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Original Research Article

Effectiveness of intermittent iron and high-dose vitamin A supplementation on cognitive development of school children in southern Ethiopia: a randomized placebo-controlled trial

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A B S T R A C T

Background: Iron is an essential mineral whose deficiency results in cognitive alteration, impaired emotional behaviors, and altered myelination and neurotransmission. In animal models, it has been shown that vitamin A (VA) could affect cognition.

Objectives: The study aimed to evaluate the effectiveness of intermittent iron and VA supplementation on cognitive development of schoolchildren, and to assess the interaction between these supplementations.

Methods: Considering a 2 × 2 factorial design, 504 children were randomly assigned to 1 of the 4 arms: placebo VA and placebo iron supplement; high-dose vitamin VA and placebo iron supplement; iron supplement and placebo VA; and iron and high-dose vitamin VA supplements. Cognitive development was assessed using Raven's Coloured Progressive Matrices, digit span, Tower of London, and visual search tasks.

Results: The mean [± standard deviation (SD)] age of the enrolled children was 9.6 (±1.6) y. One-fifth of the children had iron deficiency or anemia, whereas 2.9%, 3.9%, and 12.1% of children had low iron stores, iron deficiency anemia, and VA deficiency, respectively. Intermittent iron supplementation did not result in any significant improvement of children's cognitive development and had a negative effect on the performance index of the visual search task compared with placebo (−0.17 SD, 95% confidence interval: −0.32, −0.02). Effects were evident among children with stunting, thinness, or children coming from understimulating home environments. High-dose VA supplementation resulted in a significant improvement of digit span z-score with a mean difference of 0.30 SD (95% confidence interval: 0.14, 0.46) compared with placebo VA. VA had a more beneficial impact for girls, children infected with helminths, and those from food secure households.

Conclusion: In a population where the prevalence of iron deficiency is low, intermittent iron supplementation did not have any or negative effect on the child's cognitive development outcomes. Conversely, VA supplementation improved the child's working memory.

Trial registration number: The study is registered at clinicaltrials.gov as NCT04137354 (https://clinicaltrials.gov/study/NCT04137354).

Keywords: cognitive development, iron supplementation, schoolchildren, vitamin A supplementation

Abbreviations: AGP, alpha 1-acid glycoprotein; BMIAZ, BMI-for-age z-score; CRP, C-reactive protein; Fe arm, iron supplement and placebo vitamin A; FeVitA arm, iron supplement and high-dose vitamin A; HAZ, height-for-age z-score; HDSS, Health and Demographic Surveillance System; HEWs, health extension workers; Hb, Hemoglobin; HOME, Home Observation for Measurement of the Environment; IDA, iron deficiency anemia; LMICs, low- and middle-income countries; placebo arm, placebo vitamin A and placebo iron supplement; RDT, rapid diagnostic test; RBP, retinol-binding protein; RCPM, Raven's Coloured Progressive Matrices; SDQ, Strengths and Difficulties Questionnaire; sTfR, soluble transferrin receptor; STH, soil-transmitted helminths; ToL, Tower of London; VAD, vitamin A deficiency; VitA arm, high-dose vitamin A and placebo iron supplement.

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Introduction

In early childhood, cognitive and school outcomes are affected by genetic, biological, and psychosocial factors [1–4]. Malnutrition results in poor cognitive development and academic achievement of school age children, which are exacerbated by poor social and economic environments of the child [1,2]. In 2010, nearly 250 million children under 5 y of age in low- and middle-income countries (LMICs) have not reached their potential cognitive development because of the disadvantageous factors like undernutrition, adverse environmental conditions, and poor social interactions [5]. One in 3 children in LMICs did not reach their cognitive and socioemotional milestones in 2010, with the highest prevalence in sub-Saharan Africa [6].

Iron deficiency is associated with altered homeostasis in myelination and neurotransmission leading to altered cognitive development and impaired emotional behavior [7,8]. Moderate forms of micronutrient deficiencies including iron deficiency anemia (IDA) may remain unnoticed [9,10]. But their long-lasting consequences on health, school performance, and productivity are serious [11]. Iron deficiency and IDA in children may result in developmental delays and behavioral disturbances as evidenced by decreased motor activities, social interaction, and attention to tasks and less physical performance [12–14].

Animal model studies showed that vitamin A can affect cognition. Vitamin A deficiency (VAD) in young and adult mice induces selective memory impairment and central nervous system functional alteration, which might be a factor for cognitive deterioration [15–17]. VAD can impair the immune function, cause anemia, increase morbidities, and cause night blindness, whereas severe VAD may cause xerophthalmia and death [18,19]. Vitamin A improves iron supply to hematopoietic tissue by enhancing the mobilization of iron delivery, and hence increasing plasma iron and transferrin saturation [19–21]. However, the effect of vitamin A on cognitive development in children is less investigated [22].

Deworming and micronutrients supplementation are common interventions targeting schoolchildren to improve their nutrition, health, and development [23]. Use of intermittent iron supplementation is feasible to overcome the limitations of daily iron supplementation including side effects, low adherence, and cost [24–26]. The WHO recommends intermittent iron supplementation for the prevention of anemia and iron deficiency among schoolchildren. The evidence on preventing anemia is strong but several aspects of cognitive development, co-supplementation with other micronutrients, and adverse events especially in the context of malaria were missing [27]. High-dose vitamin A supplementation has proven effective in reducing infections and mortality in children younger than 5 y [28]. Vitamin A supplementation of other age groups including schoolchildren has not been explored to generate sufficient evidence for policy makers. In this analysis, we report on the effectiveness of intermittent iron and semestrial vitamin A supplementation on the cognitive development of schoolchildren. Secondary outcomes included the effect modifications of child's sex, nutritional status, and home environment on cognitive outcomes.

Methods

Study site and period

This interventional study was conducted in Arba Minch Health and Demographic Surveillance System (HDSS), located in Arba Minch Zuria and Gacho Baba districts, of Southern Nations, Nationalities, and Peoples' Region, Southern Ethiopia. The study was conducted between November 2020 and December 2021. It was carried out at public primary schools in the HDSS with children 7–10 y of age. There were 22 public primary schools in the HDSS across 9 kebeles (villages). At the time of recruitment (2020), 11,432 pupils (45.4% girls) enrolled in the academic year 2020–2021.

Study design

In a randomized, placebo-controlled, double-blind 2-by-2 factorial design, eligible children attending the 7 selected public primary schools in the Arba Minch HDSS were individually randomly assigned to receive *1*) placebo vitamin A and placebo iron supplement (placebo arm); *2*) high-dose vitamin A and placebo iron supplement (VitA arm); *3*) iron supplement and placebo vitamin A (Fe arm); and *4*) iron supplement and high-dose vitamin A (FeVitA arm).

Sample size calculation

Based on the findings of Leenstra et al. [29], we hypothesized that there was no interaction effect on hematological outcomes between intermittent iron supplementation and semestrial vitamin A supplementation. The sample size estimate was based on the number of children needed to detect differences in hemoglobin (Hb) between the supplemented and placebo groups with an effect size of 0.30 SD with a significance of $P < 0.05$ and a power of >80%. The effect size was based on the effect size of 0.37 SD calculated from the Cochrane systematic review by De-Regil et al. [26]. Considering a 2 × 2 factorial design, 176 participants were needed to detect the change in the mean of the main outcome between the supplemented and placebo groups after intervention. Anticipating a 30% dropout rate, this sample has been increased to 252 children per intervention arm for the total sample size of 504 pupils.

Subjects

Schoolchildren aged 7–10 y were included in this study if they met the following inclusion criteria: *1*) an informed consent was given at least by 1 parent and the child agreed to participate in the study, *2*) the parents planned to stay in the kebele during the period of the study (full academic year), and *3*) they accepted the intervention package including blood draw and home visits. Children were not included in the study if *1*) they suffered from chronic disease such as diabetes and asthma, or *2*) had night blindness (a form of VAD) or any severe form of VAD. Schoolchildren who were severely anemic (Hb concentration <8.0 g/dL) on the day of enrolment were treated following the conventional national protocol during 3 mo at nearby health centers [30]. Their Hb concentration was reassessed after the treatment period. Children whose Hb concentration >8.0 g/dL were assigned to their intervention arm, if they became eligible as described above (a total of 6 children were referred for treatment of severe anemia; all were eligible after the 3 mo and were included in the study).

Random assignment and blinding

At the start of the study, all 9 kebeles of the Arba Minch HDSS were considered, with their 22 public primary schools. Then, schools that were geographically most centrally located were selected, resulting in 9 schools that were included in the study. Because of the instability of the area in 2020, 2 schools from 2 kebeles declined to take part in the study. Eventually, the survey covered 7 public primary schools from 7 kebeles. The number of participating children per selected school was proportional to the total number of children from grades 1–4 (age 7–10 y).

The school list that included the registration number of the children with no information on their names or sex was used as a sampling frame for the study. Random numbers generated by a computer using Microsoft Excel were given and used to select children from the school list. Afterward, the same codes were subsequently used for the allocation to 1 of the 4 treatment arms. Randomization to 1 of the 4 arms was performed using the color's codes of iron/placebo and vitamin A/placebo as described below. The whole procedure was carried out by the leading author who is also the statistician of the study. Iron and iron-placebo supplements for this study were exclusively produced and donated by Metagenics (https://www.metagenics.eu/en_EU/). Iron and iron-placebo tablets were provided in boxes that were distinguished by the color of their label (blue and green). Iron and iron-placebo tablets had the same taste (strawberry) and shape. They differed slightly in their color, because of the characteristics of the products (one was darker than the other). High-dose vitamin A and placebo vitamin A were prepared by 2 research assistants from Arba Minch University, who did not participate in any other study activities. Briefly, the content of 2 capsules of vitamin A containing each 100,000 IU was transposed in 1 of the color tubes on the day of supplementation (Eppendorf® Safe-Lock microcentrifuge tubes volume 0.6 mL, assorted colors). The same 2 research assistants pipetted the same volume from commercially available corn oil (unfortified, bought from the local market in Belgium) to other colored Eppendorf tubes (red and orange). This operation was carried out in high hygienic conditions, and in dimmed light. High-dose vitamin A capsule and corn oil were similar in consistency, and slightly different in color and taste. Tubes containing vitamin A and vitamin A-placebo were only distinguished by the color of the Eppendorf tube. The color of iron/placebo and vitamin A/placebo were kept by the research assistants. The study investigators, the statistician, the participants, the teachers, the health extension workers (HEWs), and the parents were blinded for iron/iron-placebo and vitamin A/vitamin A-placebo supplements.

Supplements, interventions, and treatments

Forty-two mg of elemental iron (14 mg × 3 chewable tablets) or placebo (3 tablets) were given once weekly for 11 mo at the school, by the respective teachers [27]. Iron/placebo was supplemented from December 2020 to November 2021. The composition of the tablets containing iron (iron tablets, iron in the form of ferrous fumarate) or not (iron-placebo tablets) is provided in Table 1. Vitamin A supplements are those administered to children under 5 y of age, twice yearly as recommended by the WHO [31]. Vitamin A capsule is an oral liquid, oil-based preparation of retinyl palmitate that contains the equivalent of 100,000 IU (110 μmol) of vitamin A and stabilized with 20 IU of vitamin E. The placebo supplement (corn oil) has similar oil composition, with the exception that it does not contain vitamin A. Each child was supplemented with an equivalent of 200,000 IU of vitamin A or a vitamin A-placebo at baseline and after 5 mo provided by HEWs of the respective kebeles.

Teachers and HEWs were trained on how to provide iron and vitamin A supplements to the children according to the randomization list. Iron and iron-placebo supplements were provided as 3 chewable tablets 1 d each week by their teachers. Teachers were briefed on potential side effects and symptoms of discomfort that might be experienced by some of the participating children, and were instructed to record these signs and symptoms using a weekly checklist. During the closing of the schools (intersemestrial break and summer), HDSS workers provided iron and iron-placebo tablets weekly at the child's home. HEWs were responsible for providing vitamin A and vitamin A-placebo supplements at school.

TABLE 1
Composition of iron and placebo tablets[1]

Ingredients	Iron tables (mg)	Placebo tables (mg)
Stevia	0.53	0.53
Sucralose	0.71	0.71
Citric acid monohydrate	3.49	3.49
Silicon dioxide	4.65	4.65
Stearic acid	6.98	6.98
Guar gum	6.98	6.98
Red beet powder	9.30	9.30
Hydroxypropylcellulose	9.42	9.42
Vanilla aroma	20.93	20.93
Xylitol	25.58	25.58
Strawberry aroma	27.91	27.91
Iron	14.00	—
Palatinose	116.37	116.37
Magnesium stearate	4.65	4.65
Maldex 170 FP[2]	—	44.90

[1] All ingredients, except for iron, are excipients.
[2] It is an excipient, needed to fill-in for the volume loss resulting from leaving iron out of the formulation.

Based on the national mapping, Arba Minch Zuria and Gacho Baba districts are considered of moderate risk of soil-transmitted helminth infections [STH; *Ascaris lumbroicoides*, *Trichuris trichiura*, hookworms (*Ancylostoma duodenale*; *Necator americanus*)] [32,33]. To control the STH-attributable morbidity, WHO recommends large-scale deworming programs during which anthelminthic drugs are periodically administered to all schoolchildren [34]. Immediately after baseline data collection, all children were given albendazole (Aldaz 400 mg tablet, Bayberry Pharmaceuticals Pvt Ltd) by HEWs. Additionally, children who were diagnosed positive for *Schistosoma mansoni* or *Taenia* spp, other helminths of medical importance, were treated with praziquantel (40 mg/kg, Leben Laboratories, India Pvt Ltd). The treatment was provided by trained health professionals (BSc in Nursing/Health officer) from the closest health facility. A week posttreatment, vitamin A and vitamin A-placebo supplements were provided by HEWs. Supplementation at the schools of iron and iron-placebo supplements started a week later was done by the school teachers and lasted for 11 mo. Deworming was repeated at mid- and endpoints of the study in children who tested positive for helminths.

Data collection

Five distinct study teams collected data both at the schools and at the homes of the children. First, on the enrollment day at the school, the enrolment team collected baseline data from the child, including sociodemographic, morbidity, and dietary intake using context-adapted questionnaires. The same team performed the physical examination and measured the child's weight and height. After which, the child was handed over to the second team, who collected venous blood and stool samples. Blood samples were used to determine both Hb concentration and the presence of malaria. Stool samples were screened for the presence of helminth infections, including STH, *S. mansoni* and *Taenia* spp. The third team conducted the tests of cognitive development outcomes in a separate school room. The fourth and fifth teams visited the child's home on 2 different days, within 2 wk of enrolment. The fourth team collected additional sociodemographic data about characteristics of the child, their caregiver, and household. The fifth team collected data related to Home Observation for Measurement of the Environment (HOME) score and Strengths and Difficulties

Questionnaire (SDQ) at home. All data were collected digitally and managed using REDCap electronic data capture tools hosted at the Ghent University [35,36], offline using the REDCap Mobile App [37], except for cognitive data which were collected on paper, and then entered on the REDCap database.

Cognitive development outcome assessment

The assessment of cognitive development of children was carried out at baseline and endline, and included the following domains: fluid intelligence (nonverbal intelligence), working memory, planning and problem solving, and attention. Digit span test assesses the working memory of the children [38–41]. For assessing digit span forward, the child repeats the numbers read by the examiner and for digit span backward, the child repeats the numbers in the reverse order of the ones read. The examiner reads the digits out at the rate of 1 number per second. The test ceases when the child fails to accurately repeat 1 sequence length, 3 times, or when the maximal list length is reached (9 digits for forward and 8 digits for backward). The test minimum number of digits is 2. The respective scores of the 2 tests are the maximum number of digits that the child repeated correctly. Total digit span is the sum of digit span forward and backward scores. Tower of London (ToL) is a test used to evaluate the child's planning and problem solving [42,43]. For each ToL's item, the child is presented with "Tower" in a standard configuration (staring pattern) and then asked to rearrange the 3 colored balls on the post to the new configuration corresponding to the pattern presented on the stimulus card, with the prescribed number of moves. The score of the test includes the time to finish each item (solution time) and the number of attempts to reach the solution. The ranges of time score (9, 8, 7, 6, 5, and 0) were generated based on the solution time in seconds (less than or equal to 5, 6–10, 11–20, 21–40, 41–60, and >60, respectively). Then, the item score for the ToL was estimated by deducting the number of failed attempts from the time score of each item. The total score was the summation of the 12 items scores of the ToL [44]. For the ToL task, we only captured the number of failed attempts at baseline data collection. The Raven's Coloured Progressive Matrices (RCPM) test assesses mental ability associated with abstract reasoning, commonly named fluid intelligence [45]. RCPM test has 3 parts (A, AB, and B), consisting of 12 problems each. The questions are formed in a geometric pattern with a section missing. Based on the pattern, the child is asked to choose the missing pattern from the alternatives. Each set has its own score, and the total score is estimated from all sections. The total RCPM (out of 36) is the sum of parts A, AB, and B of the RCPM. Child's attention is assessed with vision search using cancelation task [46,47]. The child was presented with pictures (sketch) of males and females. Some are complete and the others with missing parts. The child is instructed to mark those females with full parts as fast as they can. The examiner recorded the time taken to complete the search, and the number of commissions and omissions. The performance index of the visual search task was estimated using the average of number of omissions, commissions and time to finish according to Geldmacher [Performance = (correct response/total target) × (correct response/total time)] [48].

Blood sample collection and analyses

On the enrolment and at endline, 6 mL of venous blood were drawn from the child by trained medical laboratory technicians, in serum-separating tubes. Blood samples were stored in a cool-box at 8°C (Dometic Cool Freeze CFX 5W) before transportation to the laboratory of the College of Medicine and Health Sciences of Arba Minch University. Serum-separating tubes were centrifuged at 1100 *g* for 10 min at room temperature (Advance Digital Centrifuge, LABLINE EQUIPMENTS Pvt Ltd). Serum was transferred in aliquots of 2 mL (2 mL Eppendorf tubes) and 75 µL (Capcluster tubes, Micronic TPE, Thermoplastic Elastomer). All serum samples were stored at −20°C and transported later on dry ice to the VitMin Lab for analysis of iron, vitamin A, and inflammatory biomarkers. Ferritin, soluble transferrin receptor (sTfR), retinol-binding protein (RBP), and acute phase proteins, including C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP), were analyzed in serum samples by ELISA (DBS-Tech) [49]. The coefficients of variation of the ferritin, sTfR, RBP, CRP, and AGP for a pooled serum sample were 2.25%, 3.59%, 3.61%, 5.84%, and 8.09%, respectively.

Hemoglobin concentration, malaria, and intestinal helminth infections

The remaining venous blood was used to measure Hb concentration using point-of-care Hemocue (Hemocue HB 301, HemoCue® AB) and to test malaria using a rapid diagnostic test (RDT) Histidine-rich protein II (SD BIOLINE Malaria Ag P.F/Pan, Standard Diagnostics, INC.). The RDT was carried out using the standard procedures provided by the manufacturer. Minimum of 10 g of stool sample were collected on the spot from the children, stored in a cool-box at 8°C (Dometic Cool Freeze CFX 5W) and transported to Arba Minch University's laboratory for detecting helminth eggs in duplicate Kato–Katz thick smears [50]. A child was considered infected with helminths, if at least 1 helminth egg (STH, *S. mansoni* and *Taenia* spp.) was detected in 1 of the 2 Kato–Katz thick smears.

Socioeconomic, dietary, and anthropometric measurements

Demographic characteristics of the child, sociodemographic characteristics of the parents, and household characteristics were collected at the house of the child, at baseline. Household asset was collected using the adapted version of Ethiopian Demographic and Health Survey (EDHS, 2016) questionnaire that includes durable asset ownership, access to utilities and infrastructure, and housing characteristics [51]. Household food security status was assessed using the food and nutrition technical assistance's Household Food Insecurity Access Scale [52,53].

Weight was measured in kilograms to the nearest 0.1 kg using a digital floor scale (Seca 876). Children were weighed with light clothes and without shoes. Height was measured to the nearest 0.1 cm using a portable Height-Length Measuring Short-Board (0–200 cm × 0.1 cm WEIGHT AND MEASURE, LLC). Both weight and height were measured in duplicate, and if the difference between the 2 measurements was greater than 0.1 kg for weight and 0.5 cm for height, a third measurement was completed, and the average of the closest 2values was used in the analysis.

Meal frequency, intakes of locally prepared drinks, soft drinks, milk, and food intake outside the home were collected at the school directly from the child. Additionally, at the home of the child, child's dietary intake data during the month preceding the data collection were obtained from mothers/parents using an adapted food frequency questionnaire including 75 food items [54].

The child was asked and observed for their hand washing, the cleanliness of the nails and hands, and shoe wearing practices. Recent illness 2 wk preceding data collection, bed net use, and symptoms of night blindness were checked by asking the child at the school and cross-verified by asking the caregivers during the home visit. The

physical examination of the child includes the eye, thyroid size, and skin. The eye was examined for the sign of paleness, trachoma, and other signs of severe VAD. Skin was assessed for the presence of lesion related to scabies.

HOME inventory score and SDQ

Stimulation of the child around the home environment was measured using the HOME score sheet, at baseline and endline. The components include parents encouragement to the child, praise the child gets, support provided by the parents, sphere of friends and relatives, and environment of work and play [55]. Items are normalized to make their contribution for the generation of the index equal and summed. Child's emotions, concentration, and their behavior with others were assessed by a 25-item SDQ [56]. The SDQ was categorized into 5 domains as follows: hyperactivity, emotional symptoms, conduct problems, peer problems, and prosocial scale with scores ranging between 0 and 10. The prosocial scale is considered separately, and the remaining 4 domains were used to determine the total difficulty score.

Adherence and safety assessment

A checklist was developed with the morbidity and potential side effect of iron supplementation (the presence and frequency of lack of appetite, diarrhea and vomiting, and the presence of fever) and adherence to the iron/placebo supplementation (the color code of the iron supplement, and reasons for not taking the supplement for those who did not take it). These checklists were recorded weekly before the provision of the iron/placebo supplement for the child by the teachers. The HEWs administered vitamin A/placebo supplements to the children at baseline and midline, at the schools. Children stayed for observation; however, there was no checklist on adherence or safety regarding vitamin A/placebo vitamin A supplements.

Data processing and analysis

Z-scores of body mass index-for-age (BMIAZ), and height-for-age (HAZ) were calculated using the 2006 WHO Child Growth Standards and using STATA macros [57]. Stunting and thinness were defined as BMIAZ and HAZ below −2 SD, respectively. All cognitive development outcomes were standardized by the child's school grade. Children were categorized as having low cognitive development, with moderate difficulties, and living in moderately stimulating home environment for the specific test if their cognitive development indices, total difficulty score, and HOME score, respectively, were in the 25th percentiles.

Hb concentrations were adjusted for the altitude of the child's residency based on this formula [Hb = Hb + ((–0.32) × (altitude in meters × 0.0033) + 0.22 × (altitude in meters × $0.0033)^2$)]. Hb concentrations below 11.5 g/dL categorize children as anemic [58,59]. Cutoffs of >5 mg/L for CRP and >1 g/L for AGP were used to define elevated acute phase protein(s). Inflammation phases were defined as: *1*) no inflammation if neither of acute phase proteins was elevated (CRP ≤ 5 mg/L and AGP ≤ 1 g/L), *2*) incubation if CRP was elevated (CRP > 5 mg/L and AGP ≤ 1 g/L), *3*) early convalescence if both CRP and AGP were elevated (CRP > 5 mg/L and AGP > 1 g/L), and *4*) late convalescence when only AGP was elevated (CRP ≤ 5 mg/L and AGP > 1 g/L). Serum ferritin, sTfR, and RBP concentrations were adjusted for inflammation using the ratio between the arithmetic mean of the indicator in the respective inflammation category and its mean in the reference group, that is, with no inflammation [60,61]. Low iron stores, iron deficiency, and VAD were defined based on adjusted serum concentrations in ferritin less than 15 μg/L [62], sTfR more than 8.3 mg/L, and RBP below 0.7 μmol/L, respectively [49]. IDA was defined as the presence of anemia and iron deficiency.

Household Food Insecurity Access Prevalence categorizes households into food secure or food insecure. Food insecure households are those with scores ranging from mild, moderately to severely food insecure [52]. The wealth index was developed based on the household characteristics, asset, and ownership of farm animals using principal component analysis [63]. Food ingredients were assigned to 10 food groups to estimate the dietary diversity score of the children, which was categorized as adequate if the child consumed 5 and more food groups per day [64]. Meal frequency was based on the report of the child for the previous 24-h recall. Those children who reportedly had illness 2 wk preceding the data collection were considered with illness. Good hygienic practices were defined if the child had clean hands, and trimmed and clean nails. Moderate practices were defined if the child's hands seemed washed recently and nails were trimmed but not clean, or not trimmed but clean. Poor hygienic practices were considered if the child's hands were not clean, and their nails were neither trimmed nor clean.

We used the Student's *t*-test and Chi-square test to compare the baseline characteristics and cognitive scores between 2 groups: those who provided data at the endpoint and were included in this analysis, and those who dropped out. Intervention effects on cognitive outcomes were estimated using mixed-effects model. We considered the schools as random effects. Furthermore, the models were adjusted for respective baseline value of the cognitive development outcome, in addition to the baseline variables of the child, caregiver, and household that were significantly associated at the $P < 0.2$ level with the outcome in correlation analysis. These variables were child's sex and age, adherence to the supplement, household characteristics (wealth score, caregiver's education, occupation, and age, household head educational status if different from caregiver, household food security status, prosocial scale, total difficulty score, and HOME score), baseline nutritional status (Hb concentration, HAZ, and BMIAZ), and helminth infection. In the absence of any interaction between the effects of iron and vitamin A, the effect of the intervention (iron and vitamin A) on cognitive outcomes was performed using a factorial approach. We assessed the effects of iron supplementation and high-dose vitamin A supplementation separately considering the marginal analysis. The cognitive development outcomes were analyzed in children who were assessed at both baseline and endline.

Data analysis was performed using Stata Version 14 (StataCorp). Normality of residuals were tested using Shapiro–Wilk W test for normality and the *P* values were >0.05. For nonnormal distributed residuals, dependent variables were checked again for the normality of the transformed values. Digit span forward, performance index of visual search task, RCPM (part A, AB, B, and total), and ToL outcomes were transformed following a 2-step approach [65]. Briefly, the 2-step method encompasses, in step 1, transforming the variable into a percentile rank to achieve uniform probabilities. Then, in step 2, an inverse-normal transformation is applied to yield a normally distributed variable with z-scores. Collinearity was checked for independent variables by estimating variable inflation factor. Variables resulting variable inflation factor more than 5 were checked and dropped. Homoscedasticity of residuals were checked using Breusch–Pagan/Cook–Weisberg test. We did not adjust the analyses of cognitive development outcomes for multiple comparisons.

In secondary analysis, child's sex, nutritional status (stunting, thinness, anemia, iron deficiency, and VAD), helminth infections, and

low cognitive development for respective outcome, more than moderate behavioral difficulties, less than moderately stimulating home environment and food insecurity of the household at baseline were tested as possible effect modifiers of treatment effects. These variables were selected based on literature [66–69]. However, we did not consider baseline low iron stores (adjusted serum ferritin <15 μg/L) as a potential effect modifier because of the small size. Interaction between the intervention and potential effect modifiers was tested using mixed-effects model and the cutoff P value < 0.10 was considered to define a significant interaction. Secondary analyses were not adjusted for multiple comparisons. They were intended to give insights into the patterns and hypotheses on potential target groups.

Ethical considerations

Ethical clearance was obtained from the Commission on Medical Ethics of Ghent University (EC/2019/1289) and National Research Ethics Review Committee of Ministry of Science and Higher Education Ethiopia (P.S.M/14.1/505/20). Subsequently, it has been registered at clinicaltrials.gov (reference number: NCT04137354). Formal letters were submitted to the district administration, district health and education office, kebele's offices, and schools to get their permission to conduct the study. The directors of the selected schools were contacted for authorization. Sensitization workshops were organized to explain the study objectives and methods to the school directors, the teachers and the parents. Children were enrolled only if their parents provided an informed written consent and children agreed to participate in the study.

Results

Characteristics of participants included in the analysis and those who dropped out

Of the 504 children enrolled, 84.1% (n = 424) participated in the endline assessment and 83.7% (n = 422) provided data on cognitive development. At midpoint, 88.7% (n = 447) of the children participated in the data collection, whereas vitamin A/placebo supplement was provided to 460 children (91.3%). More children completed the study in the VitA arm (88.1%), whereas the least children were observed in the Fe arm (81.0%) (Figure 1). Baseline characteristics between children included in the cognitive outcomes analysis and those who dropped out showed no significant differences. Additionally, there were no significant differences in baseline performance index, RCPM scores, and the total number of failed attempts of ToL tasks between the completers and the dropouts. However, children who dropped out exhibited a significantly lower total digit span (t = −2.29, P = 0.023), higher total difficulty score (t = 2.04, P = 0.042), and lower prosocial scale (t = −3.03, P = 0.003) when compared with those who were included in this analysis (Table 2).

Characteristics of the schoolchildren and their household

None of the children were in the incubation phase for inflammation, whereas 6.8% and 51.6% were in early and late convalescence phases, respectively. Slightly more than half of the children (50.6%) were boys, and the mean (±SD) age was 9.6 (±1.6) y at baseline. Nearly 20% of the children had iron deficiency (adjusted sTfR concentrations >8.3 mg/L) and 3% had low iron stores (adjusted serum ferritin concentrations <15 μg/L). IDA was present in 4% of the children, and VAD was found in 12% of the children. The prevalence of stunting was 21.8%, whereas 8.3% of the children had BMIAZ <−2 SD. The mean (±SD) dietary diversity score of the children for a day was 3.6 (± 1.5) out of 10 food groups and dietary diversity was adequate in 20.1% of the children. On average, iron was supplemented for 38.2 wk (from the total of 48 wk) and 68.9% of the children had at least 80% of iron/placebo supplement doses. Around half (52.4%) of the children experienced at least one of the symptoms, including lack of appetite, diarrhea and vomiting, and fever, throughout the course of supplementation. The overall prevalence of helminth infection was 54.6% (n = 275) (Table 3). The mean (±SD) age of the caregiver and head of the household were 38.3 (±9.2) and 43.4 (±9.6) y, respectively. Agriculture was the main activity in 41.1% of the households. Around half (47.8%) of the caregivers and 45.6% of the household's heads were illiterate. Half (51.2%) of the households were food insecure (Table 4).

Effects of intermittent iron supplementation and high-dose vitamin A on cognitive development outcomes

The interaction effects of intermittent iron supplementation and semestrial vitamin A supplementation were not significant on standardized total digit span (P = 0.895), performance index of visual search task (P = 0.240), RCPM total (P = 0.665), and ToL (P = 0.940). We were able to conduct a factorial analysis to examine the effects of iron and vitamin A supplementations on cognitive outcomes. Iron supplementation once weekly for 11 mo did not have any effect on cognitive development assessed by the digit span (forward, backward, and total), RCPM (Part A, AB, B, and total), and ToL. However, intermittent iron supplementation of schoolchildren had a significant negative effect on the performance index of visual search task (−0.17, 95% CI: −0.32, −0.02; P = 0.029) (Table 5). Semestrial vitamin A supplementation of schoolchildren resulted in a significant improvement in digit span forward, digit span backward, and total digit span with mean differences of 0.19 SD (95% CI: 0.01, 0.36; P = 0.035), 0.28 SD (95% CI: 0.11, 0.45; P = 0.001), and 0.30 SD (95% CI: 0.14, 0.46; P < 0.001), respectively. No effects on other cognitive outcomes were found (Table 6).

Analysis of potential modifiers of iron supplementation effects on cognitive outcomes

Iron supplementation improved digit span by 0.39 SD (95% CI: 0.05, 0.74) among children with stunting (HAZ <−2 SD) whereas no effect was found among children without stunting (Supplemental Table 1). None of the tested variables modified the effects of iron supplementation on the performance index of the visual search task (Supplemental Table 2).

Baseline iron status and thinness had interactions with iron supplementation for RCPM. Schoolchildren with iron deficiency had a lower standardized RCPM score (−0.49 SD; 95% CI: −0.95, −0.04) after iron supplementation compared with children with iron deficiency who received placebo. RCPM improved by 0.81 SD (95% CI: 0.11, 1.52) in children with thinness who received iron supplements compared with those who received placebo (Supplemental Table 3). Baseline anemia, behavioral difficulties, and HOME score had interactions with iron supplementation for the ToL task. The effect of intermittent iron supplementation was higher on the ToL task of children from understimulating home environments compared with placebo (0.44 SD; 95% CI: 0.09, 0.80) (Supplemental Table 4).

Analysis of potential modifiers of vitamin A supplementation effects on cognitive outcomes

Child's sex, and baseline anemia, vitamin A status, thinness, helminth infections, HOME score, and food security status of the household

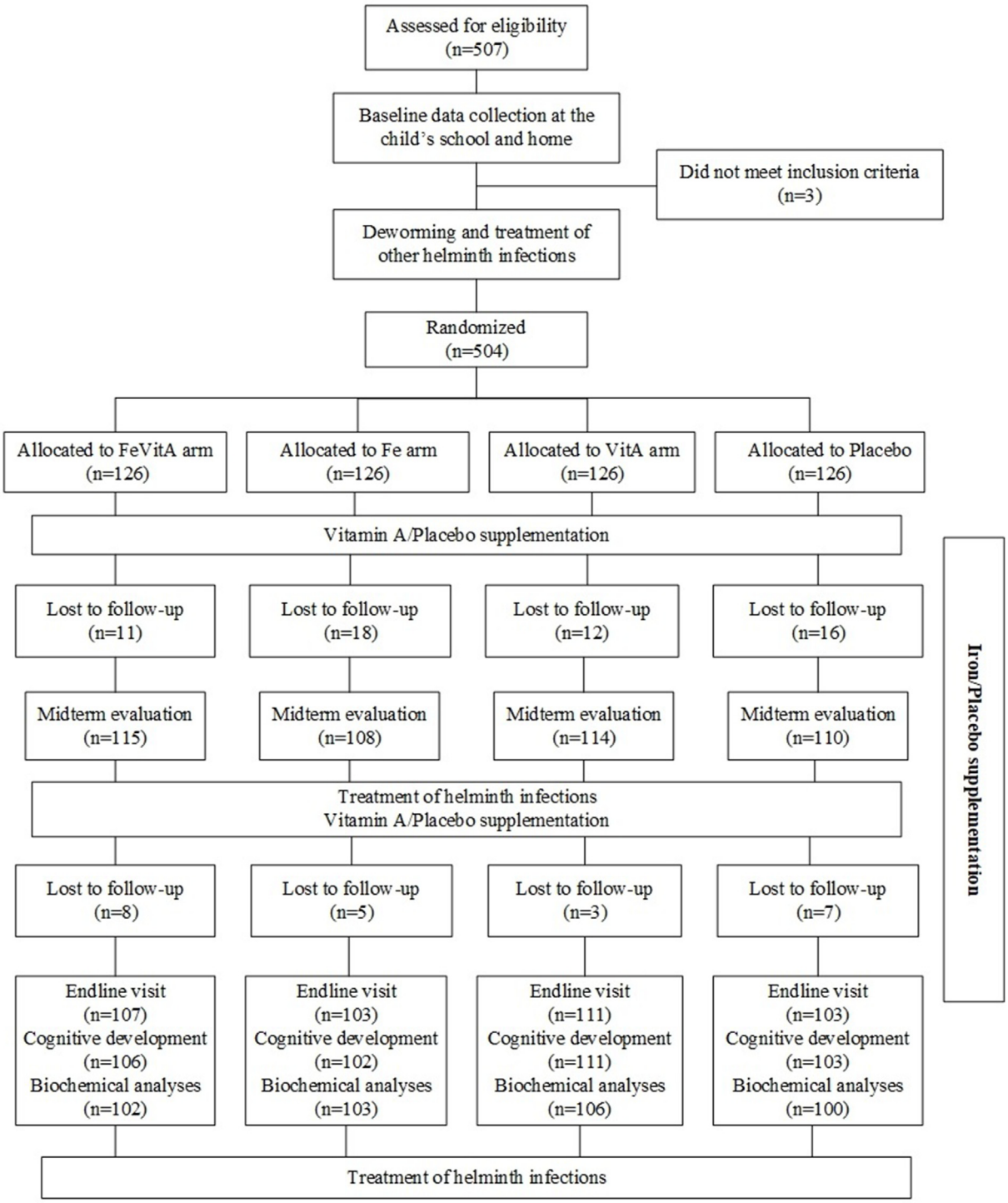

Figure 1. Participants flowchart. FeVitA arm: iron supplement and high-dose vitamin A supplement; Fe arm: iron supplement and placebo vitamin A; VitA arm: high-dose vitamin A and placebo iron supplement; Placebo: placebo vitamin A and placebo iron supplement.

modified the effect of vitamin A supplementation on digit span (total). Schoolgirls who received vitamin A showed significant improvement in digit span z-score (0.49 SD; 95% CI: 0.27, 0.72) compared with girls who received placebo. The effect of vitamin A supplementation on digit span z-score was significant among children infected with at least 1 of the helminths (0.44 SD; 95% CI: 0.22, 0.67) compared with those children infected with helminth who received placebo. Digit span z-score was significantly higher among children without anemia who received vitamin A compared with children without anemia who received placebo (0.37 SD; 95% CI: 0.19, 0.55). Similarly, vitamin A supplemented schoolchildren who had healthy- to overweight (BMIAZ >-2 SD) had higher digit span z-score compared with those who received placebo (0.36 SD; 95% CI: 0.19, 0.52). Likewise, the effect of vitamin A supplementation on digit span was significantly higher among VAD (0.94 SD; 95% CI: 0.46, 1.42) compared with children whose vitamin A status was sufficient (0.22 SD; 95% CI: 0.05, 0.40). Vitamin A supplementation improved the digit span of schoolchildren from food secure households compared with those who received placebo vitamin A (0.48

TABLE 2
Comparison of baseline characteristics and cognitive development scores between participants included in the analysis and dropouts ($N = 504$)

Characteristics	Dropouts	Completers	P value[1]
Number of children (%)	82 (16.27)	422 (83.73)	
Child's sex (%)			
Male	48 (18.82)	207 (81.18)	0.116
Female	34 (13.65)	215 (86.35)	
At least one helminth infection (%)			
No	34 (14.85)	195 (85.15)	0.430
Yes	48 (17.45)	227 (82.55)	
Child's age [mean (SD)]	9.60 (1.63)	9.57 (1.56)	0.861
HAZ [mean (SD)]	−0.92 (1.51)	−1.08 (1.23)	0.300
BMIAZ [mean (SD)]	−0.74 (0.89)	−0.75 (0.85)	0.861
Hb concentration in g/dL [mean (SD)]	12.51 (1.34)	12.45 (1.35)	0.729
Wealth score [mean (SD)]	−0.36 (2.84)	0.05 (2.47)	0.180
Food secure (%)			
No	48 (18.60)	210 (81.40)	0.157
Yes	34 (13.93)	210 (86.07)	
Caregivers' educational status (%)			
Illiterate	42 (17.43)	199 (82.57)	0.721
1–8	32 (15.76)	171 (84.24)	
9 and above	8 (13.33)	52 (86.67)	
Household head educational status (%)			
Illiterate	34 (14.78)	196 (85.22)	0.639
1–8	32 (16.84)	158 (83.16)	
9 and above	16 (19.05)	68 (80.95)	
Caregiver occupation (%)			
Agricultural	35 (19.91)	172 (83.09)	0.785
Nonagricultural	18 (17.65)	84 (82.35)	
Not employed	29 (14.87)	166 (85.13)	
HOME score [mean (SD)]	12.55 (4.66)	13.09 (4.33)	0.308
Total difficulties score [mean (SD)]	10.55 (6.43)	9.14 (5.56)	0.042
Prosocial scale [mean (SD)]	6.91 (2.47)	7.71 (2.12)	0.003
Digit span Forward [mean (SD)]	−0.17 (0.94)	0.03 (1.01)	0.086
Digit span Backward [mean (SD)]	−0.19 (1.01)	0.03 (0.99)	0.060
Digit span (total) [mean (SD)]	−0.23 (0.93)	0.04 (1.00)	0.023
Performance index [mean (SD)]	−0.11 (0.94)	0.02 (1.01)	0.288
RCPM part A [mean (SD)]	0.07 (0.98)	−0.01 (1.00)	0.485
RCPM part AB [mean (SD)]	−0.07 (1.03)	0.01 (0.99)	0.514
RCPM part B [mean (SD)]	0.02 (0.98)	−0.004 (1.00)	0.865
RCPM total [mean (SD)]	0.02 (0.99)	−0.002 (1.00)	0.889
Failed attempts for the Tower of London [mean (SD)]	−0.03 (0.98)	0.004 (1.00)	0.797

Abbreviations: BMIAZ, body mass index-for-age z-score; HAZ, height-for-age z-score; Hb, hemoglobin; HOME, Home Observation for Measurement of the Environment; RCPM, Raven's Coloured Progressive Matrices.
[1] Student's *t*-test and Chi-square test were used to compare continuous and categorical variables, respectively.

SD; 95% CI: 0.25, 0.71). Contrarily, vitamin A supplementation did not affect digit span of boys, children without helminths, children with anemia, children with thinness, and children from food insecure households. The effect of vitamin A supplementation on children who were living in less stimulating home environments regarding their digit span z-score was higher (0.56 SD; 95% CI: 0.23, 0.89) compared with those who live in stimulating environments (0.22 SD; 95% CI: 0.03, 0.40) (Supplemental Table 5).

Baseline iron and vitamin A status, and HOME score modified the effect of vitamin A supplementation on the performance index of the visual search task. Vitamin A supplementation significantly increased the performance index of VAD children compared with placebo (0.75 SD; 95% CI: 0.31, 1.19). Similarly, the effect of vitamin A supplementation was greater on the performance index of children from understimulating home environment compared with placebo (0.36 SD; 95% CI: 0.05, 0.66) (Supplemental Table 6).

Finally, baseline vitamin A status and thinness significantly modified the effect of vitamin A supplementation on RCPM z-scores. Vitamin A supplemented children with VAD (0.78 SD (95% CI: 0.22, 1.34) and with healthy- to overweight (0.22 SD (95% CI: 0.02, 0.42) had improved RCPM z-scores compared with placebo. On the contrary, vitamin A supplementation decreased RCPM z-scores in children with thinness compared with those who received placebo (−0.90 SD; 95% CI: −1.59, −0.20). Children whose vitamin A status was sufficient did not benefit from vitamin A supplementation (Supplemental Table 7). None of the tested variables modified the effect of vitamin A supplementation on ToL task z-scores (Supplemental Table 8).

Discussion

Weekly iron supplementation for 11 mo had no effects on working memory, nonverbal intelligence, and planning and problem solving of

TABLE 3
Baseline characteristics of the schoolchildren aged 7–10 y by intervention arm ($N = 504$)

Characteristic	FeVitA arm	Fe arm	VitA arm	Placebo arm	Total
Total (*N*)	126	126	126	126	504
Sex (male, %)	49.2	46.8	52.4	54.0	50.6
Age in year [mean (SD)]	9.7 (1.7)	9.6 (1.7)	9.5 (1.5)	9.5 (1.4)	9.6 (1.6)
School grade of the child (%)					
1	27.8	31.0	27.8	23.8	27.6
2	38.1	35.7	44.4	36.5	38.7
3	23.0	20.6	19.8	25.4	22.2
4	11.1	12.7	7.9	14.3	11.5
Dietary diversity [mean (SD)]	3.6 (1.5)	3.7 (1.5)	3.7 (1.5)	3.6 (1.5)	3.6 (1.5)
Child hygienic practice (%)					
Good	38.9	46.0	43.6	38.1	41.7
Moderate	31.7	29.4	28.6	34.9	31.1
Poor	29.4	24.6	27.8	27.0	27.2
Inflammation phases (%)[1]					
No inflammation	42.2	42.7	39.6	42.2	41.7
Early convalescence	8.8	7.8	4.7	5.9	6.8
Late convalescence	49	49.5	55.7	52.0	51.6
Recent history of illness (%)	13.5	13.5	11.9	11.9	12.7
HAZ [mean (SD)]	−1.1 (1.3)	−1.1 (1.4)	−1.0 (1.3)	−1.1 (1.1)	−1.1 (1.3)
BMIAZ [mean (SD)]	−0.7 (0.8)	−0. 8 (0.9)	−0.7 (0.9)	−0.8 (0.7)	−0.8 (0.9)
Hb concentration in g/dL [mean (SD)]	12.4(1.3)	12. 5 (1.2)	12.6 (1.2)	12.4 (1.6)	12.5 (1.4)
Stunted (HAZ <−2SD) (%)	21.4	27.8	21.4	16.7	21.8
Thin (BMIAZ < −2SD) (%)	5.6	11.9	11.1	4.8	8.3
Anemic (Hb <11.5 g/dL) (%)	17.5	20.6	15.9	23.8	19.4
Low iron stores (ferritin <15 μg/L) (%)[1]	2.9	1.9	0.9	5.9	2.9
Iron deficiency (sTfR >8.3 mg/L) (%)[1]	18.6	17.5	19.8	21.6	19.4
IDA (%)[1]	2.0	3.9	2.8	6.9	3.9
VAD (RBP <0.7 μg/L) (%)[1]	11.8	9.7	13.2	13.7	12.1
Weeks of iron supplementation [mean (SD)]	38.5 (9.1)	36.9 (11.4)	39.6 (7.6)	37.9 (10.7)	38.2 (9.8)
At least one episode reported (%)					
Lack of appetite	29.4	31.0	34.1	29.4	83.0
Diarrhea	19.0	22.2	23.0	24.6	22.2
Vomiting	11.9	13.5	18.3	17.5	15.3
Fever	34.1	31.7	27.0	33.3	31.6
Helminth infection (%)					
Ascaris lumbricoides	21.4	18.3	20.6	15.9	19.1
Trichuris trichiura	15.1	11.1	15.9	13.5	13.9
Hookworms	13.5	6.4	8.7	6.4	8.7
Schistosoma mansoni	29.4	23.8	27.0	26.2	26.6
Other helminths[2]	8.7	7.1	10.3	7.1	8.3
Total infection	60.3	48.4	57.1	52.4	54.6

Abbreviations: BMIAZ, body mass index-for-age z-score; HAZ, height-for-age z-score; Hb, hemoglobin; IDA, iron deficiency anemia; RBP, retinol-binding protein; sTfR, serum transferrin receptor; VAD, vitamin A deficiency.
No inflammation: CRP ≤5 mg/L and AGP ≤1 g/L; incubation phase: CRP >5 mg/L and AGP ≤1 g/L; early convalescence: CRP >5 mg/L and AGP >1 g/L; late convalescence: CRP ≤5 mg/L and AGP >1 g/L.
[1] The number of participants, $n = 422$.
[2] Other infections include *E. vermicularis*, *H. nana*, and *Taenia* spp.

schoolchildren 7–10 y of age in Southern Ethiopia. Contrarily, iron supplementation had a negative effect on the attention and concentration domain of schoolchildren in Southern Ethiopia. Iron is required for proper myelination and metabolism of the neurotransmitters [8,70]. Our results contradict other supplementation studies showing that iron supplementation improved the intelligence [71–75], attention and concentration [72,73,75–77], and memory [72,73,78] of schoolchildren. Furthermore, there were no other studies that documented an adverse effect on the attention and concentration of schoolchildren as a result of iron supplementation. However, our results are in line with other studies that have reported no significant improvement of intelligence [75,76,78–82] and memory [71] of schoolchildren after iron supplementation. Another relatively recent systematic review and meta-analysis examined the effect of oral iron supplementation. It found no evidence supporting the effect of intermittent iron supplementation on the intelligence, memory, and attention and concentration of schoolchildren [83].

Several factors could affect the effectiveness of iron supplementation on cognitive development outcomes including frequency, dose and duration of iron supplementation, co-supplementation with folic acid [75], vitamin B12 or vitamin C, child's demographic and nutritional characteristics, child's morbidity, and home environment. Twice weekly iron-folate supplementation improved significantly all cognitive domains of schoolgirls. However, once weekly iron supplementation improved the schoolgirls' Maze Test and Visual Memory test scores [75]. Increased homocysteine concentration and reduction in the synthesis of S-adenosylmethionine, which are related to folic acid deficiency, are associated with poor cognitive performance [84–86]. The lack of the effect of weekly iron supplementation on cognitive outcomes in our study could be because of the very low prevalence of low iron stores and IDA in our

TABLE 4
Household and caregivers baseline characteristics by intervention arm ($N = 504$)

Characteristic	FeVitA arm	Fe arm	VitA arm	Placebo arm	Total
Total (*N*)	126	126	126	126	504
Wealth score [mean (SD)]	0.06 (2.44)	−0.04 (2.63)	−0.06 (2.48)	−0.02 (2.61)	−0.01 (2.54)
Caregivers' educational status (%)					
Illiterate	48.41	46.03	49.21	47.62	47.82
1–8	42.06	40.48	38.1	40.48	40.28
9 and above	9.52	13.49	12.7	11.9	11.9
Household head educational status (%)					
Illiterate	41.27	47.62	47.62	46.03	45.63
1–8	42.06	36.51	35.71	36.51	37.7
9 and above	16.67	15.87	16.67	17.46	16.67
Caregiver occupation (%)					
Agricultural	39.68	38.89	38.89	46.83	41.07
Nonagricultural	20.63	23.02	22.22	15.08	20.24
Not employed	39.68	38.1	38.89	38.1	38.69
Caregivers' age [mean (SD)]	37.93 (8.59)	37.45 (8.56)	38.99 (10.12)	38.79 (9.61)	38.29 (9.24)
Household heads' age [mean (SD)]	43.2 (8.94)	42.64 (8.66)	44.45 (10.98)	43.45 (9.83)	43.44 (9.64)
Food security status (%)					
Secure	40.48	58.73	42.86	53.17	48.81
Mildly insecure	6.35	7.14	4.76	5.56	5.95
Moderately insecure	46.03	26.19	44.44	29.37	36.51
Severely insecure	7.14	7.94	7.94	11.9	8.73
HOME score [mean (SD)]	12.67 (4.07)	13.15 (4.47)	12.85 (4.62)	13.33 (4.39)	13 (4.39)
Total difficulties score [mean (SD)]	10.05 (6.19)	8.74 (4.95)	9.44 (5.9)	9.25 (5.8)	9.37 (5.73)
Prosocial scale [mean (SD)]	7.68 (2.28)	7.63 (2.18)	7.43 (2.21)	7.59 (2.15)	7.58 (2.2)

Abbreviation: HOME, Home Observation for Measurement of the Environment.

study population [82,87–89]. The negative effect of iron supplementation on the attention and concentration of the children might be the result of excess iron intake in situations where iron status (expressed in iron stores) and IDA were not a concern. Excess iron can cause oxidative stress and impacts the functionality of the cognition of the children [90,91], which was supported by studies based on randomized controlled trials [92–94]. Our report showed that the baseline low iron stores and IDA among the study participants were less than 3% and 4%, respectively. Similar to this finding, a randomized, placebo-controlled trial among schoolchildren in southern Thailand indicated that daily and weekly iron supplementation was not superior in improving the cognition of the children where the study population had a low prevalence of IDA (~6%) [82,87].

Intermittent iron supplementation improved the working memory among children with stunting, the nonverbal intelligence among children with thinness, and the planning and problem solving among children from

TABLE 5
Effect of school-based intermittent iron supplementation on schoolchildren cognitive development ($N = 422$)

Outcome	Control Mean (SE)	Intervention Mean (SE)	Unadjusted difference (95% CI)	Adjusted difference[1] (95% CI)
Digit span forward[2]	−0.19 (0.11)	−0.15 (0.11)	0.04 (−0.16, 0.23)	0.09 (−0.09, 0.26)
Digit span backward[3]	0.24 (0.08)	0.16 (0.08)	−0.07 (−0.26, 0.11)	−0.13 (−0.30, 0.04)
Digit span (total)[4]	0.17 (0.10)	0.14 (0.10)	−0.03 (−0.22, 0.15)	−0.05 (−0.21, 0.11)
Performance index[5]	0.63 (0.08)	0.53 (0.08)	−0.10 (−0.26, 0.06)	−0.17 (−0.32, −0.02)
RCPM part A[6]	0.12 (0.09)	0.13 (0.09)	0.02 (−0.17, 0.20)	−3.6E-04 (−0.18, 0.18)
RCPM part AB[7]	0.27 (0.09)	0.22 (0.09)	−0.05 (−0.24, 0.15)	−0.07 (−0.26, 0.12)
RCPM part B[8]	0.20 (0.11)	0.19 (0.11)	−0.01 (−0.21, 0.20)	−0.03 (−0.23, 0.18)
RCPM total[9]	0.30 (0.10)	0.29 (0.10)	−0.006(−0.21, 0.20)	−0.04 (−0.23, 0.16)
Tower of London[10]	0.004 (0.10)	−0.009 (0.10)	−0.005 (−0.20, 0.19)	−0.004 (−0.17, 0.18)

Unadjusted and adjusted differences (95% CI) were estimated and tested using mixed-effects model with school as a random effect.
Abbreviations: HOME, Home Observation for Measurement of the Environment; RCPM, Raven's Coloured Progressive Matrices.
[1]The cognitive outcomes were adjusted for the following respective covariates: baseline cognitive development[2–10], child sex (male/female)[2,6–9] and age (years)[1], baseline height-for-age z-score[2–7,9,10], BMI for age z-score[2], Hb concentration (g/dL)[2,4,5], helminth infection (Yes/No)[5] (*Ascaris lumbricoides*[2–4,8,9], *Trichuris trichiura*, hookworm and *Schistosoma mansoni*[2,7] infections) of the child, weeks of iron/placebo supplementation[10], educational status of the caregivers (Illiterate/1–8/9 and above)[3–7,9,10], occupation of the caregivers (Agricultural/Nonagricultural/Unemployed)[2,5–9], educational status of the head of the household (Illiterate/1–8/9 and above)[2–10], wealth index[2–4,6,7,9,10], food security (Secure/Insecure)[5], prosocial scale[2,5,10], total difficulties score[3,4,10], and HOME score[3,4,6,10].-
Unadjusted and adjusted differences (95% CI) were estimated and tested using mixed-effects model with school as a random effect.
Abbreviations: HOME, Home Observation for Measurement of the Environment; RCPM, Raven's Coloured Progressive Matrices.
[1]The cognitive outcomes were adjusted for the following respective covariates: baseline cognitive development[2–10], child sex (male and female)[2,6–9] and age (years)[2], baseline height-for-age z-score[2–7,9,10], BMI for age z-score[2], Hb concentration (g/dL)[2,4,5], helminth infection (Yes/No)[5] (*Ascaris lumbricoides*[2-4,8,9], *Trichuris trichiura*, hookworm and *Schistosoma mansoni*[2,7] infections) of the child, weeks of iron/placebo supplementation[10], educational status of the caregivers (illiterate/1–8/9 and above)[3–7,9,10], occupation of the caregivers (Agriculture/Nonagriculture/Unemployed)[2,5–9], educational status of the head of the household (illiterate/1–8/9 and above)[2–10], wealth index[2–4,6,7,9,10], food security (Secure/Insecure)[5], prosocial scale[2,5,10], total difficulties score[3,4,10], and HOME score[3,4,6,10].

TABLE 6
Effect of school-based vitamin A supplementation on schoolchildren cognitive development ($N = 422$)

Outcome	Control Mean (SE)	Intervention Mean (SE)	Unadjusted difference (95% CI)	Adjusted difference[1] (95% CI)
Digit span forward[2]	−0.27 (0.11)	−0.08 (0.10)	0.19 (0.002, 0.39)	0.19 (0.01, 0.36)
Digit span backward[3]	0.05 (0.08)	0.35 (0.08)	0.29 (0.11, 0.48)	0.28 (0.11, 0.45)
Digit span (total)[4]	−0.002 (0.10)	0.31 (0.10)	0.31 (0.12, 0.49)	0.30 (0.14, 0.46)
Performance index[5]	0.52 (0.08)	0.65 (0.08)	0.13 (−0.03, 0.29)	0.11 (−0.05, 0.26)
RCPM part A[6]	0.06 (0.09)	0.19 (0.09)	0.13 (−0.06, 0.31)	0.15 (−0.03, 0.34)
RCPM part AB[7]	0.22 (0.09)	0.27 (0.09)	0.05 (−0.14, 0.25)	0.03 (−0.16, 0.22)
RCPM part B[8]	0.16 (0.11)	0.23 (0.10)	0.07 (−0.15, 0.28)	0.04 (−0.16, 0.25)
RCPM total[9]	0.23 (0.10)	0.36 (0.10)	0.13 (−0.081, 0.33)	0.13 (−0.06, 0.33)
Tower of London[10]	−0.05 (0.09)	0.04 (0.09)	0.09 (−0.10, 0.28)	0.07 (−0.10, 0.25)

Unadjusted and adjusted differences (95% CI) were estimated and tested using mixed-effects model with school as a random effect.
Abbreviations: HOME, Home Observation for Measurement of the Environment; RCPM, Raven's Coloured Progressive Matrices.
[1]The cognitive outcomes were adjusted for the following respective covariates: baseline cognitive development[2–10], child sex (male/female)[2,6–9] and age (years)[1], baseline height-for-age z-score[2–7,9,10], BMI for age z-score[2], Hb concentration (g/dL)[2,4,5], helminth infection (Yes/No)[5] (*Ascaris lumbricoides*[2–4,8,9], *Trichuris trichiura*, hookworm and *Schistosoma mansoni*[2,7] infections) of the child, weeks of iron/placebo supplementation[10], educational status of the caregivers (Illiterate/1–8/9 and above)[3–7,9,10], occupation of the caregivers (Agricultural/Nonagricultural/Unemployed)[2,5–9], educational status of the head of the household (Illiterate/1–8/9 and above)[2–10], wealth index[2–4,6,7,9,10], food security (Secure/Insecure)[5], prosocial scale[2,5,10], total difficulties score[3,4,10], and HOME score[3,4,6,10].-
Unadjusted and adjusted differences (95% CI) were estimated and tested using mixed-effects model with school as a random effect.
Abbreviations: HOME, Home Observation for Measurement of the Environment; RCPM, Raven's Coloured Progressive Matrices.
[1]The cognitive outcomes were adjusted for the following respective covariates: baseline cognitive development[2–10], child sex (male and female)[2,6–9] and age (years)[2], baseline height-for-age z-score[2–7,9,10], BMI for age z-score[2], Hb concentration (g/dL)[2,4,5], helminth infection (Yes/No)[5] (*Ascaris lumbricoides*[2–4,8,9], *Trichuris trichiura*, hookworm and *Schistosoma mansoni*[2,7] infections) of the child, weeks of iron/placebo supplementation[10], educational status of the caregivers (illiterate/1–8/9 and above)[3–7,9,10], occupation of the caregivers (Agriculture/Nonagriculture/Unemployed)[2,5–9], educational status of the head of the household (illiterate/1–8/9 and above)[2–10], wealth index[2–4,6,7,9,10], food security (Secure/Insecure)[5], prosocial scale[2,5,10], total difficulties score[3,4,10], and HOME score[3,4,6,10].

understimulating home environments. In addition, schoolchildren with iron deficiency who were supplemented with iron scored lower in nonverbal intelligence compared with those who received placebo. Serum transferrin receptor primary reflects the intensity of red blood cell formation, erythropoiesis, and the demand for iron, which are related to functional iron deficiency [95,96]. In a population where low iron stores is not a concern but iron deficiency is prevalent, iron supplementation might not affect the functionality of iron. The evidence is strong that stunting is associated with cognitive development of children [97], including children in this study population [98].

Semestrial vitamin A supplementation of schoolchildren improved their working memory but not their nonverbal intelligence, planning and problem solving, and attention and concentration. Vitamin A affects cognition through retinoic acid, which is a crucial signaling molecule of the central nervous system and is related to memory [99]. A trial evaluating the impact of supplemental zinc, vitamin A, and glutamine on 167 Brazilian children aged 5–9 y indicated that vitamin A alone had no effect on verbal learning, whereas vitamin A provided in combination with other micronutrients including glutamine and zinc improved verbal learning. Vitamin A provided alone or in combination had no effects on nonverbal intelligence, psychomotor speed, and semantic and phonetic verbal fluency [100]. A systematic review of animal-based studies assessing the effects of vitamin A on memory showed that supplemented animals performed better, and that VAD diet was related to worse performance [99]. In general, animal-based studies showed that retinoids are required for cognitive functions including memory performance [15–17,101–103].

The working memory improved for girls, children who were infected with at least 1 of the helminths, and children from food secure households supplemented with vitamin A. In most LMICs, there is a co-endemicity for helminth infection and VAD. The findings could be explained by the role of vitamin A supplementation in reducing the reinfection with helminths [104,105]. Paradoxically, the effect of vitamin A supplementation was higher among well-nourished children (determined based on BMIAZ >-2 SD and Hb concentration over 11.5 g/dL) in our study. This could be because of the limiting factors for the optimal functioning of vitamin A, including proteins in the case of children who are malnourished [106] and other micronutrients including zinc [100].

Our study affirmed findings in different age groups and various settings, all of which highlight the significance of a stimulating home environment fostering a child's cognitive development [107,108]. It also shows that vitamin A supplementation could level up the child's cognitive development when they live in an understimulating home environment.

Strengths and limitations of the study

Our study has several strengths that ensure the reliability of the findings. First, we used an individually randomized 2 × 2 factorial design that enabled us to investigate the effects of weekly iron supplementation for 11 mo and 2 times semestrial high-dose vitamin A supplementation on the cognitive development of schoolchildren. Second, we measured 4 different cognitive domains to determine the cognitive function of the children, using tools that were adapted in similar contexts in Kenya. Third, intensive training was conducted for all the data collectors to ensure the quality of the implementation of the intervention and the data collection. Finally, the number of schools (7 out of 22 public primary schools in the HDSS) could be considered as representative of the site and the findings can be, confidently, generalizable.

Our study was not without limitations. First, the study was conducted immediately after the first COVID-19 restrictions. This meant that schoolchildren had to use face masks, observed more carefully the hygiene rules, attending the school rooms in smaller numbers, and in some cases, schoolchildren attended the class every other day (3 d/wk). Second, although cognitive development tools were adapted and pretested for the application in the area, they have their limitations. For instance, the digit span test is affected by the clarity, pitch, and rhythm in

pronunciation [109]. RCPM is a nonverbal measure of reasoning, which, on the one hand, can allow children with oral expressive language problems or spatial learners to overcome these problems, and on the other hand, might favor those children. Third, iron supplementation was given on a fixed day of the week (Tuesday or Wednesday). The pupils were not given the supplements if they were absent during that day. Fourth, deworming as per the national plan in addition to treatment of *S. mansoni* and mid-term treatment of children who tested positive for helminths can result in improving Hb concentration and could potentially dilute the effect of nutrient supplementation on cognition. Fifth, the cognitive development outcomes were analyzed based on children who were assessed at both baseline and endline. Analysis based on completers might result in a selected cohort and bias the results. However, the analysis of baseline characteristics and cognitive development scores between the completers and dropouts did not reveal any significant differences. The only exceptions were total difficulty score, prosocial scale, and total digit span score. Finally, it is important to note that our analyses of primary outcomes and potential effect modifiers were not adjusted for multiple comparisons. The absence of both Bonferroni and Hochberg corrections may result in significant results that, in reality, may not be truly significant. Specifically, in the context of this analysis, the negative effect of iron supplementation on the attention and concentration domain of schoolchildren may have been deemed statistically nonsignificant if such corrections were applied. Nevertheless, the results are of public health significance and future research is needed to replicate the study and to confirm the results.

Conclusion

Intermittent iron supplementation does not have a beneficial effect on schoolchildren's cognitive development in settings with a low prevalence of IDA. Contrarily, it negatively affects the attention and concentration of the schoolchildren who generally had adequate iron stores. However, children with stunting and thinness could benefit from intermittent iron supplementation. Intermittent iron supplementation has a negative effect on the nonverbal intelligence of schoolchildren with iron deficiency. Research is needed to confirm this result and to elucidate the underlying mechanisms. Semestrial vitamin A supplementation improved the working memory of the children. It benefited more females, children with stunting, schoolchildren infected with helminths, without anemia, with healthy- to overweight, with VAD, and schoolchildren from food secured households.

Schoolchildren who come from understimulating home environments have shown greater improvement in their working memory domain, and their attention and concentration when receiving vitamin A supplementation. Furthermore, they have improved their planning and problem-solving levels when they received iron supplementation. The effects of intermittent iron supplementation and vitamin A supplementation on the cognition of schoolchildren need further investigation in similarly low- and middle-income settings to develop and to up-scale micronutrient supplementation embedded in school programs targeting the improvement of cognition and school achievement. Schoolchildren from understimulating home environment could constitute the first target group.

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Author contributions

The authors' responsibilities were as follows—SA, SDH, BTG: were responsible for the design of the study; AA: advised on cognitive development, Strengths and Difficulties Questionnaire and Home Observation for Measurement of the Environment score tools; BL: trained the staff at Arba Minch University on the Kato–Katz technique; BTG, MBS, NDM, GEY, THZ: conducted the research; SA, SDH, BL, AA, BTG: supervised data collection; BTG: completed the statistical analyses and drafted the manuscript; SA: contributed to the writing of the manuscript; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data used in this analysis are available at https://osf.io/85vms.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.11.005.

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