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Cognitive Performance in Indian School-Going Adolescents Is Positively Affected by Consumption of Iron-Biofortified Pearl Millet: A 6-Month Randomized Controlled Efficacy Trial

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Abstract

Background: Iron deficiency remains the most prevalent micronutrient deficiency globally, but few studies have examined how iron status relates to cognition in adolescents. Iron biofortification of staple food crops is being scaled up, yet it is unknown whether consuming biofortified crops can benefit cognition.

Objective: Our objective was to determine the efficacy of iron-biofortified pearl millet in improving attention and memory in Indian school-going adolescents.

Methods: A double-blind, randomized, intervention study was conducted in 140 Indian boys and girls, aged 12–16 y, who were assigned to consume iron-biofortified [Fe = 86 parts per million (ppm)] or conventional (Fe = 21–52 ppm) pearl millet. Hemoglobin, ferritin, and transferrin receptor (TfR) were measured and body iron (BI) was calculated at baseline and after 4 and 6 mo. Five measures of cognitive function were obtained at baseline and 6 mo: simple reaction time (SRT), Go/No-Go (GNG) task, Attentional Network Task (ANT), Composite Face Effect (CFE) task, and Cued Recognition Task (CRT). Intention-to-treat analysis was used.

Results: Daily iron intake from pearl millet was higher in those consuming biofortified compared with conventional pearl millet (19.6 compared with 4.8 mg/d). Effects on ferritin, TfR, and BI at 4 mo, and on TfR at 6 mo (all $P < 0.05$), indicated efficacy of biofortified pearl millet over conventional pearl millet in improving iron status. Compared with conventional pearl millet, the consumption of biofortified pearl millet resulted in greater improvement in attention (SRT, GNG, and ANT) and memory (CFE and CRT). Reaction time decreased twice as much from 0 to 6 mo in those consuming biofortified compared with conventional pearl millet on attention tasks (SRT: −123 compared with −63 ms; GNG: −67 compared with −30 ms; ANT double cue: −74 compared with −32 ms; all $P < 0.01$).

Conclusion: Consuming iron-biofortified pearl millet improves iron status and some measures of cognitive performance in Indian adolescents. This trial was registered at www.clinicaltrials.gov as NCT02152150. *J Nutr* 2018;148:1–10.

Keywords: biofortification, iron, cognition, India, adolescent

Introduction

Iron deficiency is the most prevalent nutrient deficiency globally, and iron deficiency anemia is the leading determinant of years lived with disability among children and adolescents (1). Adolescents are particularly prone to iron deficiency and anemia. Iron requirements double from ages 7–10 y to ages 11–14 y (2), and iron intake among Indian adolescents is far below recommended levels (3). According to India's fourth National Family Health Survey data from 2015–2016, among adolescents aged 15–19 y ($n = 169{,}228$), 52% of girls and 29% of boys were anemic (4). It is alarming, although unsurprising given that adolescents are mostly ignored by policies and programs aimed at reducing anemia, that these numbers have not improved in the past 10 y; in 2006, 53% of girls and 27% of boys aged 15–19 y were anemic (5). A 2014–2015 survey of 1010 girls aged 13–17 y in Maharashtra state, where the current study was conducted and also in a rural part of the state where malaria is not common, found that 87% of girls were anemic

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(6), underscoring the magnitude of the public health issue in this particular region. Iron deficiency is a key causal factor of anemia and has been estimated to account for 25–50% of anemia cases (7, 8).

Among its many roles, iron is critical for brain development and function, with neurophysiologic and behavioral outcomes showing relations to iron status at several life stages (9). Adolescence is a period when brain structure, neurochemistry, and cognitive function are maturing (10), yet it has largely been ignored in research linking iron status to neural and behavioral outcomes. Girls in India often marry during adolescence and have children soon thereafter; from the fourth National Family Health Survey, the mean ages at marriage and first birth were 18.7 and 20.5 y, respectively, perpetuating the cycle of the effects of iron deficiency on the next generation (4). For their own cognitive development, the infants of these young mothers depend on iron stores at birth and a positive maternal-infant interaction, both of which are dependent, among other factors, on maternal iron status (11). Thus, adolescence should be a focal point for iron interventions.

A meta-analysis of iron supplementation trials among adolescents and young adults found some evidence that supplementation improved attention and concentration, but called for further evidence (12). Supplementation, however, may not be the most effective and sustainable strategy to ameliorate iron deficiency in certain contexts. Addressing iron deficiency in food-insecure areas with low access to health care and diets dominated by a few staples is challenging. Moreover, although there are only a few studies on the efficacy of a fortified-food vehicle on cognition and no studies, to our knowledge, on the effectiveness of fortification on cognition, limited access to value-added foods would most likely render food fortification ineffective in these areas (13–15). One promising solution is the delivery of iron through biofortified staple food crops (16, 17). Although the evidence for the effects of consumption of biofortified crops on cognition is extremely sparse, we recently found that consumption of iron-biofortified beans for 128 d among young women in Rwanda improved iron status (18) and performance on memory tasks (14).

This study investigates whether the consumption of iron-biofortified pearl millet, a drought-tolerant grain grown widely across central India and Africa, could benefit iron status and performance on tests of attention and memory. We previously found that biofortified pearl millet consumed at midday and evening meals by 246 adolescents (ages 12–16 y) for 6 mo resolved iron deficiency 1.6 times faster than a popular commercial (conventional) variety of pearl millet (19). Within this randomized controlled trial, tests of cognitive (computer-based tasks of behavior), neurophysiologic (electroencephalography), and physical function (cycle ergometer and accelerometer tests) were conducted on a subset of individuals selected for low initial iron status. The current investigation focuses on cognitive outcomes. We hypothesized that, compared with the conventional variety of pearl millet, the consumption of iron-biofortified pearl millet for 6 mo would result in greater cognitive improvement relative to a nonbiofortified conventional pearl millet.

Methods

Participants. Participants were students (aged 12–16 y) from economically disadvantaged families attending a rural boarding school in the Ahmednagar district of Maharashtra, India. The school was selected on the basis of the high prevalence of anemia (>25%) found in a prescreening survey and on its capacity to support the efficacy trial. To be eligible for the study, participants had to be in general good health without chronic disease or acute illness, not severely anemic (those with hemoglobin <85 g/L were not eligible and were referred to a doctor for follow-up), not taking iron supplements or medications that would interfere with iron absorption, and residing full time at the boarding school. Of the 288 individuals screened, 42 were ineligible and 246 were enrolled in the parent efficacy trial in September 2011 (19) (**Figure 1**). Anthelminthic treatment (200 mg albendazole) was provided to participants 4 wk before the baseline assessment and at the study midpoint. A subset of 146 participants was chosen to undergo functional testing—including tests of both physical (not described here) and cognitive function—on the basis of having the lowest ranked ferritin concentrations, because it was thought that these individuals would have the greatest potential to benefit from the intervention and show cognitive change. Ranking of ferritin concentrations was done by ordering the screening ferritin values from lowest to highest. This subset selection method unexpectedly resulted in unbalanced groups, with 93 participants in the biofortified group and 53 in the control group undergoing cognitive testing; as a result of blinding the treatment to investigators until after data collection was completed, this misdistribution could not be corrected. Data for 6 participants were incomplete due to technical issues or only being available at a single time point; thus, we report here on the subset of 140 individuals with complete baseline and endline cognitive data (Figure 1). A sample size calculation, assuming a 2-tailed 5% type I error rate and 90% power and based on a previous iron intervention with behavioral measures in young adult women (20) showed that a sample size of 60/group would be sufficient to detect differences in cognitive outcomes.

Study design. The study was a double-blind randomized efficacy trial. Participants were stratified by hostel (residence hall at the school). There were 3 hostels; 1 all-male, 1 all-female, and 1 co-ed. We screened children from all 3 hostels and then, within a hostel, randomly assigned eligible children to receive either conventional pearl millet [21 parts per million (ppm) Fe from baseline to 4 mo; variety DG9444; 52 ppm Fe from 4 to 6 mo; variety JKBH778] or biofortified high-iron pearl millet (86 ppm Fe; variety ICTP8203 consumed for the entire 6 mo) flour in the form of *bhakri*, a local flatbread consumed with midday and evening meals, and *shev*, a savory snack made from extruded pearl millet flour. Due to a planning error, the supply of conventional pearl millet (DG9444) was exhausted after 4 mo and replaced with another variety available in the local market (JKBH778). Post hoc laboratory analysis showed that the replacement pearl millet variety contained higher contents of iron than the original pearl millet (52 ppm Fe instead of 21 ppm Fe). The *shev* snack was introduced at the same time as the conventional pearl millet replacement (i.e., after 4 mo). Full methodologic details of the randomization procedures, pearl millet characteristics, storage, transport, preparation, and feeding strategy have previously been reported (19).

Laboratory analysis. Whole-blood samples were collected at baseline and at 4 and 6 mo after feeding initiation by a trained

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Supplemental Text 1 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: AGP, α1-acid glycoprotein; ANT, Attentional Network Task; BI, body iron; CFE, Composite Face Effect; CRP, C-reactive protein; CRT, Cued Recognition Task; GNG, Go/No-Go; ppm, parts per million; RT, reaction time; SRT, simple reaction time; TfR, transferrin receptor.

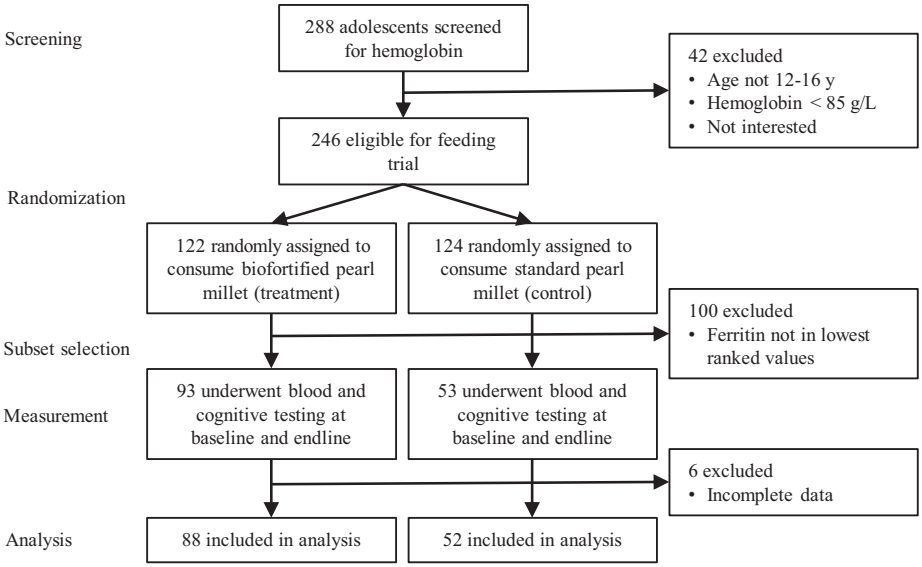

FIGURE 1 Screening, randomization, subset selection, measurement, and analysis flow diagram for a study in Indian adolescents randomly assigned to consume conventional or biofortified pearl millet.

phlebotomist. Samples were analyzed within 6 h for hemoglobin (HemoCue) by Sanjeevani Laboratory in Ahmednagar. Whole blood was collected in a heparin-coated tube and serum was separated by centrifuged at 1660 × *g* for 10 min at room temperature, divided into aliquots, and stored at −20°C. Within 72 h, serum samples were transported on ice to Mumbai and stored at −80°C in Metropolis Labs, where serum ferritin, serum transferrin receptor (TfR), C-reactive protein (CRP), and α1-acid glycoprotein (AGP) concentrations were analyzed. A 2-site sandwich immunoassay (ADVIA Centaur system) was used to assess ferritin and TfR. Body iron (BI) was estimated as the ratio of TfR and ferritin according to Cook's equation (21). Because this equation uses TfR as determined by the Ramco ELISA kit, we converted the Metropolis-derived TfR values to Ramco values by using a regression prediction equation ($R^2 = 0.97$) derived from 35 random duplicate samples as follows:

$$\mathrm{TfR_{Ramco}} = (4.17 \times \mathrm{TfR_{Metropolis}}) - 0.081 \quad (1)$$

CRP was assessed with the use of a quantitative turbidimetric immunoassay [Tulip Diagnostics (P) Ltd.] and AGP was assessed by radial immunodiffusion procedure (Kent Labs).

Ferritin values were not adjusted due to the low prevalence of inflammation (**Table 1**) and a nonparametric 2-sample Kolmogorov-Smirnov test for equality of distribution functions was used to confirm that individuals with and without inflammation did not differ in terms of ferritin (Kolmogorov-Smirnov test statistic D = 0.681, exact $P = 0.139$). Within-run plus between-run CVs for the assays were ferritin 3.7%, TfR 1.9%, AGP 2.9%, and CRP 3.0%.

Cognitive testing sessions. Each participant underwent an ∽1-h cognitive testing session at baseline and after 6 mo of consuming pearl millet. One of 5 trained research assistants gave a standard set of test instructions in the local language (Marathi). Participants were seated at an unconstrained distance of ∽76 cm from the computer screen in a comfortable position, and efforts were taken to minimize environmental distractions during testing.

Computerized tests of cognition. Five widely used cognitive/behavioral tasks—3 attention tasks and 2 memory tasks—were administered on laptop computers to assess aspects of behavior with hypothesized relations to iron status; procedural details for each of the tasks are presented in **Supplemental Text 1**. DMDX (22) software was used to conduct the tasks, which were developed and programmed by

TABLE 1 Baseline prevalence of anemia, iron deficiency, and inflammatory status in Indian adolescents by treatment group and sex[1]

		Control		Biofortified		z[2]	
	Overall ($n = 140$)	Males ($n = 21$)	Females ($n = 31$)	Males ($n = 54$)	Females ($n = 34$)	Treatment difference	Sex difference
Anemic	46 (33)	4 (19)	13 (42)	12 (22)	17 (50)	−0.3	−1.8
Iron-deficient							
Ferritin <15 μg/L	70 (50)	14 (67)	16 (52)	21 (39)	19 (56)	−0.8	−1.1
TfR >8.3 mg/L	14 (10)	4 (19)	1 (3)	7 (13)	2 (6)	0.0	1.2
Body iron <0 mg/kg[3]	35 (25)	10 (48)	9 (29)	7 (13)	9 (26)	−2.2*	−1.9
Iron-deficient anemic							
Anemic + ferritin<15 μg/L	22 (16)	2 (10)	7 (23)	5 (9)	8 (26)	−0.3	−2.0*
Non–iron-deficient anemic							
Anemic + ferritin ≥15 μg/L	48 (34)	12 (57)	9 (29)	16 (30)	11 (32)	−0.1	−0.2
Any inflammation							
AGP >1.0 g/L or CRP >5.0 mg/L	6 (4)	0 (0)	2 (6)	3 (6)	1 (3)	1.3	−0.8

[1] Values are *n* (%). Anemia was defined as hemoglobin <120 g/L for males aged <15 y and all females and <130 g/L for males aged ≥15 y. *$P < 0.05$. AGP, α1-acid glycoprotein; CRP, C-reactive protein; TfR, transferrin receptor.
[2] Tests of proportions were used and *z* statistics are shown.
[3] Calculated by using Cook's method (21).

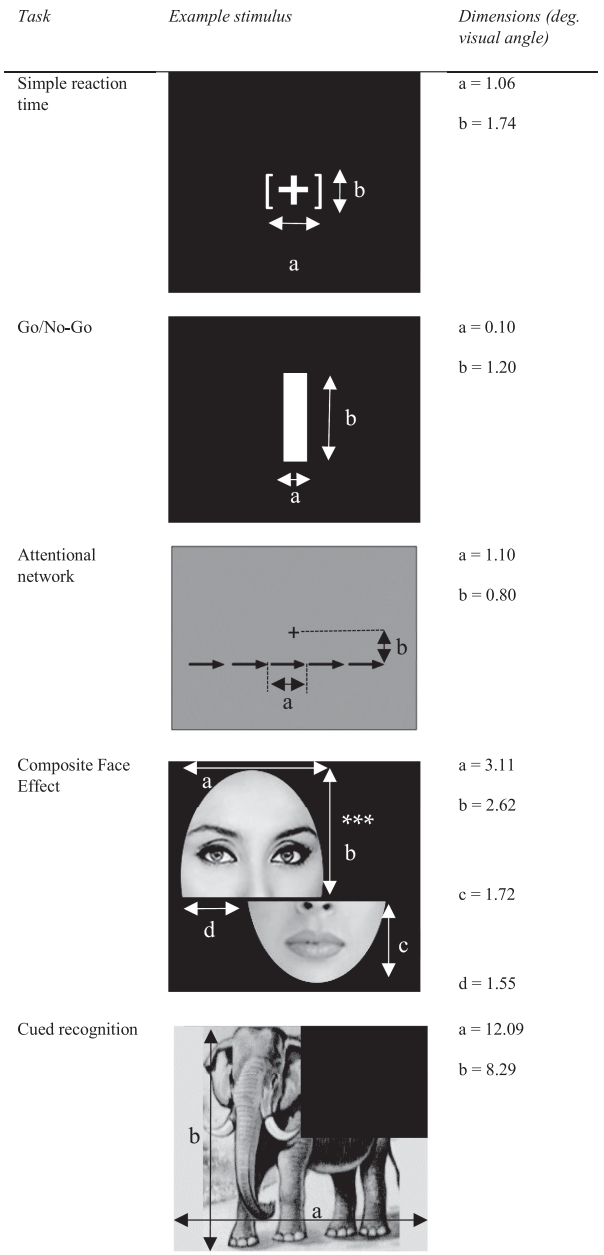

FIGURE 2 Example stimuli and stimuli dimensions for the 5 cognitive tasks administered to Indian adolescents randomly assigned to consume conventional or biofortified pearl millet. deg., degree.

MJW, and all programs and stimuli are freely available on request. The tasks were presented on a set of Windows-based laptop computers with 36-cm (diagonal) displays, running at 2.5 GHz, with ≥4 GB of RAM and ≥320 GB of hard-disk storage. Stimulus onsets were synchronized to the vertical refresh rate of the monitor and keyboard responses were timed to ±1 ms. Stimuli for the tasks were either grayscale images or white text on a black background (**Figure 2**). The tasks were presented in the following fixed order:

1) The Simple Reaction Time (SRT) task was the most basic task and assessed processing speed absent significant attentional or memory demands. Participants responded with a single key-press to a symbol that appeared in the center of the screen.
2) The Go/No-Go (GNG) task provided an estimate of the effectiveness of sustained attention and the speed of simple attentional capture. Participants were required to make a decision about 1 of 2 possible stimuli, a vertical or horizontal bar that were randomly assigned to be either a Go or a No-Go stimulus. Participants were instructed to respond using a button press as quickly as possible when seeing the Go stimulus (which appeared 20% of the time) and not to respond when seeing the No-Go stimulus (which appeared 80% of the time).
3) The Attentional Network Task (ANT) was a modified flanker task (23) assessing 3 distinct functions of attention: alerting (low-level attentional capture), orienting (midlevel spatial selective attention), and conflict (24) (high-level selection or control). On each trial, the participant was presented with either an informative or uninformative cue as to the location of an upcoming test stimulus display (above or below fixation) and was required to press a button to indicate whether a centrally presented arrow in the display pointed to the left or right, while disregarding flanking elements (congruent, incongruent, or neutral distractors) on either side of the stimulus. Participants were instructed to respond as quickly as possible by pressing one key with their left index finger if the central arrow pointed left and another key with their right index finger if the central arrow pointed right.
4) In the Composite Face Effect (CFE) task, participants learned to identify (using 2 different buttons) 2 familiar (famous celebrities) faces and 2 unfamiliar faces. They were then shown images in which the top and bottom half of the face could be from the same or different faces and were either aligned or misaligned. Participants were instructed to respond only to the cued half of the face and to ignore the other half.
5) Finally, in the Cued Recognition Task (CRT), participants were first shown a set of 24 culturally appropriate pictures, one at a time for 3 s each, to commit to memory. Next, they viewed a set of 48 pictures, 24 from the memorized set and 24 new pictures. Participants were instructed to identify whether the picture was from the memorized set or not (i.e., was "old" or "new" to them). The amount of visual information available was varied by covering picture quadrants with black squares, such that 50%, 75%, or 100% of any given picture in the second set of 48 was visible. This task was included due to evidence that the integrity of neural circuits supporting recognition memory, particularly those involving the hippocampus, is correlated with the amount of work that can be accomplished during recognition (25).

Ethics. Informed written consent was obtained from all participants and their guardians and institution heads at screening and again at baseline. The Intersystem Biomedical Ethics Committee in Mumbai, India, and the institutional review boards of The Pennsylvania State University, Cornell University, and The University of Oklahoma approved the research protocol. Procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Statistical analysis. All of the statistical analyses were performed with the use of Statistical Analysis Software version 9.4 (SAS Institute). The outcomes for each of the tasks are defined in **Supplemental Table 1**. All reaction times (RTs) were summarized for each participant at each time point as the median of the RTs for correct responses only. Furthermore, RTs were censored to exclude trials faster than 200 ms (anticipatory response) and slower than 2000 ms (lapse of attention). With the exception of the data from the CFE task and CRT, we chose to focus on measures of latency rather than accuracy due to low variability (performance ceilings) in the majority of the accuracy variables.

Baseline treatment group and sex differences for anemia, iron deficiency, iron deficiency anemia, iron deficiency without anemia, and inflammation were assessed with the use of tests of proportions. Ferritin was log-transformed before inclusion in regression models, and normality of the transformed variables was confirmed using the Shapiro-Wilk test.

Given the unbalanced design after subset selection on the basis of ranked ferritin values, intention-to-treat analyses were conducted with the use of PROC GLM (statistically robust to unequal group sizes). The general form of the model included the endline (6 mo) value as the dependent variable, treatment group as the independent variable, and baseline value as a covariate. Sex and age were included as covariates when related to the outcome at the $P < 0.05$ level using t tests. This

TABLE 2 Summary statistics for blood measures in Indian adolescents randomly assigned to conventional (control) or biofortified pearl millet at each of the 3 time points[1]

Variable[2]	Control ($n = 52$)	Biofortified ($n = 88$)
Hemoglobin, g/L		
0 mo	123 ± 2	124 ± 1
4 mo	133 ± 2	137 ± 1
6 mo	123 ± 1	125 ± 1
Ferritin, μg/L		
0 mo	14 ± 2	19 ± 1
4 mo	20 ± 2	25 ± 2
6 mo	22 ± 3	30 ± 2
Ferritin(log), μg/L		
0 mo	2.4 ± 0.1	2.7 ± 0.1
4 mo	2.7 ± 0.1	3.0 ± 0.1
6 mo	2.9 ± 0.1	3.2 ± 0.1
TfR, mg/L		
0 mo	6.2 ± 0.2	6.5 ± 0.2
4 mo	7.4 ± 0.3	6.8 ± 0.3
6 mo	7.2 ± 0.2	6.7 ± 0.1
Body iron,[3] mg/kg		
0 mo	0.7 ± 0.5	1.7 ± 0.3
4 mo	1.4 ± 0.4	2.6 ± 0.3
6 mo	2.2 ± 0.3	3.1 ± 0.4

[1]Values are means ± SEs (0 mo) or least-square means ± SEs. TfR, transferrin receptor.
[2]Months elapsed since start of feeding trial.
[3]Calculated by using Cook's method (21).

model structure accounted for group differences in sample size, sex ratio, and baseline iron status. For the blood outcomes, additional models were conducted using the midpoint (4 mo) measure as the dependent variable to assess early treatment effects.

For all regressions, highly influential points (Cook's distance >1) were examined on a case-by-case basis and removed if the point was judged to be implausible (e.g., RTs well outside the 95% CI for the data). Less than 0.1% of points were removed. This trial was registered at www.clinicaltrials.gov as NCT02152150.

Results

Sample characteristics. At baseline, participants were 13.7 y old, on average, and 54% were male. The biofortified group had a higher proportion of males than did the control group (61% compared with 40%). One-third of the sample were anemic (hemoglobin <120 g/L for females and males <15 y, and hemoglobin <130 g/L for males aged ≥15 y), half were iron-deficient (ferritin <15 μg/L), and one-quarter had negative BI (<0 mg/kg) (Table 1). As would be expected, given the onset of menstruation and thus additional iron losses in female adolescents, iron deficiency anemia was 2–3 times more prevalent in females compared with males. Inflammation was uncommon, with only 6 individuals having either elevated CRP or AGP, so ferritin and BI values were not adjusted for inflammation. The biofortified group had better iron status as indicated by fewer individuals with negative BI; thus, baseline iron status was controlled for in the intention-to-treat analysis. Summary statistics for continuous variables at all time points are shown in Table 2 for blood outcomes, Table 3 for attention outcomes, and Table 4 for memory outcomes. There were no treatment group differences at baseline on any of the cognitive measures.

TABLE 3 Summary statistics for attention tasks in Indian adolescents randomly assigned to conventional (control) or biofortified pearl millet at baseline and endline, along with the change between baseline and endline[1]

Outcome	Better[2]	Control	Biofortified
SRT RT, ms	↓		
0 mo		1089 ± 20	1098 ± 13
6 mo		1026 ± 14	975 ± 12
0- to 6-mo change		−63 ± 22	−123 ± 16
GNG RT, ms	↓		
0 mo		617 ± 14	611 ± 10
6 mo		587 ± 10	544 ± 8
0- to 6-mo change		−30 ± 17	−67 ± 10
ANT			
RT 0 cues, ms	↓		
0 mo		676 ± 13	667 ± 12
6 mo		678 ± 10	676 ± 10
0- to 6-mo change		2 ± 12	8 ± 9
RT 2 cues, ms	↓		
0 mo		647 ± 13	655 ± 13
6 mo		619 ± 11	582 ± 9
0- to 6-mo change		−32 ± 14	−74 ± 13
Alerting, ms	↑		
0 mo		29 ± 10	12 ± 11
6 mo		64 ± 8	94 ± 6
0- to 6-mo change		32 ± 12	82 ± 12
RT center cue, ms	↓		
0 mo		681 ± 13	671 ± 12
6 mo		627 ± 10	627 ± 10
0- to 6-mo change		−53 ± 12	−45 ± 10
RT spatial cues, ms	↓		
0 mo		588 ± 10	605 ± 11
6 mo		586 ± 11	548 ± 8
0- to 6-mo change		−9 ± 13	−57 ± 11
Orienting, ms	↑		
0 mo		93 ± 15	67 ± 11
6 mo		47 ± 9	79 ± 5
0- to 6-mo change		−45 ± 18	13 ± 12
RT consistent flankers, ms	↓		
0 mo		690 ± 12	675 ± 11
6 mo		672 ± 10	674 ± 10
0- to 6-mo change		−17 ± 12	−1 ± 9
RT inconsistent flankers, ms	↓		
0 mo		806 ± 16	788 ± 14
6 mo		812 ± 11	727 ± 11
0- to 6-mo change		7 ± 15	−62 ± 11
Conflict, ms	↓		
0 mo		116 ± 12	113 ± 9
6 mo		140 ± 9	52 ± 8
0- to 6-mo change		24 ± 16	−61 ± 10

[1]Values are means ± SEs (0 mo) or least-square means ± SEs; $n = 140$. The results of intention-to-treat analysis of treatment group differences over times are shown in Table 5. There were no differences between control and biofortified groups at baseline (all $P > 0.05$). ANT, Attentional Network Task; GNG, Go/No-Go task; RT, reaction time; SRT, simple reaction time.
[2]Arrows indicate which direction (higher or lower) indicate better performance.

Effect of the pearl millet intervention. Treatment effects were seen by 4 mo for ferritin, TfR, and BI and at 6 mo for TfR (Table 5). A significant group-by-age interaction was found for TfR at 4 mo; in the control group, TfR increased more from 0 to 4 mo in older children (+2.6 mg/L) compared with younger children (+0.0 mg/L). Otherwise, no significant

TABLE 4 Summary statistics for memory tasks in Indian adolescents randomly assigned to conventional (control) or biofortified pearl millet at baseline and endline, along with the change between baseline and endline[1]

Outcome	Better[2]	Control	Biofortified
CFE			
RT, ms	↑		
0 mo		41 ± 14	56 ± 14
6 mo		39 ± 14	119 ± 12
0- to 6-mo change		−1 ± 21	63 ± 19
Hit rate, proportion	↑		
0 mo		−0.03 ± 0.02	0.00 ± 0.01
6 mo		−0.00 ± 0.01	0.03 ± 0.01
0- to 6-mo change		0.02 ± 0.02	0.03 ± 0.02
False alarm rate, proportion	↑		
0 mo		−0.01 ± 0.02	0.02 ± 0.02
6 mo		0.01 ± 0.02	−0.01 ± 0.01
0- to 6-mo change		0.02 ± 0.03	−0.03 ± 0.02
Sensitivity (d'), SD	↑		
0 mo		0.14 ± 0.12	0.18 ± 0.11
6 mo		0.06 ± 0.06	0.41 ± 0.03
0- to 6-mo change		−0.08 ± 0.13	0.23 ± 0.11
Bias (c), SD	↓↑[3]		
0 mo		0.03 ± 0.07	0.08 ± 0.06
6 mo		−0.00 ± 0.04	−0.03 ± 0.02
0- to 6-mo change		−0.04 ± 0.07	−0.11 ± 0.06
CRT			
RT new items, ms	↓		
0 mo		905 ± 24	902 ± 19
6 mo		855 ± 26	865 ± 14
0- to 6-mo change		−50 ± 30	−37 ± 19
RT old items, ms	↓		
0 mo		737 ± 16	747 ± 13
6 mo		735 ± 15	666 ± 13
0- to 6-mo change		−2 ± 20	−82 ± 15
Sensitivity (d'), SD	↑		
0 mo		3.5 ± 0.2	3.5 ± 0.1
6 mo		3.4 ± 0.1	3.4 ± 0.1
0- to 6-mo change		−0.0 ± 0.2	−0.1 ± 0.2
Bias (c), SD	↓↑[3]		
0 mo		0.01 ± 0.06	−0.00 ± 0.05
6 mo		−0.01 ± 0.07	0.00 ± 0.05
0- to 6-mo change		−0.02 ± 0.09	0.01 ± 0.07
PCC, %	↑		
0 mo		32 ± 2	31 ± 2
6 mo		42 ± 4	78 ± 3
0- to 6-mo change		10 ± 4	46 ± 3

[1] Values are means ± SEs (0 mo) or least square means ± SEs; $n = 140$. Values for the variables in the CFE are mean interaction contrasts rather than means. The results of intention-to-treat analysis of treatment group differences over times are shown in Table 5. There were no differences between control and biofortified groups at baseline (all $P > 0.05$). CFE, Composite Face Effect task; CRT, Cued Recognition Task; PCC, percentage change in capacity; RT, reaction time.

[2] Arrows indicate which direction (higher or lower) indicates better performance.

[3] Optimal or unbiased performance is near zero; deviation from zero in either direction indicates either a conservative (<0) or liberal (>0) response bias.

treatment group-by-age or treatment group-by-sex interactions were found.

Cognitive performance generally improved across the trial, as hypothesized (Tables 3 and 4). On the attention tasks, significant treatment effects were observed for RT on SRT and GNG tasks, and on 5 outcomes in the ANT (Table 5). Compared with the control group, the biofortified group became faster by endline on the most basic cognitive task (SRT), the simple attentional task (GNG), and the 2-cue and inconsistent flanker conditions in the ANT. Attentional improvement in the biofortified group was strongly supported by significant treatment effects on all 3 ANT difference measures: alerting, orienting, and conflict. Performance in the control group actually declined for orienting and conflict (Table 3). A large proportion of variance in endline performance for RT on inconsistent flanker conditions ($\eta^2_p = 0.24$) and conflict score ($\eta^2_p = 0.35$) was explained by treatment group (Table 5).

In terms of memory, significant treatment effects were seen for 4 outcomes across the 2 tasks, with all effects favoring the biofortified group (Table 5). On the CFE, each score was an interaction contrast for the interaction of memory (familiarity) and attention (alignment), and was scaled so that positive values indicate a cost due to the increased demands of efficient memory retrieval on selective attention. The cost of improved memory retrieval was seen in terms of speed, accuracy (hit rate), and sensitivity to face identity. Treatment group accounted for 25% of the variance in change in sensitivity. On the CRT, participants in the biofortified group showed better ability to adapt to increasing workload, as indicated by a greater percentage change in capacity from baseline to endline. The effect for percentage change in capacity was large (group $\eta^2_p = 0.38$), with the biofortified group increasing from 31% to 78%, compared with a change from 32% to 42% in the control group (Table 4).

Discussion

The literature on interventions to improve adolescent health and well-being is limited and investment in research around adolescent health is vital for achieving the UN's Sustainable Development Goals (26, 27). Our findings indicate a benefit of consuming iron-biofortified pearl millet over commercially available, nonbiofortified pearl millet both in terms of iron status and cognition. Consistent with results obtained with iron supplements (20, 28), we showed cognitive benefits in an iron intervention, but notably did so using a lower-dose intervention that did not require changes in eating behavior (the adolescents in this setting were already consuming pearl millet every day at school). Thus, our findings are highly meaningful to populations with poor iron status—and not necessarily to those with good iron status—who may not have access to dietary supplements and commercially fortified foods.

There have been very few iron interventions in adolescents that have measured cognitive outcomes and all, to our knowledge, were iron supplementation trials. Bruner et al. (28), in their placebo-controlled iron supplementation trial, reported treatment effects on verbal learning and memory in girls aged 13–18 y in the United States. With the use of a similar design, Lambert et al. (29) found benefits of iron supplementation on verbal working memory in girls aged 12–19 y in New Zealand. In Indian school girls aged 8–15 y, iron supplementation twice during the school year improved concentration, discrimination, perception, and visual motor coordination (30). Only the Lambert et al. study used a computer-based task (of perceptual speed), but did not find a treatment effect on this outcome; all other cognitive tests in these 3 studies (28–30) were paper-based.

We recently conducted a feeding trial in college-aged females in Rwanda and administered 4 of the same tasks—SRT, GNG, ANT, and CRT—as in the current study (14). The students

TABLE 5 Intention-to-treat analysis: effect of baseline status/performance and pearl millet treatment group on blood outcomes at 4 and 6 mo and cognitive outcomes at 6 mo in Indian adolescents[1]

	Baseline effect			Group effect			
Outcome	*F*	MSE	η^2_p	*F*	MSE	η^2_p	Covariates
Hemoglobin, g/L							
4 mo	82.0***	100.2	0.39	2.5	3.1	0.01	Sex
6 mo	143.6***	80.6	0.53	0.5	0.3	0.00	Age
Ferritin, μg/L							
4 mo	29.3***	13.6	0.20	4.0*	1.9	0.02	—
6 mo	58.3***	17.8	0.31	3.0	0.9	0.01	Sex
TfR, mg/L							
4 mo	74.2***	310.7	0.39	4.8*	20.2	0.02	Age
6 mo	61.9***	108.7	0.32	4.6*	8.1	0.02	—
Body iron,[2] mg/kg							
4 mo	71.3***	398.0	0.37	5.9*	32.7	0.02	—
6 mo	97.4***	467.9	0.43	2.5	12.0	0.01	Sex
SRT RT, ms	3.3	32,701	0.03	7.9**	78,253	0.06	—
GNG RT, ms	7.4**	30,245	0.06	8.5**	34,963	0.07	Sex
ANT							
RT 0 cues, ms	71.3***	293,043	0.35	2.4	9845	0.02	Age
RT 2 cues, ms	21.3***	123,175	0.14	9.2**	53,173	0.07	Sex
RT alerting, ms	1.9	5297	0.01	11.0**	31,543	0.08	—
RT center cue, ms	81.9***	356,967	0.37	0.1	433	0.00	—
RT spatial cues, ms	22.4***	107,360	0.15	0.0	13	0.00	Age
RT orienting, ms	0.0	0.1	0.00	12.2***	31,599	0.08	—
RT consistent flankers, ms	72.9***	311,208	0.35	0.3	1105	0.00	—
RT inconsistent flankers, ms	52.6***	286,929	0.28	42.7***	233,040	0.24	—
RT conflict, ms	0.0	24	0.00	73.7***	285,278	0.35	—
CFE							
RT, ms	0.4	3460	0.00	15.2***	139,070	0.11	Sex
Hit rate, proportion	0.5	0.0	0.00	5.5*	0.02	0.04	—
False alarm rate, proportion	0.0	0.00	0.00	0.0	0.00	0.00	—
Sensitivity (d'), SD	0.9	0.1	0.01	41.4***	4.2	0.25	—
Bias (c), SD	2.6	0.1	0.02	0.7	0.03	0.01	—
CRT							
RT, new items, ms	9.2**	129,655	0.07	0.6	8722	0.01	Age
RT, old items, ms	10.0**	106,561	0.08	0.5	5495	0.00	Sex, age
Sensitivity (d'), SD	0.4	0.3	0.00	0.1	0.1	0.00	—
Bias (c), SD	1.4	0.3	0.01	0.0	0.0	0.00	—
PCC, %	1.2	647	0.01	76.6***	41,923	0.38	—

[1] $n = 140$. ANOVA with 4- or 6-mo values as the dependent variable, group as the independent variable, baseline value as a covariate, plus age and/or sex as covariates if associated with the outcome at the 5% level was used. All comparisons were single degree-of-freedom tests. Values for CFE are interaction contrasts (see Table 1 and Supplemental Text 1). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ANT, Attentional Network Task; CFE, Composite Face Effect task; CRT, Cued Recognition Task; GNG, Go/No-Go task; MSE, mean squared error; PCC, percentage change in capacity; RT, reaction time; TfR, transferrin receptor; η^2_p, partial η^2 (the proportion of total variance in the outcome accounted for by baseline status or group assignment).

[2] Calculated by using Cook's method (21).

in Rwanda were randomly assigned to consume either iron-biofortified or conventional beans with regular midday and evening meals for 18 wk, and treatment effects were seen for both attention and memory measures, with the relation being stronger for memory. Overall baseline performance in the Rwandan sample was much higher than in the current sample (e.g., mean SRT RT of 280 ms in Rwanda compared with 1100 ms in India), which is expected given the age and education advantage in the Rwandan students. In fact, performance was so good in Rwanda that floor effects in RT (i.e., most women responded very quickly) were observed for the simplest tasks (SRT and GNG), explaining the limited treatment effects on these outcomes. On the memory task (CRT), the capacity measure was significant in both studies, but other CRT measures (RT for new and old items, sensitivity, bias) were significant only in the Rwandan study. Study differences in terms of age, nutritional status, sex, food vehicle, environment, and culture make it difficult to attribute the different findings to a single factor.

Despite being underpowered in our treatment group based on sample size calculations with the use of data from an earlier study, we found treatment effects on iron status outcomes at 4 mo that were reduced by 6 mo. Due to exhaustion and replacement of the conventional pearl millet, the group iron differential was reduced from 65 ppm in the first 4 mos (21 ppm in the control compared with 86 ppm in the biofortified group) to 34 ppm in the last 2 mo (52 compared with 86 ppm). In addition, the savory snack, *shev*, made from extruded pearl millet was introduced in both groups at 4 mo and this, combined with the replacement conventional variety having higher iron content than the original conventional variety, resulted in the control group's iron intake nearly doubling. In the parent trial,

a treatment effect on blood outcomes was seen after 4 mo, but disappeared by 6 mo due to a plateau effect after 4 mo in the biofortified group and continued improvement in the control group (19). In our subsample, a similar pattern was observed.

Because cognitive assessments were only conducted at baseline and 6 mo, we are unable to characterize the magnitude of change in cognitive performance for the first 4 mo compared with the last 2 mo. The fact that we did observe treatment effects on cognitive outcomes at 6 mo despite the disappearance of effects on blood outcomes by 6 mo suggests a possible lag effect, whereby brain iron is repleted after peripheral iron. Youdim et al. (31) showed this in iron-deficient mice, where liver iron was repleted faster than brain iron. It could also reflect homeostatic mechanisms to preserve brain iron once it has been transported into the brain. Both of these hypotheses, however, require further study around the timing of brain compared with peripheral iron repletion and depletion.

The fact that treatment effects were observed on all 5 cognitive tasks administered suggests that consuming iron-rich pearl millet may have wide-ranging cognitive benefits. We selected tasks on the basis of their hypothesized sensitivity to changes in brain iron status and their ability to measure multiple components of behavior within the broad domains of attention and memory. Given that effects were observed for all domains, one might speculate that improved communication among brain regions (i.e., higher signal-to-noise ratio) is at work. The plausibility of this hypothesis is supported by literature linking iron to neurotransmitter homeostasis (32), and findings from the CFE task in particular offer support. Evidence of greater influence of semantic memory (facial recognition) on selective attention with the consumption of biofortified pearl millet may reflect higher-integrity signaling among networks linking these 2 systems, which are known to interact (33). Note that although this hypothesis is plausible, more direct evidence is needed to advance this potential explanation.

We should note that the tasks were selected to assess changes in basic cognitive function and were not selected as measures to be used for clinical categorization. Each of the tasks has a long history in the study of cognition, attesting to their high construct validity, and allowing for assessment of external validity with respect to the extant literature in cognitive and behavioral neuroscience. In interpreting the results, it should be kept in mind that these measures assess the elementary components of complex cognition and behavior that are each executed numerous times in daily life. For example, something as simple as walking from one place to another is dependent on the ability to shift and control attention numerous times while excluding irrelevant or conflicting information. The ability to speak a sentence is dependent on numerous retrievals from memory. So although the absolute change observed in any one of our tasks may appear small, the cumulative effects can be substantial. Indeed, Scott et al. (34) recently showed that iron status (in conjunction with aerobic fitness) is related to both basic aspects of memory and grade-point average. Finally, it should also be noted that some version of each one of the tasks used here is used in a variety of common clinical neuropsychological screening tools have been used to make clinical distinctions (35, 36).

Biofortified pearl millet may contain higher amounts of other nutrients with demonstrated relations to brain function, such as zinc (37). We measured zinc contents both in the pearl millet and blood. Although zinc was 2–3 times higher in the biofortified pearl millet compared with the conventional varieties (44 ppm compared with 15 and 22 ppm), no treatment effects on plasma zinc were observed (change from baseline to endline: 44 ± 4 μg/dL in the biofortified group compared with 41 ± 7 μg/dL in the control group; $P = 0.705$), although this could be due to poor sensitivity of plasma zinc as a biomarker for zinc status (38). Second, given evidence that iron interventions are able to benefit physical fitness (39), and given associations between fitness and cognition (34, 40), biofortified millet may benefit the brain and behavior via enhanced physical fitness. Because our study participants were physically active, growing adolescents, it is plausible that gains in fitness were achieved during the 6-mo intervention. This link between fitness and cognition could be further explored in our sample, because we also measured fitness using cycle ergometers and accelerometers.

Strengths of the study included the randomized controlled trial design, inclusion of participants with a high prevalence (50%) of iron deficiency, low inflammation rates, use of a sustainable food-based intervention, and use of a battery of widely-used tests of memory and attention the selection of which was based on hypothesized sensitivity to changes in brain iron status. The use of a high-yield, locally adapted, and high-iron variety of pearl millet, a drought-resistant crop, makes cultural sense and builds on traditional agricultural preferences and practices, thus increasing the potential impact on food and nutrition security in rural populations who already grow and consume lower-performing and less-iron-dense varieties.

The primary weaknesses were the subset group differences at baseline, which were addressed with the use of statistical techniques, and the substitution of a new variety of conventional pearl millet two-thirds of the way through the trial. Had there not been a substitution in the control group at 4 mo, we might have observed even larger group differences in outcomes by 6 mo, although in real-world settings in which consumers are purchasing grains from markets, it is likely that they would be consuming different varieties throughout the year that vary in iron content. In addition, given that it was a boarding school and the parents were not available to interview, we could not control for parental and household factors that may influence child cognition, such as assets, wealth, and parents' education and intelligence. Finally, we did not measure the consumption of food items eaten outside of the meals served at school. It is possible that groups differed in terms of snacks purchased from shops nearby the school, and that these food items could contain nutrients important for brain function. Given the randomized design, it is unlikely, but possible, that our results may be biased by group differences in such factors.

In future work, it would be useful to measure outcomes that could benefit from improved cognition such as academic or job-related performance. Additional work is also needed to elucidate the causal chain from food to behavior. Our findings operate within the framework that iron consumption leads to higher systemic iron concentrations, brain iron concentrations are proportional to systemic iron concentrationss, and iron in the brain is used for processes (e.g., neurotransmission) that support behavioral function. We also collected concurrent electroencephalographic data on a subset of the participants who received the cognitive test, and adding these data may allow us to better assess the links between consumption, systemic iron, brain activity, and behavior.

Biofortification is a promising tool to alleviate the large burden of iron deficiency among the rural poor. As plant breeders continue to release improved high-yielding pearl millet varieties with higher absorbable iron, the benefit of biofortified pearl millet over other varieties is expected to grow. In future evaluations of the efficacy of biofortified crops, we

recommend considering not only effects on biomarkers but also on functional outcomes with direct relevance to well-being and quality of life. Here, we have shown for the first time, to our knowledge, that consumption of a biofortified iron-rich staple food results in measurable improvements in perceptual, attention, and mnemonic abilities, which are highly pertinent to adolescents in school settings.

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References

1. Kyu HH, Pinho C, Wagner JA, Brown JC, Bertozzi-Villa A, Charlson FJ, Coffeng LE, Dandona L, Erskine HE, Ferrari AJ, et al. Global and national burden of diseases and injuries among children and adolescents between 1990 and 2013: findings from the Global Burden of Disease 2013 study. JAMA Pediatr 2016;170:267–87.
2. WHO. Prevention of iron deficiency anaemia in adolescents: role of weekly iron and folic acid supplementation. SEA-CAH-02. [Internet]. 2011. http://apps.who.int/iris/handle/10665/205656 (accessed January 1, 2018).
3. Bureau NNM. Diet and nutritional status of rural population. Indian Council of Medical Research; 2002. (Technical report no. 21.) National Nutrition Monitoring Bureau, Jamai-Osmania PO, Hyderabad
4. International Institute for Population Sciences. National Family Health Survey 4 (NFHS-4), 2015–2016. 2017. International Institute for Population Sciences, Deonar, Mumbai. http://rchiips.org/NFHS/NFHS-4Reports/India.pdf (accessed January 1, 2018).
5. International Institute for Population Sciences (IIPS) and Macro International. 2007. National Family Health Survey (NFHS-3), 2005–06: India: Volume I. Mumbai: IIPS. http://rchiips.org/NFHS/NFHS-3%20Data/VOL-1/India_volume_I_corrected_17oct08.pdf (accessed January 1, 2018).
6. Ahankari AS, Myles PR, Fogarty AW, Dixit JV, Tata LJ. Prevalence of iron-deficiency anaemia and risk factors in 1010 adolescent girls from rural Maharashtra, India: a cross-sectional survey. Public Health 2017;142:159–66.
7. Petry N, Olofin I, Hurrell R, Boy E, Wirth J, Moursi M, Donahue Angel M, Rohner F. The proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: a systematic analysis of national surveys. Nutrients 2016;8:693.
8. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014;123:615–24.
9. Murray-Kolb LE. Iron and brain functions. Curr Opin Clin Nutr Metab Care 2013;16:703–7.
10. Keshavan MS, Giedd J, Lau JYF, Lewis DA, Paus T. Changes in the adolescent brain and the pathophysiology of psychotic disorders. Lancet Psychiatr 2014;1:549–58.
11. Murray-Kolb LE, Beard JL. Iron deficiency and child and maternal health. Am J Clin Nutr 2009;89(Suppl):946S–50S.
12. Falkingham M, Abdelhamid A, Curtis P, Fairweather-Tait S, Dye L, Hooper L. The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta-analysis. Nutr J 2010;9:4.
13. Wenger MJ, Murray-Kolb LE, Nevins JE, Venkatramanan S, Reinhart GA, Wesley A, Haas JD. Consumption of a double-fortified salt affects perceptual, attentional, and mnemonic functioning in women in a randomized controlled trial in India. J Nutr 2017;147:2297–308.
14. Murray-Kolb LE, Wenger MJ, Scott SP, Rhoten SE, Lung'aho MG, Haas JD. Consumption of iron-biofortified beans positively affects cognitive performance in 18- to 27-year-old Rwandan female college students in an 18-week randomized controlled efficacy trial. J Nutr 2017;147:2109–2117.
15. Bouhouch RR, El-Fadeli S, Andersson M, Aboussad A, Chabaa L, Zeder C, Kippler M, Baumgartner J, Sedki A, Zimmermann MB. Effects of wheat-flour biscuits fortified with iron and EDTA, alone and in combination, on blood lead concentration, iron status, and cognition in children: a double-blind randomized controlled trial. Am J Clin Nutr 2016;104:1318–26.
16. Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 2011;32:S31–40.
17. Vasconcelos MW, Gruissem W, Bhullar NK. Iron biofortification in the 21st century: setting realistic targets, overcoming obstacles, and new strategies for healthy nutrition. Curr Opin Biotechnol 2017;44:8–15.
18. Haas JD, Luna SV, Lung'aho MG, Wenger MJ, Murray-Kolb LE, Beebe S, Gahutu J-B, Egli IM. Consuming iron biofortified beans increases iron status in Rwandan women after 128 days in a randomized controlled feeding trial. J Nutr 2016;146:1586–92.
19. Finkelstein JL, Mehta S, Udipi SA, Ghugre PS, Luna SV, Wenger MJ, Murray-Kolb LE, Przybyszewski EM, Haas JD. A randomized trial of iron-biofortified pearl millet in school children in India. J Nutr 2015;145:1576–81.
20. Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. Am J Clin Nutr 2007;85:778–87.
21. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–63.
22. Forster KI, Forster JC. DMDX: a windows display program with millisecond accuracy. Behav Res Methods Instrum Comput 2003;35:116–24.
23. Eriksen CW. The flankers task and response competition: a useful tool for investigating a variety of cognitive problems. Vis Cogn 1995;2:101–18.
24. Fan J, McCandliss BD, Sommer T, Raz A, Posner MI. Testing the efficiency and independence of attentional networks. J Cogn Neurosci 2002;14:340–7.
25. Wenger MK, Negash S, Petersen RC, Petersen L. Modeling and estimating recall processing capacity: sensitivity and diagnostic utility in application to mild cognitive impairment. J Math Psychol 2010;54:73–89.
26. Salam RA, Hooda M, Das JK, Arshad A, Lassi ZS, Middleton P, Bhutta ZA. Interventions to improve adolescent nutrition: a systematic review and meta-analysis. J Adolesc Heal 2016;59:S29–39.
27. Sheehan P, Sweeny K, Rasmussen B, Wils A, Friedman HS, Mahon J, Patton GC, Sawyer SM, Howard E, Symons J, et al. Building the foundations for sustainable development: a case for global investment in the capabilities of adolescents. Lancet 2017;390:1792–806.
28. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet 1996;348:992–6.
29. Lambert A, Knaggs K, Scragg R, Metcalf P, Schaaf D. Effects of iron treatment on cognitive performance and working memory in non-anaemic, iron-deficient girls. NZ J Psychol 2002;31:19–28.
30. Kashyap P, Gopaldas T. Impact of hematinic supplementation on cognitive function in underprivileged school girls (8–15 yrs of age). Nutr Res 1987;7:1117–26.
31. Youdim MB, Ben-Shachar D, Yehuda S. Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. Am J Clin Nutr 1989;50:607–615.
32. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. J Nutr Biochem 2014;25:1101–7.
33. Malcolm GL, Rattinger M, Shomstein S. Intrusive effects of semantic information on visual selective attention. Atten Percept Psychophys 2016;78:2066–78.
34. Scott SP, De Souza MJ, Koehler K, Murray-Kolb LE. Combined iron deficiency and low aerobic fitness doubly burden academic performance among women attending university. J Nutr 2017;147:104–9.

35. Cambridge Cognition. CANTAB Cognitive assessment softw are. [Internet]. 2017. http://www.cambridgecognition.com/cantab/ (accessed January 1, 2018).
36. Weiss LG. WAIS-IV clinical use and interpretation: scientist-practitioner perspectives. Academic Press, London, UK; 2010.
37. Rahman MT. Dietary zinc and the brain. In: Preedy Victor R., Watson Ronald Ross, Martin Colin R., editors, Handbook of behavior, food and nutrition. New York: Springer; 2011. p. 2357–73.
38. Wieringa F, Dijkhuizen M, Fiorentino M, Laillou A, Berger J. Determination of zinc status in humans: which indicator should we use? Nutrients 2015;7:3252–63.
39. Pasricha S-R, Low M, Thompson J, Farrell A, De-Regil L-M. Iron supplementation benefits physical performance in women of reproductive age: a systematic review and meta-analysis. J Nutr 2014;144:906–14.
40. Gomez-Pinilla F, Hillman C. The influence of exercise on cognitive abilities. Compr Physiol 2013;3:403–28.