SYSTEMATIC REVIEW

Effects of iron intake on neurobehavioural outcomes in African children: a systematic review and meta-analysis of randomised controlled trials [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Iron deficiency and developmental delay are common in African children. While experimental studies indicate an important role of iron in brain development, effects of iron on child development remain unclear. We aimed to evaluate the effects of iron supplementation or fortification on neurobehavioural outcomes in African children and further summarise these effects in children living in non-African countries for comparison.

Methods: We searched PubMed, EMBASE, PsycINFO, Scopus and Cochrane Library for studies published up to 9th March 2021. We included randomised controlled trials (RCTs) evaluating effects of iron supplementation or fortification on neurobehavioural outcomes in children. Due to heterogeneity in study methods, we analysed the studies qualitatively and only seven RCTs with 11 arms were meta-analysed.

Results: We identified 2155 studies and included 34 studies (n=9808) in the systematic review. Only five studies (n=1294) included African children while 29 (n=8514) included children living in non-African countries. Of the five African studies, two (n=647) reported beneficial effects of iron supplementation on neurobehavioural outcomes in anaemic children while three (n=647) found no beneficial effects. Of 29 studies in children living in non-African countries, nine (n=2925) reported beneficial effects of iron supplementation or fortification on

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neurobehavioural outcomes, seven (n=786) reported beneficial effects only in children who had iron deficiency, iron deficiency anaemia or anaemia while 13 (n=4803) reported no beneficial effects. Meta-analysis of seven studies (n=775) in non-African countries showed no beneficial effects of iron supplementation on cognitive or motor development in children.

Conclusions: There are few studies in African children despite the high burden of iron deficiency and developmental delay in this population. Evidence on the effects of iron supplementation on neurobehavioural outcomes remains unclear and there is need for further well-powered studies evaluating these effects in African populations.

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Keywords

Iron deficiency, iron deficiency anaemia, African children, cognitive, motor, language, behaviour, development.

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Introduction

Brain development begins at conception and continues into early adulthood¹. During this period and particularly in the first five years of life, children living in Africa are vulnerable to impaired neurobehavioural development as a result of exposure to different risk factors including poverty, malnutrition and infectious diseases^{2,3}. About a third of pre-school children in low and middle-income countries (LMICs) are unlikely to reach their cognitive and/or socioemotional milestones and 44% of these children live in sub-Saharan Africa⁴. Long-term consequences of impaired child development include poor educational performance, low incomes and poor family planning, contributing to the cycle of poverty in LMICs³. Iron deficiency may be an important risk factor for impaired brain development in childhood^{5,6}.

Iron deficiency is the most common micronutrient deficiency affecting about two billion individuals globally and accounting for over 40% of all cases of anaemia⁷⁻⁹. Children living in Africa disproportionately bear the highest burden of anaemia and iron deficiency. Approximately 43% of pre-school children are anaemic globally and over 60% of these children live in Africa^{7,10}. A recent study reported a prevalence of 52% for iron deficiency in African children after correcting for inflammation and malaria¹¹. Additionally, iron deficiency anaemia is among the leading causes of years lived with disability in sub-Saharan Africa likely due to long-term effects on brain development⁹.

Epidemiological studies provide inconclusive evidence for the effects of iron supplementation or fortification on neurobehavioural outcomes despite consistent evidence from animal and *in vitro* studies indicating that iron plays an important role in neurotransmission, DNA synthesis and myelinogenesis¹²⁻¹⁴. Iron is important for the synthesis of tryptophan hydroxylase and tyrosine hydroxylase, enzymes that are involved in the synthesis of serotonin, dopamine and norepinephrine, which are important for neurobehavioural processes in the brain^{14,15}. Iron deficiency is associated with long-term behavioural abnormalities and impaired dopaminergic-dependent synaptic plasticity in the hippocampus, which may result in learning and memory deficits^{16,17}.

Despite the high prevalence of both iron deficiency and developmental delay, the effects of iron on neurobehavioural outcomes in African children are inadequately studied. In this systematic review and meta-analysis, our objective was to evaluate the effects of iron supplementation or fortification on neurobehavioural outcomes in children living in Africa. For comparison, we further summarised evidence from randomised controlled trials on the effects of iron supplementation on neurobehavioural outcomes in non-African countries.

Methods

Reporting guidelines

Our systematic review and meta-analysis was guided by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines¹⁸ and the protocol was registered on the PROSPERO database on 20th March 2018 (registration number CRD42018091278).

Search strategy and eligibility criteria

We searched PubMed, EMBASE, PsycINFO, Scopus and Cochrane Library for studies published up to 9th March 2021. Additionally, we scanned reference lists of identified studies and previous systematic reviews. We conducted searches for RCTs using a search strategy combining Medical Subject Heading terms for [iron] AND [neurobehavioural outcomes] AND [children] AND [RCT 'publication type']. We modified the search strategy as appropriate for each of the specific databases (Extended data, file 1¹⁸). The search was not restricted by language.

We included studies that met the following criteria: (i) included participants aged below 18 years; (ii) randomised controlled trials (RCTs) of iron supplementation or fortification in children or pregnant mothers; (iii) assessed neurobehavioural outcomes in children including cognitive or motor development, intelligence quotient, attention, behaviour, educational achievement or language development. We excluded studies assessing neurobehavioural outcomes in adult participants and RCTs involving iron supplementation/fortification alongside other micronutrients or macronutrients that did not separately evaluate the effects of iron. We also excluded observational studies, reviews, case studies, abstracts, comments and study protocols.

Study selection, data extraction and quality appraisal

Two authors (AMM and KM) independently screened titles and abstracts of all identified studies against the inclusion criteria and then screened identified full texts to determine eligibility for inclusion. Disagreements between reviewers were resolved through discussion. We extracted the following variables: study author(s) and year of publication, country, sample size, baseline iron status, age at iron supplementation and neurobehavioural assessment, neurobehavioural domain assessed and the tools used, definition of iron status and findings of the study.

We used the revised Cochrane risk-of-bias tool for randomised trials (RoB 2) to assess for risk of bias for the RCTs included in the review¹⁹. RoB 2 assesses five domains of bias including bias from the randomization process, deviations from intended interventions, missing outcome data, and bias in measurement of the outcome and selection of the reported result. We used the revised Cochrane risk of bias tool for randomised trials with additional considerations for cluster-randomised trials to assess risk of bias in two cluster RCTs²⁰.

Synthesis of included studies

The large degree of diversity in the study variables necessitated narrative synthesis of the study findings. We grouped and discussed the studies based on the neurobehavioural domain assessed and summarised study characteristics and findings (Table 1 and Extended data, file 2¹⁸). We compared study findings of studies from African and non-African countries and also studies that evaluated the effect of iron-fortified foods compared to non-fortified foods. We further compared study findings based on age (studies in infants versus older children) and baseline iron status (normal iron status versus iron deficiency, iron deficiency anaemia or anaemia).

Table 1. Summary of studies assessing the effect of iron supplementation or fortification on neurobehavioural outcomes in African children: characteristics and findings.

Author, year (country)	Sample	Baseline iron status	Age at iron supplementation	Age at neuroassessment	Domain (assessment tool)	Intervention (duration)	Definition of iron status	Results
Ssemata, 2020 (Uganda) ²¹	N=145 with cerebral malaria (75 received iron concurrently and 70 received iron 28 days after antimalarial treatment)	All children had ID	18-58.8 months	At 3 timepoints: 18 months-4.9 years, 24-64.8 months and 30-70.8 months	Cognitive Executive function Sustained attention memory Socioemotional behaviour (MSEL, ECVT, COAT, CBCL, BRIEF-P, BRS)	Ferrous sulphate 2mg/kg/day either concurrently with antimalarial treatment or 28 days after receiving antimalarial treatment (3 months)	ID: ZnPP $\geq$ 80 μ mol/mol heme	No difference in neurobehavioural scores between children who received iron supplementation concurrently or 28 days after antimalarial treatment.
Bouhouch, 2016 (Morocco) ²²	n=455 lead exposed children (110 received iron, 116 received iron+ EDTA, 112 received EDTA, and 117 received placebo)	Anaemia: 21% ID: 32%, 7% or 34% as defined by SF, TfR, or ZnPP	3 to 14 years	At 2 time-points: at baseline (3 to 14 years) and after supplementation.	Cognitive Memory (KABC-II, HVLIT)	2-3 biscuits (depending on body weight) containing 8 mg ferrous sulphate, 8 mg ferrous sulphate + 41 mg EDTA, 41 mg EDTA, or placebo (28 weeks)	ID: SF <12 mg/L for children <5 years, SF <15 mg/L for children $\geq$ 5 years, or TfR >8.3 mg/L with (CRP $\leq$ 5 mg/L, α 1-acid glycoprotein $\leq$ 51 g/L) Anaemia: Hb <11.0 g/dL for children <5 years, Hb <11.5 g/dL for children 5-11 years, Hb <12.0 g/dL for children 12 years	No difference in cognitive or memory scores between children who received iron supplementation or placebo.

Author, year (country)	Sample	Baseline iron status	Age at iron supplementation	Age at neuroassessment	Domain (assessment tool)	Intervention (duration)	Definition of iron status	Results
Baumgartner, 2012 (South Africa) ²³	n=288 (70 received iron+ placebo, 72 placebo + DHA/EPA, 73 iron+ DHA/EPA, and 73 placebo + placebo)	Anaemia: 20.6-21.1% ID: 6.2% to 16%	6 to 11 years	At 2 time-points: at baseline (6 to 11 years) and after supplementation.	Cognitive Memory (HVLIT KABC)	50 mg iron sulphate + DHA/EPA (420/80 mg), 50 mg iron sulphate+ placebo, placebo+ DHA/EPA, or placebo + placebo (8.5 months)	ID: SF<15 µg/L excluding children with CRP >5 mg/L or ZnPP >70 µmol/mol or TfR >8.3 mg/L Anaemia: Hb <11.5 g/dL IDA: anaemia + SF<15 µg/L	Anaemic children who received iron supplementation+ placebo had higher cognitive and memory scores compared to children who received placebo + placebo No difference in cognitive scores in children who received iron+ DHA/EPA compared to children who received placebo + placebo
Stoltzfus, 2001 (Zanzibar) ²⁴	n=359 (183 received iron and 176 placebo)	Anaemia: 97% Severe anaemia: 18%	6 to 59 months	At 2 timepoints: 6 to 59 months and 18 to 71 months	Language Motor (Parents reported motor and language milestones)	20 mg ferrous sulphate or placebo (12 months)	Anaemia: Hb<110g/l Severe anaemia: Hb<70g/l ID: SF<12 mg/L	Children who received iron supplementation had improved language scores compared to children who received placebo and children with baseline Hb<90 g/l who received iron supplementation had improved motor scores compared to children who received placebo.
Boivin, 1993 (Zaire) ²⁵	N=47 (17 children received both anthelmintics and iron, 7 only iron, 8 only anthelmintics, and 15 did not receive either intervention)	Not indicated	23 boys (mean age=7.7, SD=0.8 years) and 24 girls (mean age=8.0, SD=1.8 years)	At baseline (mean age for boys=7.7, SD=0.8 years) and mean age for girls=8.0, SD=1.8 years) and 4 weeks after the 1st assessment	Cognitive (KABC)	20 mg iron (4 weeks)	Anaemia: Hb<12 g/dL	No difference in cognitive scores between children who received only iron supplementation or placebo.

BRIEF-P, Behavior Rating Inventory of Executive Functioning, Preschool edition; BRS, Behavior Rating Scales; CBLC, Child Behaviour checklist; CRP, C-reactive protein; COAT, Color Object Association Test; DHA/EPA, docosahexaenoic acid and eicosapentaenoic acid; ECVI, Early Childhood Vigilance Test; EDTA, ethylenediaminetetraacetic acid; Hb, haemoglobin; HVLIT, Hopkins Verbal Learning Test; ID, iron deficiency; KABC, Kaufman Assessment Battery for Children; SD, standard deviation; SF, serum ferritin; TfR, transferrin receptor; ZnPP, zinc protoporphyrin.

Due to the substantial variation in study methods, we did a meta-analysis for only seven RCTs that used the Bayley Scales of Infant Development to assess cognitive and motor development in children living in non-African countries. In these studies, mean cognitive and motor development scores and standard deviations were reported. For each of the two domains, we generated forest plots to show the mean differences (MDs) and the weight of each study and the pooled effect size with their corresponding 95% confidence intervals (CIs). Heterogeneity between the studies was assessed using the I^2 statistic. We applied random-effects meta-analysis since the I^2 values were

> 40%. All analyses were conducted using [STATA](#) version 15.1 (StataCorp, College Station, TX 77845, USA).

Results

Study selection

We identified a total of 2137 papers from the database searches and an additional 18 papers from screening references of eligible papers ([Figure 1](#)). We removed 500 duplicates and after screening titles and abstracts, excluded 1579 papers that were not relevant to our study. We further excluded 13 randomised controlled trials (RCTs) because participants were randomised

Figure 1. PRISMA flow chart showing the selection process for studies included in the review and meta-analysis.

to multiple micronutrient powders or other nutritional supplements together with iron and the effect of iron supplementation was not evaluated separately from the other supplements. We excluded seven observational studies, four RCTs in adults and 15 papers that were literature reviews, study protocols, comments or abstracts. We excluded one study that did not have a placebo group as all participants received a single iron-dextran intramuscular injection. We further excluded one RCT in low birthweight children (<2500g) and another in premature children (born at 27 to 30 gestational weeks).

Study characteristics and outcomes

We included a total of 34 RCTs published between 1978 and 2020 (Figure 2). In total, five were in African countries while 29 RCTs were in non-African countries. Of the 34 RCT studies: 25 evaluated the effect of iron supplementation compared to placebo or no treatment; five the effect of iron-fortified foods compared to non-fortified foods; one the effect of fortification of formula milk with high compared to low dosages of iron; one the effect of immediate iron supplementation given concurrently or 28 days after antimalarial treatment on development in children with severe malaria and one the effect of varying and consistent doses of iron supplementation compared to placebo; two the effects of maternal iron supplementation on neurobehavioural outcomes in children after birth.

Out of the 34 studies, two were in lead-exposed children at baseline. Overall, 11 studies were carried out in high-income countries and 23 in low and middle-income countries. The sample sizes ranged from 16 to 1358 and the RCT studies provided varying forms of iron supplementation in varying dosages over periods ranging from 1 day to 15 months. The studies evaluated various neurobehavioural outcomes including cognitive, motor, language and behavioural development and educational achievement using a wide range of neuroassessment tools, the most common being the Bayley Scales of Infant Development. Iron status and anaemia were defined differently in the studies using varying iron biomarkers and haemoglobin cut-offs. The characteristics of the included studies are shown in Table 1 and Extended data, file 2¹⁸. Of the 34 RCTs, 18 studies showed a low risk of bias, one study showed a high risk of bias while 15 studies were judged to raise some concerns (Extended data, files 3 and 4¹⁸). Some common limitations included a lack of description of the randomization process and missing outcome data.

Studies in African countries versus studies in non-African countries

Five RCTs including a total of 1294 children evaluated the effect of iron supplementation or fortification on cognitive (n=4), motor (n=1), behaviour (n=1) and language (n=1) outcomes in

Figure 2. Studies on the effect of iron supplementation or fortification on neurobehavioural outcomes in children. Five studies (n=1294) were in Africa, 14 studies (n=5989) were in Asia, four studies (n=695) were in Europe, nine studies (n=1332) were in North America, one study (n=196) was in South America and one study (n=302) was in Australia.

African children. Only three out of the five studies were in Sub-Saharan Africa and of the three one did not have a placebo group. Out of the five studies, two (n=647) reported beneficial effects on cognitive, motor or language outcomes only in anaemic children^{23,24} while three studies (n=647) found no beneficial effects in children^{21,22,25}. Of the three studies that reported no beneficial effects of iron supplementation, one included only children with iron deficiency²¹, one study had a prevalence of 21% of anaemia²² while one study did not report baseline iron status²⁵. Among children living in non-African countries, 29 RCTs including a total of 8514 participants investigated the effect of iron supplementation or fortification on cognitive (n=24), motor (n=17), behaviour (n=7) or educational achievement (n=5) outcomes. Out of the 29 studies, nine (n=2925) reported beneficial effects on neurobehavioural outcomes, seven studies (n=786) reported beneficial effects only in children with iron deficiency, iron deficiency anaemia or anaemia at baseline while 13 studies (n=4803) found no beneficial effects^{26–54}.

Cognitive development

A total of 28 RCTs (n=7503) investigated the effect of iron supplementation (n=23) or fortification (n=5) on cognitive development. Out of these studies, four (n=935) were in children living in African countries including two among children living in sub-Saharan Africa (n=433). One RCT of 288 South African children aged six to 11 years reported improved cognitive and memory scores among anaemic children (haemoglobin (Hb) <11.5 g/dL) who received iron supplementation for 8.5 months compared to children who received placebo²³. However, two RCTs, one of 455 lead-exposed Moroccan children aged three to 14 years and another of 47 Zairean school children aged seven to nine years, reported that iron supplementation or fortification for four to 28 weeks was not beneficial for cognitive development^{22,25}. One RCT (n=145) without a placebo arm reported no improvement in cognitive development in Ugandan pre-school children with severe malaria who received iron supplementation concurrently or 28 days after antimalarial treatment²¹.

A total of 24 RCTs (n=6568) investigated the effect of iron supplementation or fortification on cognitive development in children living in non-African countries. Out of these 24 studies, three reported beneficial effects on cognitive development, six reported beneficial effects only in children with iron deficiency anaemia (IDA) at baseline while 15 found no beneficial effects. Due to substantial heterogeneity in study methods, only seven (n=775) out of 24 studies that used the Bayley Scales of Infant Development (BSID) to assess cognitive development were included in a meta-analysis and they provided limited evidence of beneficial effects of iron supplementation in pre-school children (MD=1.73, 95% CI, -1.05, 4.52) (Extended data, file 5¹⁸). Out of 24 studies, three (n=604) reported beneficial effects of iron supplementation or fortification on cognitive development. In school-aged children, one small RCT of 73 adolescent girls aged 13 to 18 years in the USA reported improved verbal learning and memory after iron supplementation for eight weeks compared to

placebo⁴². Another RCT of 391 Thai school children reported improved intelligence quotient (IQ) scores in children who received once-a-week iron supplementation compared to children who received daily iron supplementation or placebo for 16 weeks³². One RCT of 140 Indian children aged 12 to 16 years reported improved cognition in children who received iron-biofortified pearl millet compared to children who received conventional pearl millet²⁷. Six studies (n=678) out of 24 studies reported improved cognitive development after iron supplementation among children with IDA or anaemia at baseline. Among pre-school children, three RCTs, one in Costa Rica (n=191) and two in Indonesia (n=295) reported improved cognitive development in children with IDA or iron depletion at baseline after iron supplementation for two to four months^{44,46,49}. One small RCT of 49 children aged three to four years in Greece reported improved aspects of cognitive development among anaemic children who received iron supplementation for two months compared to children who received placebo³³. Another small RCT in 24 American children aged nine to 26 months, who had IDA at baseline reported improved cognitive development among children who received a single iron-dextran complex injection compared to children who received a single sterile saline injection⁵³. In school children, one RCT of 119 Indonesian children reported improved IQ among children with IDA at baseline who received iron supplementation for three months compared to children who received placebo⁵¹.

In total, 15 studies (n=5286) out of 24 studies found no beneficial effects of iron supplementation or fortification on cognitive development. Among pre-school children, two RCTs in Indonesia (n=655) and Bangladesh (n=221) reported no beneficial effects of iron supplementation for six months on cognitive development^{34,35}. Three other RCTs in Chile (n=196), Costa Rica (n=86) and Guatemala (n=68) found that iron supplementation for one week to six months was not beneficial for cognitive development^{41,45,52}. Additionally, two RCTs in 124 Turkish children aged six to 30 months reported that iron supplementation for three months was not beneficial for cognitive development^{36,38}. Two RCTs, one in Canada (n=225) and another in the UK (n=428) reported no beneficial effects of iron-fortified formula on cognitive development in children aged six to nine months compared to unfortified formula or cow's milk^{40,43}. One RCT of 133 Spanish children aged six months reported no beneficial effects of high-iron formula milk on cognitive development compared to low-iron formula milk²⁶. Among school children, two large RCTs in Thailand (n=1358) and Sri-Lanka (n=1190) reported that iron supplementation for four to six months was not beneficial for cognition in children aged eight to 11 years^{29,48}. Another RCT of 130 Indonesian children aged 8.1 to 11.6 years reported no beneficial effects of iron supplementation for three months on IQ⁴⁷. One RCT of 170 Indian children aged eight to 13.4 years found no beneficial effect of daily iron-fortified wheat-based meals for seven months on cognition compared to unfortified wheat-based meals³⁰. Another RCT in 302 Australian mother-child pairs reported no beneficial effects of iron supplementation during pregnancy on IQ at four years⁵⁴.

Motor development

A total of 18 RCTs (n=4258) investigated the effect of iron supplementation (n=15) or fortification (n=3) on motor outcomes. Out of these studies, only one RCT (n=359) was in African children. One RCT of 359 children in Zanzibar reported improved motor and language scores in anaemic children at baseline who received iron supplementation for 12 months compared to children who received placebo²⁴.

A total of 17 RCTs (n=3899) investigated the effect of iron supplementation or fortification on motor development in children living in non-African countries. Out of these 17 studies, six reported beneficial effects on motor development, two reported beneficial effects only in children with IDA at baseline while nine studies found no beneficial effects. Due to substantial heterogeneity in study methods, seven (n=775) out of 17 studies that used the BSID to assess motor development were included in a meta-analysis and they showed limited evidence of beneficial effects of iron supplementation in pre-school children (MD=1.99, 95% CI, -0.97, 4.95) (Extended data, file 6¹⁸). Out of 17 studies, six (n=2299) reported beneficial effects of iron supplementation or fortification on motor development. In pre-school children, one large RCT of 1196 Chinese children aged six weeks whose mothers also received iron supplementation during pregnancy reported that iron supplementation for 7.5 months, with or without iron supplementation in pregnancy, improved gross motor development compared to placebo²⁸. Two other RCTs, one in 97 American children and another in 41 Canadian children reported beneficial effects of iron supplementation for two to five months on motor development compared to placebo^{37,50}. Additionally, one RCT in 655 6-month old Indonesian children reported improved motor development after iron supplementation for six months³⁴. Two RCTs, one in the UK (n=85) and another in Canada (n=225) reported improved motor development in children aged six to eight months who received iron-fortified formula milk for 10 to 15 months compared to children who received regular formula or cow's milk^{39,43}. Out of 17 studies, two (n=310) reported improved motor development after iron supplementation among children with IDA or anaemia at baseline. In pre-school children, one RCT of 191 Costa Rican children aged 12 to 23 months reported that children with IDA who received iron supplementation for three months had improved motor development compared to children who received placebo⁴⁹. Another RCT in 119 Indonesian children reported improved motor development in children with IDA at baseline after iron supplementation for four months⁴⁴.

Out of the 17 studies, nine (n=1290) found no beneficial effects of iron supplementation or fortification on motor development in pre-school children. Two RCTs, one in 196 Chilean children below 15 months of age and another of 221 Bangladeshi six-month-old children, reported no beneficial effect of three to six months of iron supplementation compared to placebo^{35,45}. Two RCTs, one in 96 Costa Rican children aged 12 to 24 months and another in 68 Guatemalan children aged six to 24 months, reported no beneficial effect of iron supplementation for six months on motor development compared to placebo^{41,52}.

Additionally, two RCTs in 124 Turkish children aged six to 30 months reported that iron supplementation for three months was not beneficial for cognitive development^{36,38}. Another small RCT in 24 American children aged nine to 26 months, who had IDA at baseline reported no beneficial effects of a single iron-dextran complex injection on motor development⁵³. One RCT of 428 nine-month-old children in the UK reported no beneficial effects of iron-fortified formula milk on motor development compared to unfortified formula or cow's milk while another RCT of 133 Spanish six-month-old children reported no beneficial effects of high-iron compared to low-iron formula milk on motor development^{26,40}.

Behavioural functioning

In total, eight RCTs (n=2295) investigated the effect of iron supplementation (n=7) or fortification (n=1) on behavioural functioning in children. Of these eight studies, only one (n=145) was in African children. One RCT of 145 Ugandan children aged 18 to 58.8 months with severe malaria reported no improvement in behavioural functioning in children who received iron supplementation concurrently or 28 days after antimalarial treatment²¹. A total of seven RCTs (n=2150) investigated the effect of iron supplementation or fortification on behavioural functioning in children living in non-African countries. Of these seven studies, one study (n=24) reported beneficial effects on behavioural functioning in children with IDA, while six studies (n=2126) reported no beneficial effects. One small RCT of 24 American children aged nine to 26 months, who had IDA at baseline reported improved behavioural functioning among children who received a single iron-dextran complex injection compared to children who received a single sterile saline injection⁵³. Six studies (n=2126) out of seven found no beneficial effects of iron supplementation or fortification on behavioural functioning in pre-school children. Three RCTs in Indonesia (n=655), Bangladesh (n=221) and Chile (n=196) found no beneficial effects of iron supplementation for three to six months on behavioural functioning in children up to six months of age^{34,35,45}. One RCT of 527 Mexican children who were exposed to lead at baseline similarly reported no beneficial effect of iron supplementation for six months on behavioural functioning³¹. Another RCT of 225 Canadian children aged six months found no beneficial effects of iron-fortified formula for 15 months on behaviour compared to regular formula⁴³. One RCT in 302 Australian mother-child pairs reported no beneficial effects of iron supplementation during pregnancy on child behaviour at four years⁵⁴.

Educational achievement

Overall, five RCTs (n=3188) investigated the effect of iron supplementation on educational achievement in children living in non-African countries. There were no studies in African countries. Out of these five studies, one study (n=130) reported beneficial effects in anaemic children, while four studies (n=3058) reported no beneficial effects. One RCT of 130 Indonesian children aged 8.1 to 11.6 years reported improved educational achievement in children who were anaemic at baseline and who received iron supplementation for three months compared to children who received placebo⁴⁷. Out of the

five studies, four studies (n=3058) found no beneficial effects of iron supplementation on educational achievement in school children. Three RCTs in Sri Lanka (n=1190), Thailand (n=1358) and Indonesia (n=119) found no beneficial effects of iron supplementation for three to six months on educational achievement in school children^{29,48,51}. Another RCT including 391 school children in Thailand found no beneficial effect of iron supplementation on educational achievement in children who received once-a-week compared to daily iron supplementation for 16 weeks³².

Effect of iron supplementation based on baseline iron status

Sub-group meta-analysis based on baseline iron status indicated that iron supplementation was not beneficial for cognitive development in 250 children with iron deficiency (ID), iron deficiency anaemia (IDA) or anaemia (MD=2.63, 95% CI, -4.65, 9.90) or in 103 children with sufficient iron levels at baseline (MD=0.48, 95% CI, -1.77, 2.73) (Extended data, file 5¹⁸). Similarly, sub-group meta-analysis showed that iron supplementation was not beneficial for motor development in 250 children with ID, IDA or anaemia (MD=3.42, 95% CI, -3.52, 10.36) or in 103 children with sufficient iron levels at baseline (MD=-0.19, 95% CI, -2.25, 1.87) (Extended data, file 6¹⁸). Eleven studies (n=2769) evaluated the effect of iron supplementation in groups of children with ID, IDA or anaemia compared to children with normal iron status at baseline, five studies (n=698) included all children with ID, IDA or anaemia at baseline while two studies (n=57) included all children with sufficient iron status at baseline. Of the 11 studies that compared the effect of iron supplementation in groups of children with ID, IDA or anaemia and children with normal iron status at baseline, six studies (n=953) reported beneficial effects of iron supplementation on neurobehavioural outcomes^{23,33,44,46,47,49} while five studies (n=1816) reported no beneficial effects^{36,41,45,48,52}. Of the five studies that included all children with ID, IDA or anaemia at baseline, four studies (n=553) reported beneficial effects of iron supplementation on neurobehavioural outcomes^{24,42,50,53} while one study (n=145) found no beneficial effects²¹. Of the two studies that included children that only had sufficient iron status at baseline, one study (n=41) reported that iron supplementation was beneficial for motor development³⁷ while one study of 16 children reported no beneficial effects of iron supplementation on cognitive or motor development³⁸.

Effect of iron supplementation or fortification in children during infancy versus older age

Overall, nine RCTs including a total of 3000 children evaluated the effect of iron supplementation (n=5) or fortification (n=4) on neurobehavioural outcomes during infancy and of these studies, five (n=2202) reported beneficial effects of iron supplementation (n=3) or fortification (n=2) on neurobehavioural outcomes^{28,34,37,39,45} while four studies (n=798) found no beneficial effects of iron supplementation (n=2) or fortification (n=2)^{26,35,38,40}. In children above one year of age, 24 RCTs including a total of 6506 children evaluated the effects of iron supplementation (n=22) or fortification (n=2) on neurobehavioural outcomes and of these, 13 studies

(n=2156) reported a beneficial effect of iron supplementation (n=12) or fortification (n=1) on neurobehavioural outcomes^{23,24,27,32,33,42,44,46,47,49,50,51,53} while 11 studies (n=4350) reported no beneficial effects of iron supplementation (n=11) or fortification (n=1)^{21,22,25,29-31,36,41,45,48,52}.

Effect of duration of supplementation or fortification

In total, seven studies (n=534) investigated the effect of iron supplementation for less than three months and of these studies, five studies (n=419) reported beneficial effects of iron supplementation on neurobehavioural outcomes^{33,42,46,50} while two studies (n=115) reported no beneficial effects^{25,52}. Out of 26 studies (n=8972) that evaluated the effect of iron supplementation (n=20) or fortification (n=6) for three months or more, 13 studies (n=3939) reported beneficial effects of iron supplementation (n=10) or fortification (n=3) on neurobehavioural outcomes^{23,24,27,28,32,34,37,39,43,44,47,49,51} and 13 studies (n=5033) reported no beneficial effects of iron supplementation (n=10) or fortification (n=3)^{21,22,26,29-31,35,36,38,40,41,45,48}.

Discussion

In this systematic review and meta-analysis, we found mixed evidence for the effects of iron supplementation or fortification on neurobehavioural outcomes in children. We found few studies that investigated the effects of iron supplementation on neurobehavioural outcomes in African children despite the high burden of both iron deficiency and developmental delay. Evidence from other regions on the effects of iron on neurobehavioural outcomes may not be generalisable to African children as these effects may be mediated by different risk factors such as malnutrition and a high burden of infectious diseases including malaria, HIV, tuberculosis and helminthic infections⁵⁵. Of five studies in African children only three included children living in Sub-Saharan Africa, which has the highest prevalence of malaria⁵⁶, and one of these studies had no placebo arm²¹. Additionally, only six observational studies have evaluated the associations between iron status and neurobehavioural outcomes in African children and their findings are inconsistent⁵⁷⁻⁶². The few iron supplementation studies in African children may be due to concern that iron supplementation may increase the risk of malaria and other infections or delay malaria parasite clearance⁶³⁻⁶⁵. While the World Health Organization recommends iron supplementation together with effective malaria treatment and prevention in children living in malaria-endemic areas, evidence on the optimal time for iron supplementation and the effects on neurobehavioural outcomes in African children is limited⁶⁶.

Only five studies assessed the effect of iron supplementation or fortification on cognitive and language development and no studies included educational achievement in African children. We found mixed evidence among the five studies; the two studies that reported improved cognitive or language development included only anaemic African children, while the three studies reporting lack of beneficial effects did not evaluate the effects of iron supplementation based on baseline iron status. Evidence from observational studies in African children is also limited with only three observational studies

in Ethiopia, Egypt and Benin reporting no association between child or maternal iron status and cognitive or language development in young children^{58,60,62}. Lack of associations in these studies may be explained by the sufficient iron status of the participants. Similarly, evidence for the effects of iron supplementation on cognitive development in children living in non-African countries was limited with seven of 19 RCTs reporting beneficial effects. It is possible that some of the tools used to assess cognitive development detect broad aspects of cognition and may have limited sensitivity to slight changes resulting from nutritional effects in specific elements of cognition such as attention and information processing speed⁶⁷. Effects of iron on aspects of cognition including concentration, memory, attention and IQ may mediate the reported improved educational achievement following iron supplementation in one study while the lack of beneficial effects observed in four studies may be explained by the low prevalence of iron deficiency anaemia at baseline^{29,48}. In contrast to epidemiological studies, evidence from animal studies consistently suggests that iron may impact cognitive and language development through its roles in myelination, dopamine metabolism and the structure and function of the hippocampus, the centre for memory and learning processes^{13,68,69}.

A single study evaluated the effects of iron supplementation on motor development in African children and reported improved motor development in anaemic African children. Improved motor development following iron supplementation may be attributed to improved iron status. Iron deficiency is associated with low cellular oxygen-carrying capacity of blood in tissues causing low muscle energy production which may limit independent effort and balance delaying acquisition of motor skills in children^{70,71}. Evidence from observational studies in African children also shows associations between iron status and motor development. Three observational studies in Zanzibar and Ghana reported associations between ID and/or IDA and poor motor development in children^{57,59,61}. About half of the RCT studies in children living in non-African countries reported beneficial effects of iron supplementation on motor development. The mixed findings may be explained by differences in study methods such as sample sizes and baseline iron status. Animal studies provide mechanistic evidence of how iron might influence motor development. Iron is important for myelination in the corticospinal and corticostriatal tracts, the main pathways for motor signals from the brain to the limbs⁷². Additionally, iron plays an important role in dopamine function in the basal ganglia, an important area in the brain for motor function⁷³.

We found little evidence for the effects of iron supplementation on behavioural functioning. There was only one study in African children, and it found no evidence of improved behavioural functioning in children who received iron supplementation concurrently or 28 days after antimalarial treatment. This study may be limited by the small sample size and lack of a placebo group that did not receive iron supplementation. In children living in non-African countries, only one of five RCTs reported beneficial effects in American children

with IDA. Children with IDA have been observed to be clumsy, inattentive, irritable and withdrawn, traits that are consistent with impaired behavioural functioning⁷⁴. The lack of beneficial effects of iron observed in some studies may be attributed to the small proportion of children with iron deficiency anaemia at baseline as mild iron deficiency may not result in depletion of iron in body tissues that would manifest in behavioural changes^{34,45}. Evidence from animal studies indicate that iron may influence behavioural functioning through its role in dopaminergic neurotransmission that is key in behaviour activation and behaviour inhibition and reward seeking behaviour⁷⁵.

The mixed findings for the effects of iron supplementation on child development in our review may be attributed to differences in study methods, sensitivity of outcome measures used and populations. Based on baseline iron status, iron supplementation was mostly beneficial for development in children with iron deficiency or iron deficiency anaemia. Improvement of symptoms of iron deficiency or iron deficiency anaemia, including lethargy and withdrawal, after iron supplementation may result in improved neurobehavioural outcomes in these children⁷⁶. Also, children with iron deficiency anaemia are likely to be fussy and clingy to their caregivers and their caregivers are likely to respond by holding them which may delay the child's independent exploration and interaction with their environment and consequently delay neurobehavioural development⁷⁷. We observed little difference when comparing findings between studies that gave iron supplementation during or after infancy. Evidence indicates that iron supplementation may be more beneficial to child development in early childhood when there is rapid brain development but beneficial effects of iron supplementation on neurobehavioural outcomes have also been reported in older children^{78–80}. Only two studies evaluated the effects of maternal iron supplementation during pregnancy and they did not report beneficial effects on neurobehavioural outcomes in children after delivery^{28,54}. One of the studies reported that maternal iron supplementation did not improve iron status in the newborn as indicated by cord blood ferritin at delivery, which may explain the lack of beneficial effects on child development⁸¹. Further studies are necessary to evaluate the effects of maternal iron supplementation on neurobehavioural outcomes in children. We found little difference when comparing studies that gave iron supplementation for less than three months and studies that gave iron supplementation for three months or more. The World Health Organization recommends iron supplementation for three consecutive months in children living in areas with a high prevalence of anaemia for prevention of iron deficiency and anaemia but it is unclear if this duration is adequate to improve neurobehavioural outcomes in children⁶⁶.

We identified five systematic reviews and meta-analyses of the effects of iron on neurobehavioural outcomes in children. Consistent with our review, one recent systematic review of 25 RCTs and 26 observational studies reported inconsistent findings on the effects of maternal or child iron supplementation or iron status on neurobehavioural outcomes in children⁸².

This systematic review only included studies with children below four years of age. Another systematic review and meta-analysis of 33 RCTs evaluating the effects of daily iron supplementation on child health reported no beneficial effects of iron supplementation on mental or psychomotor development in children aged four to 23 months⁸³. Of the 33 included studies, only six studies evaluated mental and psychomotor development in children. One systematic review and meta-analysis of 32 RCTs investigating the effects of daily supplementation on child health reported beneficial effects of iron supplementation on cognition, IQ among anaemic children and aspects of attention and concentration in children aged five to 12 years⁸⁰. Of the eligible 32 RCTs, only 12 assessed aspects of cognition in children and unlike our review, did not include studies that assessed other neurobehavioural domains. One Cochrane systematic review of eight RCTs assessing the effects of iron supplementation in children below three years of age who had iron deficiency reported no beneficial effects of iron supplementation on mental or psychomotor development⁸⁴. Another systematic review and meta-analysis of 14 RCTs evaluating the effects of oral iron supplementation in older school children and women reported beneficial effects of iron supplementation on attention, concentration and IQ but not memory, psychomotor function or school achievement⁷⁸.

Strengths and limitations

Strengths of our review include a very comprehensive search strategy of five databases without geographical location, language or date limitations. To our knowledge, our review is the first to summarise evidence on the effects of iron supplementation on neurobehavioural outcomes in African children in comparison to evidence in children living in non-African countries. Additionally, our review included children up to the age of 18 years giving an overview of neurobehavioural outcomes across childhood. Limitations of our review were the inability to conduct a quantitative meta-analysis of the African studies due to the substantial heterogeneity in study populations and methods including the tools used to assess neurobehavioural outcomes and definitions for iron status. The majority of studies are from non-African countries and only three studies have been conducted in sub-Saharan Africa.

Conclusions

We found conflicting evidence for the effects of iron supplementation or fortification on neurobehavioural outcomes in children and there were very few studies in African children. Further, well-powered randomised controlled trials on the effects of iron supplementation on neurobehavioural outcomes in African children are required considering the high burden of both iron deficiency and developmental delays in these

populations. These studies further need to consider the impact of other risk factors such as infections and malnutrition on the relationship between iron and neurobehavioural outcomes in African children. Additionally, well-validated and standardised tools for assessing neurobehavioural outcomes across all age groups in childhood would help in comparison of findings in studies.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Figshare: Effects of vitamin D deficiency on neurobehavioural outcomes in children: a systematic review-supplementary files. <https://doi.org/10.6084/m9.figshare.14473077.v3>¹⁸.

This project contains the following extended data:

- Extended datafile 1: Search terms.
- Extended datafile 2: Summary of studies assessing the effect of iron supplementation or fortification on neurobehavioural outcomes in children living in non-African countries: characteristics and findings.
- Extended datafile 3: Assessment of risk of bias in randomized parallel-group trials included in the review using the revised Cochrane risk-of-bias tool for randomized trials.
- Extended datafile 4: Assessment of risk of bias in cluster-randomized parallel-group trials included in the review using the revised Cochrane risk-of-bias tool for randomized trials with additional considerations for cluster-randomized trials.
- Extended datafile 5: Forest plot for the effects of iron supplementation on cognitive development: overall effect and subgroup analyses based on baseline iron status.
- Extended datafile 6: Forest plot for the effects of iron supplementation on motor development: overall effect and subgroup analyses based on baseline iron status.

Reporting guidelines

Figshare: PRISMA checklist for 'Effects of vitamin D deficiency on neurobehavioural outcomes in children: a systematic review'. <https://doi.org/10.6084/m9.figshare.14473077.v3>¹⁸.

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Michael Mireku

School of Psychology, University of Lincoln, Lincoln, UK

Matua *et al.* have done excellent work on a very difficult topic. The authors attempted to investigate the effect of iron supplementation and fortification on neurobehavioural outcomes in children. The objective although clearly stated is very difficult to accurately investigate because of several reasons:

1. There are several domains of neurobehavioural outcomes assessed in most papers;
2. Different assessment scales are used across several studies and the scores generated from one scale are not easily interchangeable/transferable into another scale;
3. Some of these scales are adapted for use in specific countries and others are not;
4. Age-dependent dose of iron supplementation will vary for different settings. Trials that recruit only iron-deficient children may provide a relatively higher dose of iron relative to studies in a general, supposedly healthy population. Thus, the benefit of iron supplementation may be the same across groups because of the dose. the same can be said for fortification - how much of the fortified product is the child consuming?

The authors have therefore done a very good job in identifying these potential issues and providing their analysis under these specific subheadings.

My only major concern is the validity of the meta-analysis in relation to the standardisation of the scales used to assess cognitive and motor functions. The authors failed to explain how they used the scores from the different scales in the meta-analysis.

Further, findings from the meta-analysis are actually scanty for the obvious reason of heterogeneity across studies. This should be discussed further.

Minor comment: Revise the first sentence under Methods "...metanalysis were" not "...metanalysis was"

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Yes

Is the statistical analysis and its interpretation appropriate?

Partly

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Perinatal and paediatric epidemiology; Sleep health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Nov 2021

Agnes Mutua, Kenya Medical Research Institute (KEMRI), Centre for Geographic Medicine Research-Coast, KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

We are grateful to the reviewer for his insightful comments and have addressed the below:

Mutua *et al.* have done excellent work on a very difficult topic. The authors attempted to investigate the effect of iron supplementation and fortification on neurobehavioural outcomes in children. The objective although clearly stated is very difficult to accurately investigate because of several reasons:

1. There are several domains of neurobehavioural outcomes assessed in most papers;
2. Different assessment scales are used across several studies and the scores generated from one scale are not easily interchangeable/transferable into another scale;
3. Some of these scales are adapted for use in specific countries and others are not;
4. Age-dependent dose of iron supplementation will vary for different settings. Trials that recruit only iron-deficient children may provide a relatively higher dose of iron relative to studies in a general, supposedly healthy population. Thus, the benefit of iron supplementation may be the same across groups because of the dose. the same can be said for fortification - how much of the fortified product is the child consuming?

The authors have therefore done a very good job in identifying these potential issues and

providing their analysis under these specific subheadings.

My only major concern is the validity of the meta-analysis in relation to the standardisation of the scales used to assess cognitive and motor functions. The authors failed to explain how they used the scores from the different scales in the meta-analysis. Further, findings from the meta-analysis are actually scanty for the obvious reason of heterogeneity across studies. This should be discussed further.

Response: We thank the reviewer for this comment and agree that a main limitation of the review is the high level of heterogeneity in the studies which precluded a meta-analysis. We therefore analysed all studies in African and non-African countries qualitatively by summarizing the findings.

In secondary analyses we performed a meta-analysis of all studies (n=7) which used the same tool, the Bayley Scales of Infant Development, to assess cognitive and motor development. The Bayley Scales of Infant Development has standardized scales and therefore there was no need for any further standardization of the scores. We agree that these secondary analyses had a number of limitations. First, only a very limited number of studies used the Bayley Scales, which may introduce bias. Second, we were not able to meta-analyse African studies since this tool was not used and Bayley Scales are likely to be less applicable in this population. Third, although the meta-analysis included standardized assessment of neurodevelopmental outcomes many other factors were not standardized including dose and duration of iron supplementation.

We have now added a sub-heading to highlight the sub-section detailing the meta-analysis in the methods section as follows:

"Secondary meta-analysis of seven studies in non-African countries"

Due to the substantial variation in study methods, we did a secondary meta-analysis in a limited number of RCTs that all used the Bayley Scales of Infant Development (BSID) to assess cognitive and motor development in children living in non-African countries. None of the studies in African children used the BSID. In the meta-analysed studies, mean cognitive and motor development scores and standard deviations were reported. For each of the two domains, we generated forest plots to show the mean differences (MDs) and the weight of each study and the pooled effect size with their corresponding 95% confidence intervals (CIs). Heterogeneity between the studies was assessed using the I² statistic. We applied random-effects meta-analysis since the I² values were > 40%. All analyses were conducted using STATA version 15.1 (StataCorp, College Station, TX 77845, USA)."

We have summarized the results of the meta-analysis in the results section as follows:

'Cognitive development': "Secondary meta-analysis of seven studies (n=775), provided limited evidence of beneficial effects of iron supplementation on cognitive development in pre-school children (MD=1.73, 95% CI, -1.05, 4.52)."

'Motor development': "The seven studies(n=775) included in a secondary meta-analysis showed no beneficial effects of iron supplementation on motor development in pre-school children (MD=1.99, 95% CI, -0.97, 4.95) (Extended data, file 6)."

We have also noted this limitation in the Discussion section under 'Strengths and limitations':

"Limitations of our review were the inability to conduct a quantitative meta-analysis due to the substantial heterogeneity in study populations and methods including the tools used to assess neurobehavioural outcomes and definitions for iron status. Our secondary meta-analyses are likely to be limited by the small sample sizes and other unstandardized factors including age,

dosage and duration of iron supplementation."

Minor comment: Revise the first sentence under Methods "...metanalysis were" not "...metanalysis was"

Response: We have now edited the sentence under 'Methods' as follows:

"Our systematic review and meta-analyses were guided by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines and the protocol was registered on the PROSPERO database on 20th March 2018 (registration number CRD42018091278)."

Competing Interests: No competing interests were disclosed.

Reviewer Report 06 August 2021

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Patricia Kitsao-Wekulo

Maternal and Child Wellbeing Unit, African Population and Health Research Center, Nairobi, Kenya

The authors have presented data from a systematic review and meta-analysis which found mixed evidence for the effects of iron supplementation or fortification on neurobehavioural outcomes in children. This is an area on which there is limited evidence from Africa, despite the high prevalence of both iron deficiency and developmental delay. The authors are commended for the use of a comprehensive search strategy for their review which yielded important evidence on the effects of iron supplementation on neurobehavioural outcomes in African children, while at the same time comparing their findings with those from children living in non-African countries.

I have made the following comments for consideration by the authors:

Introduction

- The rationale for, and the objectives of the systematic review are clearly stated. However, I would like to suggest that the authors consider shifting some of the material in the Introduction. For instance, the second paragraph could be shifted to the beginning of this section as it gets straight to the point in providing context for the review.
- The first mention of 'randomised controlled trials' appears at the end of the introduction, and it is here that the abbreviation 'RCT' should appear after it has been written out in full. In later sections, the authors can just use the abbreviation, without having to spell it out in full again as they have done under the Methods and other sections.

Methods

- Under the subsection on the **search strategy**, the authors indicate that they 'searched...for studies published up to 9th March 2021.' It is not clear which month/ year was considered as the start point.
- 'Disagreements between reviewers were resolved through discussion.' Please clarify what the disagreements were on and the content of the discussion to get to a consensus.
- 'We used the revised Cochrane risk-of-bias tool for randomised trials...' This statement appears twice in the same paragraph. Consider revising.
- 'The large degree of diversity in the study variables necessitated narrative synthesis of the study findings.' Was this applied to all the studies included in the review, or only to those from Africa? Please clarify

Results

- '...that were not relevant to our study.' I suggest you use 'review' rather than 'study'
- The information on the time period of the publications included in the review should be provided earlier, under search strategy
- 'Of the 34 RCT studies: 25 evaluated the effect of iron supplementation compared to placebo or no treatment; five the effect of iron-fortified foods compared to non-fortified foods; one the effect of fortification of formula milk with high compared to low dosages of iron; one the effect of immediate iron supplementation given concurrently or 28 days after antimalarial treatment on development in children with severe malaria and one the effect of varying and consistent doses of iron supplementation compared to placebo; two the effects of maternal iron supplementation on neurobehavioural outcomes in children after birth.' For clarity and to avoid repetition, the number of studies under each study description could be presented as Ns e.g. 'Among the 34 RCT studies were those that studied the effect of: iron supplementation compared to placebo or no treatment (N = 25); iron-fortified foods compared to non-fortified foods (N = 5); etc
- Check consistency in the use of Sub-Saharan Africa or sub-Saharan Africa
- "Due to substantial heterogeneity in study methods, only seven (n=775) out of 24 studies that used the Bayley Scales of Infant Development (BSID) to assess cognitive development' - This statement is repeated under the subsection on **synthesis**.
- It is not clear what number of studies were included to investigate the effect of iron supplementation. Under the subsection on study characteristics, the number is indicated as 25. Later on, under 'cognitive development, the number is indicated as 23. Please clarify.
- Consider revising the following statement for improved readability as 'children' appears four times
'In school children, one RCT of 119 Indonesian children reported improved IQ among children with IDA at baseline who received iron supplementation for three months compared to children who received placebo.'

- There is a lot of repetition in the text under the Results section which makes reading the text quite tedious. For instance, under the subsection on 'Educational achievement,' a similar statement appears in the third and sixth lines 'four studies (n=3058) reported no beneficial effects.' Consider revising
- The authors tend to use the word 'studies' several times in single statements and should consider revisions. For example, the first statement under the subsection titled 'Effect of duration..'
- The second objective for the review was '**For comparison**, we further summarised evidence from randomised controlled trials on the effects of iron supplementation on neurobehavioral outcomes in non-African countries.' My sense is that the comparison does not really come out clearly in the results. The authors should consider providing a summary to provide this comparison, either at the end of each subsection on specific neurobehavioural outcomes or at the end of the Results section, if this fits within the journal guidelines.

Discussion

- In the first paragraph, there is discussion around the association between iron supplementation and the heightened risk of malaria infection. This information would fit better under the Introduction as it provides more context around why there are limited iron supplementation studies in Africa

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Yes

Is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Developmental psychology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Nov 2021

Agnes Mutua, Kenya Medical Research Institute (KEMRI), Centre for Geographic Medicine Research-Coast, KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

We thank the reviewer for her helpful comments, which we have addressed point-by-point below:

The authors have presented data from a systematic review and meta-analysis which found mixed evidence for the effects of iron supplementation or fortification on neurobehavioural outcomes in children. This is an area on which there is limited evidence from Africa, despite the high prevalence of both iron deficiency and developmental delay. The authors are commended for the use of a comprehensive search strategy for their review which yielded important evidence on the effects of iron supplementation on neurobehavioural outcomes in African children, while at the same time comparing their findings with those from children living in non-African countries.

I have made the following comments for consideration by the authors:

Introduction

The rationale for, and the objectives of the systematic review are clearly stated. However, I would like to suggest that the authors consider shifting some of the material in the Introduction. For instance, the second paragraph could be shifted to the beginning of this section as it gets straight to the point in providing context for the review.

Response: We agree with the reviewers point that the second paragraph provides a clearer context for our review and have now moved paragraph two under introduction to the beginning of the section as follows:

"Iron deficiency is the most common micronutrient deficiency affecting about two billion individuals globally and accounting for over 40% of all cases of anaemia ¹⁻³. Children living in Africa disproportionately bear the highest burden of anaemia and iron deficiency. Approximately 43% of pre-school children are anaemic globally and over 60% of these children live in Africa ^{1, 4}, while it is estimated that 52% of African children are iron deficient ⁵.

The first mention of 'randomised controlled trials' appears at the end of the introduction, and it is here that the abbreviation 'RCT' should appear after it has been written out in full. In later sections, the authors can just use the abbreviation, without having to spell it out in full again as they have done under the Methods and other sections.

Response: We have now abbreviated 'randomised controlled trials' in the last paragraph of the introduction and added the abbreviation RCT in the 'Search strategy and eligibility criteria', 'Study selection' and 'Conclusions' sections.

Methods

Under the subsection on the search strategy, the authors indicate that they 'searched...for studies published up to 9th March 2021.' It is not clear which month/

year was considered as the start point.

Response: We thank the reviewer for this comment. For comprehensiveness, we did not restrict our search by date of publication, therefore we did not have a start date for the included studies. The earliest study we included was published in 1978. For clarification, we have now added the following statement in the 'Search strategy and eligibility criteria' section:

"The search was not restricted by language or publication date."

Additionally, we repeated our search on 22nd October 2021 and found one more RCT in Indian children. We have now updated the Abstract, Results section, Figures 1 and 2, References and Supplementary materials to reflect this addition.

'Disagreements between reviewers were resolved through discussion.' Please clarify what the disagreements were on and the content of the discussion to get to a consensus.

Response: The few disagreements were on the methods in some of the older studies. For clarification, we have now revised the sentence under the sub-section 'Study selection, data extraction and quality appraisal' as follows:

"Disagreements between reviewers on study methodologies were resolved through discussion following the pre-determined inclusion and exclusion criteria."

'We used the revised Cochrane risk-of-bias tool for randomised trials...' This statement appears twice in the same paragraph. Consider revising.

Response: Thank you. The Cochrane risk of bias tool for randomised trials is only used to assess risk of bias in individually-randomised, parallel-group trials while the Cochrane risk of bias tool for randomised trials with additional considerations for cluster-randomised trials is an extended version that is only used to assess risk of bias in cluster RCTs. We have now revised the paragraph as follows:

"We used the revised Cochrane risk-of-bias tool for randomised trials (RoB 2) to assess for risk of bias for the individually-randomised, parallel-group RCTs included in the review. RoB 2 assesses five domains of bias including bias from the randomisation process, deviations from intended interventions, missing outcome data, and bias in measurement of the outcome and selection of the reported result. To assess the risk of bias in two cluster RCTs, we used the revised Cochrane risk of bias tool for randomised trials with additional considerations for cluster-randomised trials."

'The large degree of diversity in the study variables necessitated narrative synthesis of the study findings.' Was this applied to all the studies included in the review, or only to those from Africa? Please clarify

Response: We summarized all the studies narratively and performed a secondary meta-analysis for seven studies in non-African countries that used the same tool (the Bayley Scales of Infant Development). We have now clarified that the meta-analysis was a secondary analysis and revised the sentence in the Methods section under 'Synthesis of included studies' as follows:

"The large degree of diversity in the study variables necessitated narrative synthesis of the study findings for all included studies."

We have also revised the Methods section under 'Secondary meta-analysis of seven studies in non-African countries'

"Due to the substantial variation in study methods, we did a secondary meta-analysis in a limited number of RCTs that all used the Bayley Scales of Infant Development (BSID) to assess cognitive and motor development in children living in non-African countries. None of the studies in African children used the BSID."

Results

'...that were not relevant to our study.' I suggest you use 'review' rather than 'study'

Response: We have revised 'study' to 'review'.

The information on the time period of the publications included in the review should be provided earlier, under search strategy

Response: Thank you, per comment 3 above, we have now clarified in the 'Search strategy and eligibility criteria' section that we did not restrict our search by date of publication and therefore we did not have a pre-determined start date for the included studies. Hence, we reported the time period of the included studies in the Results section.

'Of the 34 RCT studies: 25 evaluated the effect of iron supplementation compared to placebo or no treatment; five the effect of iron-fortified foods compared to non-fortified foods; one the effect of fortification of formula milk with high compared to low dosages of iron; one the effect of immediate iron supplementation given concurrently or 28 days after antimalarial treatment on development in children with severe malaria and one the effect of varying and consistent doses of iron supplementation compared to placebo; two the effects of maternal iron supplementation on neurobehavioural outcomes in children after birth.'
For clarity and to avoid repetition, the number of studies under each study description could be presented as Ns e.g. 'Among the 34 RCT studies were those that studied the effect of: iron supplementation compared to placebo or no treatment (N = 25); iron-fortified foods compared to non-fortified foods (N = 5); etc

Response: We have now edited the statement under 'Study characteristics and outcomes' in the Results as follows:

"Among the 35 RCTs were those that studied the effect of: iron supplementation compared to placebo or no treatment (n= 25), iron-fortified foods compared to non-fortified foods (n=5), formula milk fortified with high compared to low dosages of iron (n=1), immediate iron supplementation given concurrently or 28 days after antimalarial treatment on development in children with severe malaria (n=1), varying and consistent doses of iron supplementation compared to placebo (n=1), and maternal iron supplementation on neurobehavioural outcomes in children after birth (n=2)."

Check consistency in the use of Sub-Saharan Africa or sub-Saharan Africa

Response: Thank you. We have now revised 'Sub-Saharan Africa' to 'sub-Saharan Africa' in the sub-section 'Comparison of studies in African countries versus non-African countries, and in the Discussion section for consistency.

"Due to substantial heterogeneity in study methods, only seven (n=775) out of 24 studies that used the Bayley Scales of Infant Development (BSID) to assess cognitive development" - This statement is repeated under the subsection on synthesis.

Response: We have rephrased the sentence in results under the 'Cognitive development' sub-section as follows:

"Secondary meta-analysis of seven studies (n=775) provided limited evidence of beneficial effects of iron supplementation on cognitive development in pre-school children (MD=1.73, 95% CI, -1.05, 4.52) (Extended data, file 5 ²¹)."

It is not clear what number of studies were included to investigate the effect of iron supplementation. Under the subsection on study characteristics, the number is indicated as 25. Later on, under 'cognitive development, the number is indicated as 23. Please clarify.

Response: We thank the reviewer for this comment. Two of the 25 studies did not investigate the effects of iron supplementation on cognitive outcomes and were therefore not included under the section on 'Cognitive development'.

Consider revising the following statement for improved readability as 'children' appears four times

'In school children, one RCT of 119 Indonesian children reported improved IQ among children with IDA at baseline who received iron supplementation for three months compared to children who received placebo.'

Response: We have revised the sentence in the 'Cognitive development' section in results as follows:

"One RCT (n=119) in Indonesia reported improved IQ among school children with IDA who received iron supplementation for three months compared to those who received placebo ⁵⁴."

There is a lot of repetition in the text under the Results section which makes reading the text quite tedious. For instance, under the subsection on 'Educational achievement,' a similar statement appears in the third and sixth lines 'four studies (n=3058) reported no beneficial effects.' Consider revising

Response: Thank you. We have now removed the repeated text under 'Educational achievement' and throughout the Results section more generally and have made the Results more concise to improve readability.

The authors tend to use the word 'studies' several times in single statements and should consider revisions. For example, the first statement under the subsection titled 'Effect of duration..'

Response: We agree with the reviewer that the term 'studies' has been repeated in the Results section and we have removed the repeated text from the Results section. For instance, we have revised the sub-section 'Effect of duration of supplementation or fortification' in the Results as follows:

"In total, seven studies (n=534) investigated the effect of iron supplementation for less than three months and of these studies, five (n=419) reported beneficial effects of iron supplementation on neurobehavioural outcomes, while two (n=115) reported no beneficial effects. Out of 26 studies (n=8972) that evaluated the effect of iron supplementation (n=20) or fortification (n=6) for three months or more, 13 (n=3939) reported beneficial effects of iron supplementation (n=10) or fortification (n=3) on neurobehavioural outcomes and 13 (n=5033) reported no beneficial effects of iron supplementation (n=10) or fortification (n=3)."

The second objective for the review was 'For comparison, we further summarised evidence from randomised controlled trials on the effects of iron supplementation on neurobehavioral outcomes in non-African countries.' My sense is that the comparison does not really come out clearly in the results. The authors should consider providing a summary to provide this comparison, either at the end of each subsection on specific neurobehavioural outcomes or at the end of the Results section, if this fits within the journal guidelines.

Response: Thank you. We included a paragraph titled 'Studies in African countries versus non-African countries' in the Results after 'Study characteristics and outcomes.' We have now edited and moved this paragraph to the end of the Results and changed the title as follows for clarification:

"Comparison of studies in African countries versus non-African countries"

Five RCTs including a total of 1294 children evaluated the effect of iron supplementation or fortification on cognitive (n=4), motor (n=1), behavioural (n=1) or language (n=1) outcomes in African children. Only three out of the five studies were in sub-Saharan Africa and of the three one did not have a placebo group. Out of the five studies, two (n=647) reported beneficial effects on cognitive, motor or language outcomes only in anaemic children ^{26, 27} while three (n=647) reported no beneficial effects in children ^{24, 25, 28}. Of the three studies that reported no beneficial effect of iron supplementation, one included only children with ID ²⁴, one had a prevalence of 21% of anaemia ²⁵ while one did not report baseline iron status ²⁵. Among children living in non-African countries, 30 RCTs including a total of 8694 participants investigated the effect of iron supplementation or fortification on neurobehavioural outcomes. Compared to studies in African children, over half of the 30 studies reported beneficial effects on neurobehavioural outcomes, mostly in children with ID, IDA or anaemia ²⁹⁻⁵⁷."

Additionally, under each neurobehavioural outcome, we discuss African studies first, then studies from non-African countries. We have now added the following information in the methods section under the subsection 'Synthesis of included studies':

"Under each neurobehavioural domain, we first summarized studies in African countries followed by studies in non-African countries."

Discussion

In the first paragraph, there is discussion around the association between iron

supplementation and the heightened risk of malaria infection. This information would fit better under the Introduction as it provides more context around why there are limited iron supplementation studies in Africa

Response: Thank you for this comment. We have now added this information to the last paragraph of the introduction section as follows:

"Despite the high prevalence of both iron deficiency and anaemia, there are few studies investigating the effects of giving iron on neurobehavioural outcomes in African children. This may be due to many factors including concern that iron supplementation may increase the risk of malaria and other infections ¹⁸⁻²⁰."

Competing Interests: No competing interests were disclosed.