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: 1 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...
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 adulthood. 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. 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 Africa. Long-term consequences of impaired child development include low incomes and poor family planning, contributing to the cycle of poverty in LMICs. Iron deficiency may be an important risk factor for impaired brain development in childhood.

Iron deficiency is the most common micronutrient deficiency affecting about two billion individuals globally and accounting for over 40% of all cases of anaemia. 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. A recent study reported a prevalence of 52% for iron deficiency in African children after correcting for inflammation and malaria. 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 development.

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 myelination. 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. 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.

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) guidelines 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 1). 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 review. 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 RCTs.

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 2). 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>
<thead>
<tr>
<th>Author, year (country)</th>
<th>Sample</th>
<th>Baseline iron status</th>
<th>Age at iron supplementation</th>
<th>Age at neuroassessment</th>
<th>Domain (assessment tool)</th>
<th>Intervention (duration)</th>
<th>Definition of iron status</th>
<th>Results</th>
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<tbody>
<tr>
<td>Ssemata, 2020 (Uganda)</td>
<td>N=145 with cerebral malaria (75 received iron concurrently and 70 received iron 28 days after antimalarial treatment)</td>
<td>All children had ID</td>
<td>18-58.8 months</td>
<td>At 3 timepoints: 18 months-4.9 years, 24-64.8 months and 30-70.8 months</td>
<td>Cognitive Executive function Sustained attention Associative memory Socioemotional behaviour (MSEL, ECVT, COAT, CBCL, BRIEF-P, BRS)</td>
<td>Ferrous sulphate 2mg/kg/ day either concurrently with antimalarial treatment or 28 days after receiving antimalarial treatment (3 months)</td>
<td>ID: ZnPP ≥ 80 µmol/mol heme</td>
<td>No difference in neurobehavioural scores between children who received iron supplementation concurrently or 28 days after antimalarial treatment.</td>
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<td>Bouhouch, 2016 (Morocco)</td>
<td>n=455 lead exposed children (110 received iron, 116 received iron+ EDTA, 112 received EDTA, and 117 received placebo)</td>
<td>Anaemia: 21% ID: 32%, 7% or 34% as defined by SF, TIR, or ZnPP</td>
<td>3 to 14 years</td>
<td>At 2 time-points: at baseline (3 to 14 years) and after supplementation.</td>
<td>Cognitive Memory (KABC-II, HVLT)</td>
<td>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)</td>
<td>ID: SF &lt;12 mg/L for children &lt;5 years, SF &lt;15 mg/L for children ≥5 years, or TIR &gt;8.3 mg/L with (CRP ≤5 mg/L, α1-acid glycoprotein ≤51 g/L) Anaemia: Hb≤11.0 g/dL for children &lt;5 years, Hb≤11.5 g/dL for children 5–11 years, Hb ≤12.0 g/dL for children 12 years</td>
<td>No difference in cognitive or memory scores between children who received iron supplementation or placebo.</td>
</tr>
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<td>Author, year (country)</td>
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<td>Baumgartner, 2012 (South Africa)23</td>
<td>n=288 (70 received iron+ placebo, 72 placebo + DHA/EPA, 73 iron+ DHA/EPA, and 73 placebo + placebo)</td>
<td>Anaemia: 20.6-21.1% ID: 6.2% to 16%</td>
<td>6 to 11 years</td>
<td>At 2 time-points: at baseline (6 to 11 years) and after supplementation.</td>
<td>Cognitive Memory (HVLT KABC)</td>
<td>50 mg iron sulphate + DHA/EPA (420/80 mg), 50 mg iron sulphate+ placebo, placebo + DHA/EPA, or placebo + placebo (8.5 months)</td>
<td>ID: SF&lt;15 µg/L excluding children with CRP &gt;5 mg/L or ZnPP &gt;70 µmol/mol or TfR &gt;8.3 mg/L Anaemia: Hb &lt;11.5 g/dL IDA: anaemia + SF&lt;15 µg/L</td>
<td>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.</td>
</tr>
<tr>
<td>Stoltzfus, 2001 (Zanzibar)14</td>
<td>n=359 (183 received iron and 176 placebo)</td>
<td>Anaemia: 97% Severe anaemia: 18%</td>
<td>6 to 59 months</td>
<td>At 2 timepoints: 6 to 59 months and 18 to 71 months</td>
<td>Language Motor (Parents reported motor and language milestones)</td>
<td>20 mg ferrous sulphate or placebo (12 months)</td>
<td>Anaemia: Hb&lt;110 g/l Severe anaemia: Hb&lt;70 g/l ID: SF&lt;12 µg/L</td>
<td>Children who received iron supplementation had improved language scores compared to children who received placebo and children with baseline Hb&lt;90 g/l who received iron supplementation had improved motor scores compared to children who received placebo.</td>
</tr>
<tr>
<td>Boivin, 1993 (Zaire)25</td>
<td>N=47 (17 children received both anthelminthics and iron, 7 only iron, 8 only anthelminthics, and 15 did not receive either intervention)</td>
<td>Not indicated</td>
<td>23 boys (mean age=7.7, SD=0.8 years) and 24 girls (mean age=8.0, SD =1.8 years)</td>
<td>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</td>
<td>Cognitive (KABC)</td>
<td>20 mg iron (4 weeks)</td>
<td>Anaemia: Hb&lt;12 g/dL</td>
<td>No difference in cognitive scores between children who received only iron supplementation or placebo.</td>
</tr>
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</table>

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; ECVT, Early Childhood Vigilance Test; EDTA, ethylenediaminetetraacetic acid; Hb, haemoglobin; HVLT, 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

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**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 18. 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 4).

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 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.

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\(^{22,24}\), while three studies (n=647) found no beneficial effects in children\(^{23,25,26}\). Of the three studies that reported no beneficial effects of iron supplementation, one included only children with iron deficiency\(^{23}\), one study had a prevalence of 21% of anaemia\(^{22}\) while one study did not report baseline iron status\(^{25}\). 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 placebo\(^{27}\). 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\(^{22,26}\). 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 treatment\(^{27}\).

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 5\(^{38}\)). 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 placebo\(^{40}\). 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 weeks\(^{32}\). 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 millet\(^{37}\). 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\(^{14,46,49}\). 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 placebo\(^{33}\). 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 injection\(^{36}\). 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\(^{31}\).

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\(^{34,50}\). 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\(^{41,43,52}\). 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 milk\(^{26}\). 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 IQ\(^{37}\). 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 meals\(^{50}\). Another RCT in 302 Australian mother-child pairs reported no beneficial effects of iron supplementation during pregnancy on IQ at four years\(^{41}\).
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 placebo\(^{34}\).

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 6\(^{16}\)). 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 placebo\(^{32}\). 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\(^{32}\). Additionally, one RCT in 655 6-month old Indonesian children reported improved motor development after iron supplementation for six months\(^{32}\). 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\(^{9,43}\). 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 placebo\(^{39}\). Another RCT in 119 Indonesian children reported improved motor development in children with IDA at baseline after iron supplementation for four months\(^{46}\).

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\(^{34,45}\). 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\(^{31,52}\). 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\(^{16,34}\). 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 development\(^{18}\). 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 treatment\(^{37}\). 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 injection\(^{39}\). Six studies (n=2126) out of seven found no beneficial effects of iron supplementation or fortification on motor development in children living in non-African countries. 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 aged 18 to 58.8 months\(^{34,35,45}\). 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 functioning\(^{34}\). 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 formula\(^{43}\). One RCT in 302 Australian mother-child pairs reported no beneficial effects of iron supplementation during pregnancy on child behaviour at four years\(^{44}\).

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 placebo\(^{49}\). 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\textsuperscript{30,48,51}. 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 weeks\textsuperscript{32}.

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 5). 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 6). 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\textsuperscript{25,33,44,46,47,49}, while five studies (n=1816) reported no beneficial effects\textsuperscript{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\textsuperscript{24,42,50,53} while one study (n=145) found no beneficial effects\textsuperscript{51}. 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 development\textsuperscript{17} while one study of 16 children reported no beneficial effects of iron supplementation on cognitive or motor development\textsuperscript{18}.

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\textsuperscript{28,34,35,39,65} while four studies (n=798) found no beneficial effects of iron supplementation (n=2) or fortification (n=2)\textsuperscript{30,35,38,41}. 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\textsuperscript{23,24,27,32,33,42,46,47,49,50,51,53} while 11 studies (n=4350) reported no beneficial effects of iron supplementation (n=11) or fortification (n=1)\textsuperscript{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 five, studies (n=419) reported beneficial effects of iron supplementation on neurobehavioural outcomes\textsuperscript{33,42,46,50} while two studies (n=115) reported no beneficial effects\textsuperscript{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\textsuperscript{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)\textsuperscript{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 infections\textsuperscript{35}. Of five studies in African children only three included children living in Sub-Saharan Africa, which has the highest prevalence of malaria\textsuperscript{46}, and one of these studies had no placebo arm\textsuperscript{29}. Additionally, only six observational studies have evaluated the associations between iron status and neurobehavioural outcomes in African children and their findings are inconsistent\textsuperscript{57–62}. 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 clearance\textsuperscript{63–65}. 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 limited\textsuperscript{66}.

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. 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 speed. 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. In contrast to epidemiological studies, evidence from animal studies consistently suggests that iron may impact cognitive and language development through its roles in myelinisation, dopamine metabolism and the structure and function of the hippocampus, the centre for memory and learning processes.

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. 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. 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 myelinisation in the corticospinal and corticostriatal tracts, the main pathways for motor signals from the brain to the limbs. Additionally, iron plays an important role in dopamine function in the basal ganglia, an important area in the brain for motor function.

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 functioning. 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. 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 behaviour.

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 children. 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 development. 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. 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. 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 development. 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 children.

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 children.
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 months. 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 years. 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 development. 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 achievement.

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

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

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).


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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 neurobehavioural 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.