important interventions to reduce anemia. This apparent disparity between the findings for serum ferritin and dietary iron may be explained by poor bioavailability of consumed iron. Inadequate absorption could result from high levels of inhibitors such as phytate and polyphenols which are present in many plant-based Ethiopian foods, or from low levels of meat, fish, and poultry consumption.⁴⁶ Fortification of staple food items with iron is a common policy intervention globally to improve iron status, but it is critical that a population-level intervention such as fortification does not result in iron overload among individuals who already consume adequate iron. Data from this study shows that 5.4% (3.3, 8.9) of women and 10.0% (5.9, 16.5) of men had excess levels of serum ferritin; in Addis Ababa and Tigray high serum ferritin was seen among approximately one in four men (**Table S3.10**). Given the potential risk of iron overload in some populations, targeted interventions to increase bioavailable iron intake in populations at high-risk for iron deficiency could be pursued. Point-of-use fortification for children and iron

supplementation programs among women of reproductive age are recommended by the WHO as alternative means of targeting populations with a high prevalence of iron deficiency.^{47,48}

Low serum folate was highly prevalent among women (41%), men (39%), and children (21%). Among women and men, low serum folate was associated with increased anemia, and was estimated to be responsible for approximately a quarter of anemia cases. Dietary assessment of folate consumption indicated that 26-42% of participants consumed inadequate quantities. As a result, food fortification with folic acid may be warranted as a national strategy to control anemia across all groups. In addition to benefits for anemia reduction, interventions to improve folate status are likely to improve other health outcomes such as neural tube defects and stroke, which are also notable public health problems in Ethiopia.⁴⁹⁻⁵³ Dietary interventions may also be considered. For example, behavior-change communication to promote cooking legumes (an important source of dietary folate) for short periods of time without pre-soaking, or the consumption of steamed as opposed to boiled vegetables.⁵⁴⁻⁵⁶

Low serum vitamin B12 was observed in 20-26% of women, men and children, and participants reported negligible dietary intake of vitamin B12 on most recall days. Prior studies have also identified that the association is heterogeneous between dietary intake and serum levels of vitamin B12.⁵⁷ Vitamin B12 is primarily found in animal food products (such as meats, eggs, and dairy), which all participant groups reported having rarely consumed. Low animal source food consumption in Ethiopia is common due to both religious fasting practices and economic constraints.⁵⁸ Vitamin B12 deficiency is an established cause of macrocytic anemia, and

therefore our finding that low serum B12 was associated with a reduced risk of anemia among women and men is counterintuitive. A potential explanation is that serum vitamin B12 concentrations were confounded by an unmeasured variable. Milk, which contains vitamin B12, has been noted as a potential cause of anemia among children – due to mechanisms that may apply to adults as well – so this may be a confounder.⁵⁹ As a sensitivity analysis, a binary indicator of milk consumption was added to the proximal models, but this did not substantially attenuate the association between serum vitamin B12 and anemia. Another alternative explanation is that households raising livestock, and hence more likely to have better vitamin B12 status, may be exposed to environmental pathogens resulting in environmental enteric dysfunction and anemia.⁶⁰ Nevertheless, the biomarker data suggest that vitamin B12 deficiency does not appear to be a major contributor to anemia in Ethiopia. Given the high burden of vitamin B12 deficiency as noted by biomarker and dietary data, research on the consequences for other health outcomes is needed.⁶¹

Inflammation (as measured by high CRP) was found among less than one in ten participants and was associated in multivariate models with an increase in the risk of anemia among women and children. Overall, 9% of anemia cases among women and 5% among children were attributed to this cause. CRP is associated with the acute phase of inflammation, may be an indication of subclinical infection, and peaks especially with bacterial infections.⁶² Tuberculosis infection can cause increased levels of CRP and anemia, but the low prevalence of active tuberculosis infections (0.3%) suggests this is not a primary cause of inflammation in the population.⁶³ Bacterial causes of bloody diarrhea (such as *E. coli* or *Shigella*) or other infections may be a more plausible explanation; this study found that cough was associated with anemia among

women and diarrhea was associated with increased anemia among children. Further research is needed to understand the determinants of inflammation in Ethiopia.

The burden of malaria and helminth infection was found to be low among men, women, and children. Malaria was strongly associated with anemia in adults, but due to the low prevalence of infection at the population-level only 3% of anemia cases in women and 8% in men were attributable to malaria. Helminth infections were not found to be associated with anemia in this study. However, prior research has shown that deworming programs are associated with significant improvements in hemoglobin.⁶⁴ Ethiopia has programs for deworming and malaria control with high levels of population coverage.^{65,66} Although malaria and helminth infections are not currently major contributors to the existing burden of anemia in the country, current programs should be maintained to prevent a potential increase in anemia due to these causes.

We also found that social determinants were important contributors to anemia risk, as has been observed in other studies.⁶⁷ Socioeconomic risk factors were associated with a significant proportion of anemia cases, including use of an unimproved water source for women and unimproved sanitation for men. These findings emphasize that poverty reduction and increased access to improved water and sanitation are potential interventions to produce reductions in anemia. Reducing anemia may also contribute to breaking poverty cycles as research has established that improvement in hematologic status is associated with higher learning in children and increased productivity in adults.^{6,68}

A major strength of this study is that it was population-based, so the distribution of risk factors represents the target population for public health interventions. Furthermore, data on multiple risk factors and covariates was collected, which enhances the ability to disentangle the individual contributions to anemia of multiple correlated risk factors. Serum nutrient data was collected in addition to dietary intake, which allowed for a comparison of results between nutrition indicators. A limitation is that dietary assessment methods which use reference values for the nutritional content of foods may be subject to error, as recipes and preparation methods can vary between households. This study used a set of standard Ethiopian recipes to calculate nutrient intake. Furthermore, serum biomarkers taken at a single time point may be subject to measurement error relative to their usual value. This study did not collect repeated samples of serum biomarkers and therefore assumes that the serum biomarker values represent usual values. Finally, tests of genetic causes of anemia were not done for this study, though the prevalence of sickle cell and thalassemias in Ethiopia is low.^{69,70}

Folate deficiency, iron deficiency, and inflammation are important contributors to anemia in Ethiopia to varying degrees among men, women and children. Folate fortification in Ethiopia could lead to enhanced folate status and result in reduced risk of anemia and other adverse health outcomes. Targeted iron supplementation, particularly for women and children, should be considered as means to address anemia. Behavior change interventions to improve dietary intake of bioavailable nutrients and address social determinants of anemia are also important. While the risk factors identified in this study are supported by prior research and are generalizable to other contexts, analyses such as this study are critical in order to identify the key factors that contribute

to anemia etiology for a specific context. As a result, other countries beyond Ethiopia will likely benefit from carrying out similar studies of anemia etiology.

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Supplemental Appendix

Table S3.1. Cutoffs used for binary indicators.

	Women	Men	Children
Low hemoglobin, i.e. anemia (g/dL)	<12	<13	<11
Mild anemia	11.0 – 11.9	11.0 – 12.9	10.0 – 10.9
Moderate anemia	8.0 – 10.9	8.0 – 10.9	7.0 – 9.9
Severe anemia	<8.0	<8.0	<7.0
Low serum ferritin (µg/L)	<15	<15	<12
High serum ferritin (µg/L)	>150	>200	--
Low serum folate (ng/mL)	<3	<3	<3
Low serum vitamin B12 (pg/mL)	<203	<203	<203
High C-reactive protein (mg/L)	>5	>5	>5
Inadequate dietary iron (mg/d)	<22.4	Age 15-17 y: <25.6 Age 18-49 y: <19.2	Age 6-11 mo: <16 Age 12-59 mo: <10
Inadequate dietary folate (µg/d)	<250	<250	Age 6-35 mo: <90 Age 36-59 mo: <110
Inadequate dietary vitamin B12 (µg/d)	<2	<2	Age 6-35 mo: <0.7 Age 36-59 mo: <1

Appendix 3.1. Methodology for inverse-probability of censoring weights

Some participants did not provide complete specimens for assessment of hemoglobin, serum biomarkers, and stool (see Figure 1). In order to account for potential bias introduced by this data loss, inverse-probability of censoring weights were applied to the data.¹

First, two binary indicator variables were defined to indicate censoring status. The first indicator was set to “1” if the participant had hemoglobin data available, and “0” otherwise; this indicator was used for calculating weights for the medial and distal etiology models. The second indicator was set to “1” if the participant had complete serum biomarker and stool data, and “0” otherwise; this indicator was used for calculating weights for the proximal etiology model.

Two logistic models were then fit – one for each of the censoring status indicators – to estimate the probability of not being censored. Predictor variables used in the censoring models included all variables used in the distal etiology model (that is: not completed primary education, unimproved water source, unimproved sanitation, binary indicator variables for quintiles of household assets, binary indicator variables for region, and season). All two-way interactions that were statistically significant ($p < 0.05$) using backwards stepwise model selection were also included as predictor variables.

The predicted probability of not being censored was then calculated based on the model's estimated coefficients. The inverse of this probability was then taken as the weight. This means that those observations with a higher probability of being censored had a larger weight in the model. The censoring weights were multiplied together with the regional sampling weights. Estimates for subsequent models were then conducted using Stata's "svyset" command.

¹ Hernán MA, Robins JM. Causal inference: What if. Boca Raton: Chapman & Hall/CRC. 2020.

Appendix 3.2. Estimation of usual dietary intake.

We used the Iowa State University (ISU) method¹ to adjust iron and folate intake distributions for within-person variation. We implemented the ISU method using the Intake Monitoring Assessment and Planning Program (IMAPP). For both nutrients, intake distributions were estimated separately for women (n=2574 recall days, including 163 repeat recalls), men (n=1074, including 62 repeats), and children (n=1528, including 84 repeats) to produce usual intakes at the individual level, which were used to estimate survey-weighted statistics including the prevalence of inadequacy based on harmonized nutrient reference values (NRVs)². Statistics were estimated separately for participant type and region strata, and participant type, region, and round strata. In estimating usual intake distributions, we also adjusted for the effects of region, round, weekday, and interview sequence. It was not possible to obtain usual intakes for vitamin B12 using the ISU method due to the high proportion of non-consumption days and resulting highly skewed distribution, so the SPADE methodology was used.³ Given the extremely small number of repeat recalls within participant type and region strata, and participant type, region, and round strata, distributions were estimated within participant types only.

Table: Variance components for iron and folate obtained using the ISU method

Group	Nutrient	Within Person Variance	Between Person Variance	Within: Between	Within: Total	4th Moment
Women	Iron	0.85	0.15	5.63	0.85	2.66 (set to 3 in IMAPP)
	Folate	0.74	0.26	2.90	0.74	2.56 (set to 3 in IMAPP)
Men	Iron	0.88	0.12	7.19	0.88	2.85 (set to 3 in IMAPP)
	Folate	0.90	0.10	8.65	0.90	2.52 (set to 3 in IMAPP)
Children	Iron	0.85	0.15	5.54	0.85	2.84 (set to 3 in IMAPP)
	Folate	0.61	0.40	1.52	0.60	3.28

1. Nusser SM, Carriquiry AL, Dodd KW, Fuller WA. A Semiparametric Transformation

Approach to Estimating Usual Daily Intake Distributions. *Journal of the American Statistical Association* 1996;91:1440-9.

2. Allen LH, Carriquiry AL, Murphy SP. Perspective: Proposed Harmonized Nutrient Reference Values for Populations. *Advances in Nutrition* 2019.

3. Dekkers AL, Verkaik-Kloosterman J, van Rossum CT, Ocké MC. SPADE, a new statistical program to estimate habitual dietary intake from multiple food sources and dietary supplements. *J Nutr.* 2014;144(12):2083-2091. doi:10.3945/jn.114.191288

Appendix 3.3. Methodology for bootstrapped confidence interval estimates of usual diet

In order to account for the uncertainty introduced by estimating usual dietary intake, a bootstrapping approach is required for confidence interval estimation. First, sampling with replacement was performed within the dietary intake dataset that includes repeat dietary recalls. Repeated sampling was performed by individual so that when an individual was selected all dietary recalls corresponding to that individual were selected. Estimates of usual diet were then calculated. In a second step, sampling with replacement was performed for the main study, and the bootstrapped estimates of usual diet were merged with the main study data. Statistical analyses are then performed using this dataset and the point estimates were saved to a separate data file.

The process above was repeated 2000 times. The 2.5th and 97.5th percentile of values in the data file containing the 2000 point estimates are then used as the lower and upper bounds of the 95% confidence interval.

Table S3.2. P-values for differences in anemia by region and season.

	Region	Season
Women	<0.01	0.23
Men	0.14	0.80
Children	0.44	0.37

Table S3.3. Prevalence of mild and moderate/severe anemia in Ethiopia among women, men and children by region.*

Participant	Region	<i>n total</i>	Mild anemia		Moderate or severe anemia	
			<i>n anemic</i>	<i>weighted % (95% CI)</i>	<i>n anemic</i>	<i>weighted % (95% CI)</i>
Women	All regions†	2229	252	10.3 (8.4, 12.5)	173	6.3 (3.5, 11.1)
	Addis	378	27	7.2 (5.0, 10.3)	12	3.2 (1.1, 8.7)
	Afar	371	74	20.3 (15.0, 26.7)	79	21.4 (18.8, 24.3)
	Amhara	357	40	11.3 (6.5, 18.9)	23	6.5 (2.3, 16.8)
	Oromia	368	37	10.3 (7.7, 13.8)	25	7.4 (2.4, 20.6)
	SNNP	404	34	8.4 (7.3, 9.6)	16	3.9 (2.6, 5.7)
	Tigray	351	40	11.4 (10.7, 12.3)	18	5.5 (2.8, 10.3)
	Men	All regions†	917	59	5.4 (4.3, 6.7)	28
Addis		125	2	1.5 (0.3, 7.0)	1	0.7 (0.1, 6.6)
Afar		187	16	8.2 (4.9, 13.3)	9	4.6 (1.4, 14.4)
Amhara		148	11	7.3 (5.5, 9.5)	3	2.1 (0.4, 9.6)
Oromia		151	4	2.5 (1.3, 4.9)	4	3.1 (0.5, 15.8)
SNNP		162	13	8.0 (5.1, 12.3)	5	2.9 (1.9, 4.3)
Tigray		144	13	9.2 (3.9, 20.2)	6	4.4 (2.1, 9.1)
Children	All regions†	1162	157	13.6 (11.1, 16.7)	110	8.3 (6.7, 10.3)
	Addis	135	12	8.6 (3.5, 19.4)	17	12.3 (8.4, 17.6)
	Afar	216	36	17.0 (10.5, 26.3)	31	14.3 (5.7, 31.6)
	Amhara	202	32	15.9 (12.8, 19.6)	15	7.5 (6.4, 8.7)
	Oromia	190	32	16.4 (10.9, 24.0)	16	8.1 (5.1, 12.6)
	SNNP	219	15	6.8 (5.2, 8.8)	17	7.8 (4.4, 13.4)
	Tigray	200	30	14.9 (7.7, 27.0)	14	7.2 (4.6, 11.1)

* Hemoglobin values adjusted for altitude using WHO-recommended regression method. Mild anemia defined as a hemoglobin concentration of 11-11.9 g/dl for women, 11-12.9 g/dl for men, and 10-10.9 g/dl for children. Moderate or severe anemia defined as hemoglobin <11 g/dl for women and men, and <10 g/dl for children.

†Average weighted by regional population size.

Table S3.4. Prevalence of iron deficiency anemia using World Health Organization definition.*

	Women	Men	Children
All regions	3.7 (2.0, 6.7)	1.3 (0.8, 2.2)	9.2 (6.0, 14.0)
Addis Ababa	2.7 (0.9, 7.5)	0.0 (--)	10.5 (4.0, 24.8)
Afar	27.6 (22.3, 33.6)	4.5 (3.8, 5.5)	20.6 (12.3, 32.6)
Amhara	1.0 (0.1, 8.3)	1.3 (0.9, 1.8)	5.4 (1.6, 16.7)
Oromia	4.4 (1.4, 13.3)	0.7 (0.1, 6.2)	12.1 (5.9, 23.1)
SNNP	3.5 (0.7, 15.2)	2.5 (1.2, 5.1)	6.5 (3.7, 11.1)
Tigray	1.1 (0.2, 4.9)	0.0 (--)	6.0 (3.3, 10.4)

* Anemia cases with serum ferritin <15 µg/L among women and men and <12 µg/L among children.

Table S3.5. Anemia risk factors among women, men, and children by region.

Region	Risk factor	Women		Men		Children	
		%	95% CI	%	95% CI	%	95% CI
Addis	Low serum ferritin	8.7	(4.9, 15.2)	0	n/e	30.7	(23.9, 38.4)
	Low serum folate	20.9	(14.6, 28.9)	21.9	(4.4, 63.4)	0	n/e
	Low serum vitamin B12	26.2	(19.2, 34.5)	22.8	(5.9, 58.1)	14.0	(0.5, 85.0)
	High C-reactive protein	18.7	(12.8, 26.6)	17.5	(5.6, 43.3)	14.3	(0.6, 81.5)
	Helminth infection	0	n/e	0	n/e	0	n/e
	Malaria	1.5	(0.7, 3.3)	1.4	(0.0, 49.5)	0	n/e
	Inadequate dietary iron	0	n/e	0	n/e	0.6	(0.0, 32.7)
	Inadequate dietary folate	42.2	(37.3, 47.3)	36.4	(21.1, 54.9)	18.4	(12.4, 26.4)
	Inadequate dietary vitamin B12	99.7	(97.9, 100.0)	86.9	(67.8, 95.4)	0	n/e
	Heavy menstruation	25.6	(21.4, 30.3)	n/a	n/a	n/a	n/a
	Diarrhea	5.0	(3.2, 7.9)	4.8	(2.4, 9.6)	11.2	(5.5, 21.7)
	Cough	18.1	(14.4, 22.4)	17.1	(10.7, 26.1)	27.9	(15.5, 45.0)
	Fever	14.4	(11.1, 18.5)	11.7	(4.3, 28.0)	17.7	(7.5, 36.4)
	Not completed primary education*	43.7	(38.7, 48.7)	53.1	(50.4, 55.8)	48.4	(22.1, 75.6)
	Unimproved water source	1.5	(0.6, 3.5)	0.0	n/e	0.9	(0.0, 40.3)
	Unimproved sanitation	42.2	(37.3, 47.3)	38.0	(12.6, 72.3)	42.5	(15.0, 75.5)
	Low serum ferritin	52.6	(43.1, 61.9)	24.0	(7.7, 54.3)	48.3	(24.9, 72.4)
	Low serum folate	77.1	(68.1, 84.1)	77.4	(58.9, 89.1)	52.7	(28.8, 75.5)
	Low serum vitamin B12	45.0	(35.8, 54.5)	42.1	(22.7, 64.2)	24.9	(16.3, 36.0)
	Afar	High C-reactive protein	10.8	(6.2, 18.2)	9.2	(2.7, 26.6)	3.3
Helminth infection		0.9	(0.1, 6.1)	1.3	(0.0, 49.7)	5.5	(0.7, 34.1)
Malaria		1.6	(0.7, 3.5)	0.5	(0.0, 34.0)	2.7	(0.3, 21.6)
Inadequate dietary iron		0.6	(0.1, 2.2)	0	n/e	0	n/e
Inadequate dietary folate		45.5	(40.5, 50.6)	35.9	(16.1, 62.1)	21.9	(15.6, 29.8)
Inadequate dietary vitamin B12		100.0	n/e	47.7	(33.8, 62.0)	0	n/e
Heavy menstruation		19.2	(15.5, 23.5)	n/a	n/a	n/a	n/a
Diarrhea		7.3	(4.9, 10.8)	4.6	(1.2, 16.0)	14.3	(10.2, 19.6)
Cough		15.5	(11.8, 20.0)	10.5	(3.0, 30.8)	26.6	(13.7, 45.2)
Fever		30.8	(25.9, 36.2)	15.6	(4.7, 40.9)	26.5	(11.9, 49.1)
Not completed primary education*		91.2	(87.8, 93.7)	93.8	(89.6, 96.4)	92.3	(70.0, 98.4)
Unimproved water source		45.5	(40.4, 50.6)	42.4	(25.6, 61.3)	49.8	(36.2, 63.4)
Unimproved sanitation	87.3	(83.5, 90.3)	85.4	(41.0, 98.0)	87.0	(67.4, 95.6)	

Table S3.5. (Continued)

Region	Risk factor	Women		Men		Children	
		%	95% CI	%	95% CI	%	95% CI
Amhara	Low serum ferritin	8.0	(4.5, 13.6)	2.5	(0.6, 9.3)	14.8	(3.4, 46.4)
	Low serum folate	40.2	(32.4, 48.5)	35.6	(18.2, 57.8)	28.3	(14.5, 47.9)
	Low serum vitamin B12	14.5	(9.6, 21.4)	10.6	(1.7, 44.8)	18.0	(16.7, 19.3)
	High C-reactive protein	7.2	(4.0, 12.6)	4.3	(0.9, 17.6)	7.3	(1.8, 25.7)
	Helminth infection	0.7	(0.1, 4.6)	5.0	(0.7, 28.3)	5.7	(1.4, 21.0)
	Malaria	0.8	(0.3, 2.6)	0.8	(0.0, 34.5)	0	n/e
	Inadequate dietary iron	0	n/e	0	n/e	0	n/e
	Inadequate dietary folate	39.9	(34.9, 45.1)	22.9	(16.3, 31.1)	26.1	(14.9, 41.8)
	Inadequate dietary vitamin B12	100.0	n/e	100.0	n/e	71.1	(54.7, 83.4)
	Heavy menstruation	20.8	(16.9, 25.4)	n/a	n/a	n/a	n/a
	Diarrhea	4.9	(3.0, 7.8)	4.2	(1.2, 13.8)	15.9	(3.5, 49.6)
	Cough	13.4	(10.1, 17.6)	11.9	(3.7, 32.3)	17.2	(6.9, 36.8)
	Fever	24.8	(20.3, 29.8)	19.2	(4.7, 53.4)	17.9	(16.4, 19.6)
	Not completed primary education*	72.5	(67.7, 76.9)	75.4	(56.8, 87.8)	73.0	(39.9, 91.7)
	Unimproved water source	15.5	(11.9, 20.0)	15.8	(3.0, 53.3)	13.0	(3.0, 41.7)
	Unimproved sanitation	66.7	(61.7, 71.4)	66.6	(32.6, 89.2)	63.0	(52.9, 72.0)
	Oromia	Low serum ferritin	9.0	(5.1, 15.5)	0.7	(0.0, 36.3)	25.6
Low serum folate		49.6	(41.7, 57.6)	45.1	(31.1, 60.0)	24.4	(10.8, 46.4)
Low serum vitamin B12		28.3	(21.8, 35.9)	23.3	(0.7, 93.4)	19.9	(3.8, 60.8)
High C-reactive protein		7.6	(4.3, 13.1)	10.5	(9.6, 11.4)	7.4	(1.8, 25.9)
Helminth infection		1.9	(0.6, 5.8)	1.4	(1.2, 1.7)	0	n/e
Malaria		2.6	(1.2, 5.5)	1.6	(0.0, 55.0)	3.0	(0.0, 72.8)
Inadequate dietary iron		0	n/e	0	n/e	0.9	(0.9, 1.0)
Inadequate dietary folate		43.3	(38.2, 48.6)	30.5	(14.1, 53.9)	24.5	(17.4, 33.5)
Inadequate dietary vitamin B12		100.0	n/e	100.0	n/e	0	n/e
Heavy menstruation		24.6	(20.4, 29.4)	n/a	n/a	n/a	n/a
Diarrhea		9.8	(7.0, 13.4)	7.7	(4.1, 14.2)	11.9	(9.6, 14.7)
Cough		17.6	(13.8, 22.3)	15.2	(12.9, 17.9)	26.1	(14.0, 43.5)
Fever		33.0	(28.1, 38.3)	22.9	(14.4, 34.3)	25.7	(22.5, 29.1)
Not completed primary education*		73.3	(68.5, 77.6)	68.3	(50.8, 81.8)	72.8	(53.3, 86.2)
Unimproved water source		24.1	(19.7, 29.1)	27.3	(7.0, 65.4)	24.4	(4.1, 70.7)
Unimproved sanitation		81.7	(77.2, 85.5)	81.7	(73.0, 88.1)	81.7	(60.3, 92.9)

Table S3.5. (Continued)

Region	Risk factor	Women		Men		Children		
		%	95% CI	%	95% CI	%	95% CI	
SNNP	Low serum ferritin	31.6	(25.2, 38.8)	13.2	(7.5, 22.2)	32.3	(6.8, 75.7)	
	Low serum folate	17.8	(12.8, 24.1)	24.7	(1.1, 90.4)	6.0	(0.2, 62.8)	
	Low serum vitamin B12	36.1	(29.4, 43.5)	40.7	(6.4, 87.3)	24.7	(3.1, 77.1)	
	High C-reactive protein	1.6	(0.5, 5.0)	3.5	(1.5, 8.2)	9.2	(1.6, 39.0)	
	Helminth infection	15.5	(10.9, 21.6)	11.5	(3.5, 32.2)	14.9	(0.8, 78.4)	
	Malaria	0.5	(0.1, 1.9)	0	n/e	0.5	(0.0, 24.1)	
	Inadequate dietary iron	2.2	(1.1, 4.2)	0	n/e	0.9	(0.8, 1.0)	
	Inadequate dietary folate	42.2	(37.5, 47.1)	34.6	(17.2, 57.3)	30.7	(16.4, 49.9)	
	Inadequate dietary vitamin B12	100.0	n/e	100.0	n/e	92.7	(82.6, 97.2)	
	Heavy menstruation	24.6	(20.6, 29.0)	n/a	n/a	n/a	n/a	
	Diarrhea	8.0	(5.6, 11.2)	4.8	(0.4, 39.8)	15.9	(10.3, 23.9)	
	Cough	14.3	(11.1, 18.3)	12.9	(7.5, 21.4)	22.5	(12.2, 37.7)	
	Fever	25.6	(21.4, 30.3)	15.8	(4.4, 43.0)	22.1	(7.3, 50.5)	
	Not completed primary education*	66.6	(61.8, 71.1)	69.8	(36.1, 90.4)	69.9	(33.2, 91.5)	
	Unimproved water source	7.5	(5.3, 10.6)	9.0	(1.8, 35.4)	7.4	(0.7, 48.0)	
	Unimproved sanitation	68.7	(64.0, 73.1)	68.4	(38.4, 88.3)	70.6	(40.6, 89.4)	
	Tigray	Low serum ferritin	4.0	(1.9, 8.2)	3.0	(0.4, 20.7)	19.6	(10.0, 34.8)
		Low serum folate	63.8	(55.6, 71.2)	62.3	(48.3, 74.5)	36.4	(20.8, 55.5)
		Low serum vitamin B12	9.7	(5.7, 16.2)	6.7	(0.9, 35.4)	20.6	(10.1, 37.5)
High C-reactive protein		3.4	(1.5, 7.5)	12.5	(7.7, 19.7)	11.8	(6.5, 20.3)	
Helminth infection		2.5	(0.9, 6.5)	2.4	(0.7, 8.5)	6.4	(4.5, 9.1)	
Malaria		0	n/e	0.6	(0.0, 33.1)	0.4	(0.0, 26.5)	
Inadequate dietary iron		0.7	(0.2, 3.1)	0	n/e	0.5	(0.0, 28.0)	
Inadequate dietary folate		42.9	(37.7, 48.3)	28.7	(16.8, 44.4)	22.4	(14.3, 33.2)	
Inadequate dietary vitamin B12		100.0	n/e	100.0	n/e	99.5	(70.5, 100.0)	
Heavy menstruation		24.4	(20.1, 29.2)	n/a	n/a	n/a	n/a	
Diarrhea		3.2	(1.7, 6.0)	5.0	(0.9, 24.1)	13.3	(4.5, 33.0)	
Cough		24.3	(19.9, 29.3)	26.9	(13.4, 46.7)	36.1	(25.4, 48.5)	
Fever		21.4	(17.2, 26.3)	20.5	(4.7, 57.2)	28.9	(10.3, 59.2)	
Not completed primary education*		57.6	(52.2, 62.7)	59.2	(38.6, 77.0)	60.5	(31.9, 83.4)	
Unimproved water source		2.5	(1.3, 4.7)	2.9	(0.1, 51.0)	2.7	(0.1, 45.5)	
Unimproved sanitation	75.3	(70.1, 79.9)	80.4	(74.3, 85.4)	79.7	(74.1, 84.4)		

* Refers to woman, man, or primary caregiver for women, men, and children respectively

Abbreviations: n/a, not applicable; n/e, not estimable; SNNP, Southern Nations Nationalities and Peoples

Table S3.6. Mean nutrient intake using IMAPP estimates of usual consumption.

	Iron (mg/d)								
	Women			Men			Children		
	Overall	Dry season	Wet season	Overall	Dry season	Wet season	Overall	Dry season	Wet season
All regions	55.2	55.9	54.5	63.3	64.3	62.3	29.6	29.9	29.2
Addis	53.2	55.1	51.3	58.3	63.1	54.4	27.4	30.0	25.5
Afar	50.7	53.5	47.9	56.6	59.8	53.8	27.8	28.7	26.8
Amhara	60.1	59.3	60.9	70.4	69.0	72.0	32.3	32.8	31.9
Oromia	52.3	54.3	50.3	60.1	63.0	57.1	28.7	28.8	28.7
SNNP	56.3	55.5	57.0	63.7	62.4	65.1	29.6	29.3	29.9
Tigray	52.9	54.1	51.8	59.2	60.8	57.4	27.1	28.5	25.9
	Folate ($\mu\text{g}/\text{d}$)								
	Women			Men			Children		
	Overall	Dry season	Wet season	Overall	Dry season	Wet season	Overall	Dry season	Wet season
All regions	304.6	306.9	302.2	363.4	361.7	365.1	174.2	177.5	171.2
Addis	307.1	290.1	323.5	352.8	349.6	355.5	180.6	182.7	179.1
Afar	300.1	297.1	303.0	357.8	360.6	355.3	169.9	173.9	165.0
Amhara	297.4	297.1	297.6	354.7	338.5	372.2	172.4	180.5	165.2
Oromia	299.2	300.0	298.5	363.8	359.8	367.9	176.2	174.3	178.1
SNNP	323.0	338.2	307.6	376.7	395.5	358.3	171.8	178.7	165.9
Tigray	306.1	306.1	306.0	365.7	372.0	358.8	169.8	178.5	162.0

Table S3.7. High dietary iron consumption (%) among women, men, and children by region.

	Women	Men	Children
All regions	0 (0, 0.3)	0 (0, 0.4)	0 (--)
Addis	0 (--)	0 (--)	0 (--)
Afar	0 (--)	0 (--)	0 (--)
Amhara	0 (--)	0 (--)	0 (--)
Oromia	0 (--)	0 (--)	0 (--)
SNNP	0 (--)	0 (--)	0 (--)
Tigray	0.5 (0.1, 4.3)	0.7 (0.1, 5.6)	0 (--)

Note: High dietary iron consumption defined according to IOM recommendations after adjustment for bioavailability. Cutoff for women and men is 162 mg/d, and for children is 144 mg/d. Nutrient values used for defining cutoffs are taken from the 24-hour dietary recall after adjustment to usual intake using IMAPP software.

Table S3.8. Combined partial population attributable risk percentages for risk factors significantly associated with increased anemia in the proximal model.

Risk factors	Combined pPAR%
Women	
Low serum folate	
High C-reactive protein	33.6 (12.9, 49.3)
Malaria	
Men	
Low serum ferritin	
Low serum folate	40.6 (23.6, 58.9)
Malaria	
Children	
Low serum ferritin	
High C-reactive protein	23.5 (8.1, 36.3)

Table S3.9. Proportion of anemia cases attributable to risk factors, using hemoglobin without correction for altitude.

	Risk ratio		Partial population attributable percent	
	<i>RR</i>	<i>95% CI</i>	<i>%</i>	<i>95% CI</i>
Women				
Proximal factors model				
Low serum ferritin	3.12	(1.58, 6.15)	20	(4, 34)
Low serum folate	1.52	(1.00, 2.33)	19	(0, 35)
Low serum vitamin B12	0.35	(0.16, 0.77)	-19	(-29, -10)
High C-reactive protein	4.55	(1.47, 14.08)	12	(-2, 24)
Malaria	4.19	(2.78, 6.34)	5	(3, 7)
Helminth infection	0.66	(0.22, 1.96)	-1	(-3, 1)
Medial factors model				
Lower three quartiles of dietary iron intake	0.59	(0.30, 1.16)	-47	(-126, 4)
Insufficient dietary folate intake	1.30	(0.70, 2.43)	11	(-15, 31)
Lower three quartiles of dietary vitamin B12 intake	1.33	(0.79, 2.24)	20	(-14, 44)
Heavy menstruation	0.71	(0.39, 1.27)	-7	(-18, 3)
Diarrhea	0.90	(0.56, 1.44)	-1	(-4, 2)
Cough	1.09	(0.77, 1.53)	1	(-4, 6)
Fever	1.41	(0.72, 2.78)	8	(-9, 23)
Distal factors model				
Not completed primary education	1.01	(0.57, 1.80)	1	(-43, 32)
Unimproved water source	2.23	(1.27, 3.92)	19	(3, 32)
Unimproved sanitation	1.11	(0.67, 1.82)	8	(-32, 36)
Lower four quintiles of household asset index	1.18	(0.51, 2.74)	15	(-72, 58)
Region				
Addis	ref			
Amhara	1.20	(0.20, 7.37)	2	(-22, 22)
Afar	7.63	(2.32, 25.09)	8	(5, 11)
Oromia	2.59	(0.72, 9.28)	32	(-2, 54)
SNNP	1.77	(0.55, 5.73)	7	(-3, 16)
Tigray	2.49	(0.73, 8.47)	4	(0, 8)
Wet season	1.73	(0.93, 3.21)	26	(-4, 48)
Men				
Proximal factors model				
Low serum ferritin	5.70	(1.84, 17.68)	8	(-2, 17)
Low serum folate	1.66	(0.96, 2.88)	21	(-3, 40)
Low serum vitamin B12	0.27	(0.12, 0.64)	-26	(-46, -9)
High C-reactive protein	4.27	(1.72, 10.56)	16	(1, 29)
Malaria	25.92	(7.66, 87.74)	15	(8, 21)
Helminth infection	0.80	(0.19, 3.40)	-1	(-7, 4)
Medial factors model				
Lower three quartiles of dietary iron intake	2.60	(0.40, 16.86)	53	(-75, 87)
Insufficient dietary folate intake	0.73	(0.36, 1.49)	-9	(-29, 7)
Lower three quartiles of dietary vitamin B12 intake	1.75	(0.54, 5.69)	37	(-57, 75)
Diarrhea	1.18	(0.36, 3.91)	1	(-6, 7)
Cough	1.02	(0.37, 2.77)	0	(-18, 16)
Fever	1.26	(0.57, 2.78)	5	(-12, 19)

Table S3.9. (Continued)

	Risk ratio		Partial population attributable percent	
	<i>RR</i>	<i>95% CI</i>	<i>%</i>	<i>95% CI</i>
Men				
Distal factors model				
Not completed primary education	0.72	(0.39, 1.33)	-24	(-78, 13)
Unimproved water source	1.63	(0.66, 4.02)	10	(-11, 27)
Unimproved sanitation	1.25	(0.57, 2.75)	16	(-48, 53)
Lower four quintiles of household asset index	2.02	(0.65, 6.27)	48	(-37, 80)
Region				
Addis				
Amhara	3.44	(0.24, 49.15)	15	(-19, 40)
Afar	8.63	(0.83, 90.01)	5	(1, 9)
Oromia	3.31	(0.30, 37.05)	23	(-25, 53)
SNNP	4.73	(0.52, 43.15)	19	(1, 33)
Tigray	10.41	(0.95, 114.59)	13	(-2, 26)
Wet season	1.97	(0.61, 6.30)	32	(-36, 66)
Children				
Proximal factors model				
Low serum ferritin	2.09	(1.06, 4.14)	25	(0, 44)
Low serum folate	2.02	(1.32, 3.08)	19	(6, 30)
Low serum vitamin B12	0.97	(0.42, 2.24)	-1	(-19, 15)
High C-reactive protein	2.06	(1.27, 3.34)	9	(2, 15)
Malaria	0.21	(0.06, 0.79)	-1	(-2, 0)
Helminth infection	0.09	(0.01, 0.65)	-4	(-6, -3)
Medial factors model				
Lower three quartiles of dietary iron intake	0.72	(0.33, 1.54)	-29	(-116, 23)
Insufficient dietary folate intake	1.23	(0.79, 1.92)	6	(-6, 16)
Lower three quartiles of dietary vitamin B12 intake	0.97	(0.57, 1.63)	-3	(-45, 28)
Diarrhea	2.44	(1.39, 4.28)	18	(6, 28)
Cough	1.43	(0.80, 2.54)	11	(-5, 25)
Fever	0.82	(0.47, 1.42)	-7	(-27, 10)
Distal factors model				
Not completed primary education	1.31	(0.94, 1.83)	18	(-3, 34)
Unimproved water source	1.54	(0.57, 4.12)	7	(-11, 23)
Unimproved sanitation	0.85	(0.43, 1.69)	-12	(-73, 27)
Lower four quintiles of household asset index	0.79	(0.23, 2.74)	-23	(-218, 52)
Region				
Addis				
Amhara	0.78	(0.24, 2.52)	-5	(-29, 15)
Afar	2.69	(0.58, 12.41)	3	(-1, 7)
Oromia	1.33	(0.52, 3.39)	11	(-20, 34)
SNNP	1.20	(0.41, 3.49)	3	(-15, 19)
Tigray	1.43	(0.64, 3.17)	2	(-2, 7)
Wet season	1.17	(0.84, 1.64)	8	(-8, 22)

Table S3.10. High serum ferritin (%) among women and men by region.

	Women	Men
All regions	5.4 (3.3, 8.9)	10.0 (5.9, 16.5)
Addis	2.3 (0.9, 6.3)	30.8 (22.0, 41.3)
Afar	1.0 (0.1, 6.3)	0.9 (0.1, 8.2)
Amhara	6.7 (5.9, 7.6)	7.1 (3.9, 12.5)
Oromia	7.2 (2.7, 17.5)	9.8 (2.5, 31.3)
SNNP	0.6 (0.1, 5.1)	3.8 (1.2, 10.9)
Tigray	9.7 (3.1, 26.4)	23.2 (15.5, 33.3)

Note: High serum ferritin defined according to WHO criteria. Cutoff for women is 150 µg/L and for men is 200 µg/L. Serum ferritin values were adjusted for inflammation using the BRINDA correction.