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[1]

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ABSTRACT

Background: Lead is a common neurotoxicant and its absorption may be increased in iron deficiency (ID). Thus, iron fortification to prevent ID in populations is a promising lead mitigation strategy. Two common fortificants are ferrous sulfate ($FeSO_4$) and ferric sodium EDTA (NaFeEDTA). EDTA can chelate iron and lead.

Objectives: Our study objective was to determine the effects of iron and EDTA, alone and in combination, on blood lead (BPb) concentration, iron status, and cognition.

Design: In this 2 × 2 factorial, double-blind placebo-controlled trial, 457 lead-exposed Moroccan children were stratified by school and grade and randomly assigned to consume biscuits (6 d/wk at school) containing *1*) ∼8 mg Fe as $FeSO_4$, *2*) ∼8 mg Fe as NaFeEDTA that contained ∼41 mg EDTA, *3*) ∼41 mg EDTA as sodium EDTA (Na_2EDTA), or *4*) placebo for 28 wk. The primary outcome was BPb concentration; secondary outcomes were iron status and cognitive outcomes from subtests of the Kaufman Assessment Battery for Children and the Hopkins Verbal Learning Test. These outcomes were measured at baseline and endpoint. All data were analyzed by intention-to-treat.

Results: The adjusted geometric mean BPb concentration at baseline was 4.3 μg/dL (95% CI: 4.2, 4.3 μg/dL), and at endpoint these values were 3.3 μg/dL (95% CI: 3.1, 3.5 μg/dL) for $FeSO_4$, 2.9 μg/dL (95% CI: 2.7, 3.0 μg/dL) for NaFeEDTA, 3.3 μg/dL (95% CI: 3.1, 3.5 μg/dL) for EDTA, and 3.7 μg/dL (95% CI: 3.5, 3.9 μg/dL) for placebo. We found an effect of iron ($P = 0.009$) and EDTA ($P = 0.012$) for reduced BPb concentrations at endpoint, but no iron × EDTA interaction. Iron fortification improved iron status, but there were no positive effects of iron or EDTA on cognitive test scores.

Conclusions: Food fortification with iron and EDTA additively reduces BPb concentrations. Our findings suggest that NaFeEDTA should be the iron fortificant of choice in lead-exposed populations. This trial was registered at clinicaltrials.gov as NCT01573013. *Am J Clin Nutr* 2016;104:1318–26.

Keywords: NaFeEDTA, cognition, fortification, iron, lead

INTRODUCTION

Lead exposure and iron deficiency anemia (IDA)[7] are major global health problems that are concentrated in young children and may irreversibly impair neurodevelopment (1, 2). Lead is a well-known neurotoxicant, and low-level lead exposure remains a public health problem not only in developing but also in developed countries (3, 4). Even low-level exposure is associated with a decrease in intelligence quotient (IQ), and the steepest decline in IQ is predicted by blood lead (BPb) concentration increments in the lower BPb range of 2.0–10 μg/dL (1, 2, 5). Recent estimations suggest that BPb concentrations as low as 0.1–1.0 μg/dL are associated with a decrease in IQ (3). As a result, the WHO has withdrawn its provisional tolerable upper intake limit for lead (6).

Lead exposure in children occurs largely through oral ingestion (2, 7). Increasing dietary iron may benefit lead-exposed populations because the absorption of lead may be increased in iron deficiency (ID), although not all studies agree (8, 9). There are likely multiple absorptive pathways for oral lead (10), but iron and lead share a common intestinal transporter—divalent metal transporter 1 (DMT1)—which is upregulated during ID (11). In addition, because DMT1 has a higher affinity for iron than lead, the presence of luminal iron may competitively inhibit this pathway of lead absorption (12, 13). A longitudinal study reported a 4- to 5-fold increased risk of subsequent lead poisoning in iron-deficient children (14). However, in an intervention trial in Mexican children, iron supplements did not reduce BPb concentrations (15). The CDC recommends an iron-rich diet for

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[7] Abbreviations used: AGP, α_1-acid glycoprotein; BPb, blood lead; CRP, C-reactive protein; DMT1, divalent metal transporter 1, $FeSO_4$, ferrous sulfate; HVLT, Hopkins Verbal Learning Test; ID, iron deficiency; IDA, iron deficiency anemia; IQ, intelligence quotient; KABC-II, Kaufman Assessment Battery for Children, Second Edition; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA; SF, serum ferritin; TfR, transferrin receptor; ZnPP, zinc protoporphyrin.

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lead-exposed children but has emphasized the need for randomized trials (16).

Sodium iron EDTA (NaFeEDTA) is a recommended iron fortificant for foods (6). EDTA is a metal chelator that binds both iron and lead (17). Given intravenously, it is an effective BPb chelator (18). Orally administered EDTA can chelate lead in the gut lumen and reduce its absorption (9, 19). Although <5% of oral EDTA is absorbed (20), small amounts of absorbed EDTA could possibly chelate BPb and increase its clearance. The affinity of EDTA for metals is pH-dependent: binding to ferric iron is favored at a low gastric pH, but in the alkaline duodenum the iron is released and in the distal gut, at near neutral pH, the affinity of EDTA is higher for other metals, including lead (17). Therefore, our study aimed to determine the effect of iron and EDTA, alone and in combination, on BPb concentration, iron status, and cognition in lead-exposed Moroccan children. We hypothesized that *1*) both iron and EDTA would reduce BPb concentrations and *2*) iron and EDTA would have an additive effect to reduce BPb concentrations.

METHODS

Study design and participants

We conducted this randomized 2 × 2 factorial, double-blind placebo-controlled trial from September 2011 to July 2012 at 2 schools near Marrakesh, Morocco (clinicaltrials.gov; identification NCT01573013). We screened 479 children for eligibility. Our inclusion criteria were as follows: *1*) preschool-aged third- through sixth-graders, who were *2*) apparently healthy, *3*) had hemoglobin concentrations >7 g/dL, and *4*) did not use iron supplements. We invited 457 children who met these criteria to join the study between September 2011 and January 2012. We obtained written informed consent from parents and verbal consent from the children. The Ministry of Health in Rabat, Morocco, and the ethical committee of ETH Zurich, Switzerland, approved the study protocol. The study protocol complied with the Declaration of Helsinki and local laws and regulations. A data safety board monitored the study.

Randomization and masking

We randomly assigned the eligible children, stratified by school and grade, by using computer-generated randomization sequences to 4 groups who received biscuits containing one of the following fortificants: *1*) ~8 mg Fe as ferrous sulfate ($FeSO_4$), *2*) ~8 mg Fe as NaFeEDTA containing ~41 mg EDTA, *3*) ~41 mg EDTA as sodium EDTA (Na_2EDTA), or *4*) placebo (**Figure 1**).

All of the biscuits (mainly composed of wheat flour, sugar, palm oil, and almonds) were identical in recipe, with the exception of the fortificant. We obtained the fortificants from Paul Lohmann GmbH, and Hug AG produced the biscuits. The iron content of the biscuits was measured by using atomic absorption spectrometry, and EDTA content was calculated from measured iron content in the fortified biscuit, taking into account the amount of native iron in the control (nonfortified) biscuit. Subjects, investigators, and sponsors were masked to group assignment.

Procedures and outcomes

The intervention lasted 28 wk, and we administered 2 or 3 biscuits/d for 6 d/wk, depending on the child's body weight, to stay within the acceptable daily intake of EDTA (1.9 mg EDTA · kg body weight^{-1} · d^{-1}) (4, 21). Trained fieldworkers directly supervised biscuit consumption and recorded compliance daily.

The primary outcome measure of this study was BPb concentration at 28 wk of the intervention. Secondary outcome measures were iron status indicators and cognitive test scores at 28 wk of the intervention. Other outcome variables included C-reactive protein (CRP), α_1-acid glycoprotein (AGP), and the prevalence of anemia, ID, and IDA, as well as the prevalence of elevated BPb concentrations.

At baseline, endpoint, and at 2 intermediate time points on the basis of a sparse random serial sampling protocol (22) (only

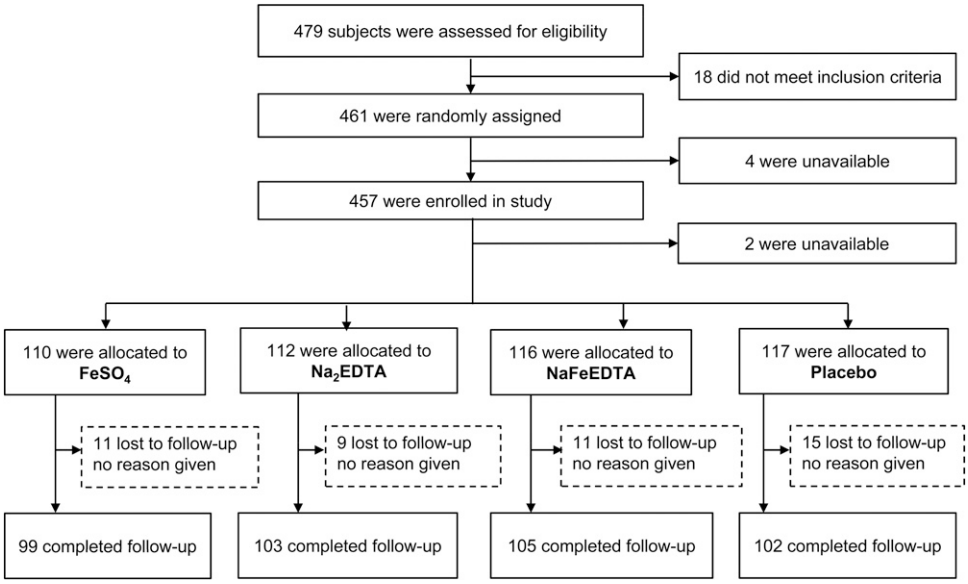

FIGURE 1 Trial profile. $FeSO_4$, ferrous sulfate; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA.

baseline and endpoint results are presented), we measured height and weight and collected a 10-mL venous blood sample into EDTA-coated trace element–free tubes (Becton Dickinson) for the analysis of BPb, iron status indicators, and inflammatory markers. We analyzed hemoglobin and mean corpuscular volume by using a Sysmex XT-2000i Automated Hematology Analyzer and the corresponding standard material (L1–L3: QC-21810810 to QC-21810812). BPb was measured in baseline and endpoint whole-blood samples at the Karolinska Institute, Sweden, by using inductively coupled plasma mass spectrometry (Agilent 7700X; Agilent Technologies), as previously described (23). Analysis of Seronorm blood reference samples [reference values (acceptance range): 1.02 μg Pb/dL (0.60–1.44 μg Pb/dL) and 31.0 μg Pb/dL (18.6–43.4 μg Pb/dL)] showed 0.97 $\pm$ 0.04 μg Pb/dL and 32.1 $\pm$ 0.76 μg Pb/dL. The limit of detection (3 times the SD of the element concentration in the calibration blank) was 0.002 μg Pb/dL, and no samples had a BPb concentration below this level. Elevated BPb concentrations were defined as BPb $>$3 μg/dL and $>$5 μg/dL to provide categorical variables of low-level lead exposure and $>$10 μg/dL based on older reference values (24). Zinc protoporphyrin (ZnPP) was analyzed on washed erythrocytes by using a hematofluorometer as described previously (25); values $\geq$70 μmol/heme may indicate iron-deficient erythropoiesis, but ZnPP can also be elevated by increased body lead (26). We measured serum concentrations of ferritin [serum ferritin (SF)], transferrin receptor (TfR), CRP, and AGP by using ELISA (27). We used SF $<$12 μg/L for children $<$5 y of age, SF $<$15 μg/L for children $\geq$5 y (28), or TfR $>$8.3 mg/L to define ID. To adjust for the elevating effect of inflammation on SF, we used correction factors suggested by Thurnham et al. (29, 30) to adjust SF concentrations by the participant's inflammation status defined as follows: no inflammation (CRP $\leq$5 mg/L, AGP $\leq$1 g/L), incubation (CRP $>$5 mg/L, AGP $\leq$1 g/L), early convalescence (CRP $>$5 mg/L, AGP $>$1 g/L), and late convalescence (CRP $\leq$5 mg/L, AGP $>$1 g/L). We defined anemia by using age-specific hemoglobin cutoffs of $<$11.0 g/dL, $<$11.5 g/dL, and $<$12.0 g/dL for children aged $<$5 y, 5–11 y, and 12 y, respectively (31). We used WHO Anthro (version 3.2.2, 2011) and WHO Anthro Plus (version 1.0.3, 2010) to calculate anthropometric scores (32).

At baseline and endpoint, we performed cognitive tests, which have been previously validated in children of this age (25), in the local Arabic or Berber dialect. We administered 4 subtests from the Kaufman Assessment Battery for Children, Second Edition (KABC-II): the Atlantis (working memory) and Atlantis delayed (long-term memory and retrieval) tests from the learning scale, the hand movement test from the sequential processing scale (short-term memory, manual activity), and the Triangles test from the simultaneous processing scale (visuospatial cognition). We also administered the Hopkins Verbal Learning Test (HVLT) (33, 34). The testing procedures are described in detail elsewhere (25, 35–37).

Statistical analysis

The sample size was estimated on the basis of the predicted improvement in BPb concentrations after iron fortification (38). The sample size calculations indicated that 85 subjects/group would be needed to detect a mean $\pm$ SD difference in BPb of 4.1 $\pm$ 8.2 μg/dL over the 28 wk (38), with an α of 0.05 and a β of 0.20. Anticipating a drop-out rate of 10%, we planned to enroll $\geq$95 children/group.

For statistical analysis, we used IBM SPSS Statistics (version 22.0.0). We checked data for normal distribution (by using Q-Q plots and Shapiro-Wilk tests) and log-transformed skewed data before analysis. Parametric data are presented as means $\pm$ SDs and nonparametric data as covariate-adjusted geometric means with 95% CIs. The latter were calculated from the estimated marginal means obtained by running separate 1-factor (treatment group as the factor) ANCOVA on all outcome variables (baseline and endpoint), including respective baseline values (only for endpoint variables), age, sex, site, and grade as covariates.

Data analysis was performed according to intention-to-treat without the removal of outliers and by using multiple imputations to treat dropouts (Figure 1) and missing data ($<$23%, $\leq$20%, and $<$15% missing data for BPb, iron status, and cognitive measures, respectively; e.g., due to refusal to draw blood, assay fails, or inadequate blood volumes to perform analysis), under the assumption that data were missing at random. Five imputations were produced by using an iterative Markov chain Monte Carlo method and a linear regression model that included the treatment groups (iron and EDTA), a treatment-interaction term (iron $\times$ EDTA), the respective baseline or endpoint variable, grade, sex, age, and site as independent predictors. For skewed variables, multiple imputations were produced on transformed data.

For each continuous outcome variable, we determined the estimated intervention effects (β values and 95% CIs) of iron (iron compared with no iron) and EDTA (EDTA compared with no EDTA), as well as their interaction (iron $\times$ EDTA), by performing a 2-factor ANCOVA on the endpoint variable (dependent variable) with the use of the respective baseline values, age, sex, site, and grade as covariates. Data were log-transformed for the ANCOVA analysis, and the intervention effects (β values and 95% CIs) are reported on the log scale. In the presence of a significant interaction on the endpoint variable, between-group comparisons were performed by using 1-factor ANCOVA with Bonferroni adjustments and controlled for respective baseline values, age, sex, site, and grade. To test whether the difference in iron dose provided to children above and below 30 kg body weight affected any of the outcomes, we performed separate 3-factor ANCOVA to determine whether there were any interactions of body weight ($<$30 kg compared with $\geq$30 kg body weight) with iron and/or EDTA treatment.

For each dichotomous outcome variable, we determined ORs and 95% CIs for each factor (iron and EDTA), as well as their interactions, by using 2-factor binary logistic regression analysis on the endpoint variable, with respective baseline values, sex, age, grade, and site controlled for. $P < 0.05$ was considered significant.

RESULTS

Study participants

Between 18 September 2011 and 15 January 2012, a total of 457 participants were enrolled in the trial (Figure 1). No serious adverse events were reported during the trial.

The mean $\pm$ SD iron content of both the NaFeEDTA and $FeSO_4$ biscuits was 3.6 $\pm$ 0.5 mg. The NaFeEDTA and Na_2EDTA biscuits both contained 18.6 $\pm$ 0.5 mg EDTA. Children with

a body weight <30 kg (n = 390) consumed 2 biscuits/d, which provided 7.2 ± 0.9 mg Fe and 37.1 ± 0.9 mg EDTA; children ≥30 kg (n = 77) consumed 3 biscuits/d, which provided 10.8 ± 1.4 mg Fe and 55.7 ± 1.4 mg EDTA. The difference in iron dose provided to children with a body weight <30 kg and ≥30 kg did not affect any of the outcome variables, because we found no significant interactions of body weight (<30 kg compared with ≥30 kg) with iron and/or EDTA (data not shown). Overall, children in the 2 iron groups consumed 7.8 ± 1.0 mg Fe/intervention day, and children in the 2 EDTA groups consumed 40.1 ± 1.0 mg EDTA/d. Compliance was high (82.2%) and did not significantly differ between groups.

Overall baseline prevalence of anemia (defined by hemoglobin) was 21%, and overall prevalences of ID defined by SF, TfR, or ZnPP were 32%, 7%, or 34%, respectively (data not shown). Baseline characteristics of the study participants in each of the 4 groups are shown in **Table 1**.

BPb concentrations at baseline and after 28 wk of intervention are shown in **Table 2**. At baseline, the overall adjusted geometric mean BPb concentration was 4.3 μg/dL (95% CI: 4.2, 4.3 μg/dL), and 2%, 29%, and 83% of children had BPb concentrations >10, >5, and >3 μg/dL, respectively. We found a significant effect of iron (P = 0.009) and EDTA (P = 0.012) for reduced BPb concentrations at endpoint, but no iron × EDTA interaction (P = 0.606). Furthermore, children who received iron had significantly lower odds (0.48; 95% CI: 0.25, 0.90) of having BPb concentrations >3 μg/dL than did those who did not receive iron (P = 0.023).

Red blood cell indexes and indicators of iron and inflammatory status at baseline and after 28 wk of the intervention are shown in **Table 3**. We found significant effects of iron fortification on all red blood cell indexes and iron status indicators (all $P < 0.05$). Furthermore, children who received iron had significantly lower odds of being iron-deficient (on the basis of TfR or SF), anemic, or iron-deficient anemic at endpoint (iron-deficient: $P < 0.001$; anemic: P = 0.002; iron-deficient anemic: P = 0.003). We also found a significant effect of EDTA fortification for higher AGP (P = 0.019) concentrations at endpoint. The children who received EDTA-fortified biscuits had a 2.5-fold higher odds of having elevated AGP concentrations at endpoint.

The KABC-II subtest and HVLT scores at baseline and endpoint are shown in **Table 4**. We found no significant effects of iron or EDTA fortification and no iron × EDTA interactions on any of the KABC-II or HVLT test scores.

DISCUSSION

The main findings of this trial are that *1*) iron and EDTA fortification additively reduce BPb concentrations in lead-exposed children and *2*) iron fortification reduces the risk of having elevated BPb concentrations (>3 μg/dL) and improves iron status. Our results indicate that iron and EDTA act independently and in an additive manner to reduce BPb, and this suggests that they reduce BPb through independent mechanisms. A likely mechanism for reduction in BPb by iron is that the improvements in iron status reduced gastrointestinal lead absorption. Iron-deficient rats absorb a greater fraction of ingested lead (39), but human studies have produced mixed results (8, 9). Improved iron status downregulates the expression of DMT1 (40) and DMT1 may transport lead, although other routes of lead absorption may be more important (10, 11, 41). In contrast, a likely mechanism for the reduction in BPb by EDTA is chelation of lead by EDTA. Because <5% of orally administered EDTA is absorbed (20, 42), the main chelating effect of EDTA in this study was likely to take place in the gut lumen. In radiotracer studies in humans, oral EDTA reduced the absorption of gastrointestinal lead (9). In 23 adults who ingested 55.8 μg ^{203}Pb without and with 1.02 mg oral Na_2EDTA, body lead retention was ~57% and 8%, respectively (19). However, in our study, it is also possible that absorbed EDTA chelated BPb; the total ingested dose of EDTA over 7 mo was ~6.5 g; if this was absorbed at 3–5%, ~200–300 mg absorbed EDTA was available to chelate BPb during the study.

Previous uncontrolled studies of iron supplementation or fortification to reduce BPb have produced conflicting results (43–46). In a controlled study in Mexican children (n = 602), 6 mo of oral iron supplementation (30 mg/d as ferrous fumarate) did not reduce

TABLE 1
Baseline characteristics of the intention-to-treat population[1]

	$FeSO_4$ (n = 110)	Na_2EDTA (n = 112)	NaFeEDTA (n = 116)	Placebo (n = 117)
Female sex, n (%)	57 (52)	66 (59)	53 (46)	66 (56)
Age, y	7.5 ± 2.9[2]	7.7 ± 2.9	7.2 ± 2.7	7.1 ± 2.7
Height, cm	116.3 ± 16.7	118.6 ± 16.5	115.7 ± 14.8	115.8 ± 16.4
Weight, kg	21.0 (16.0–25.0)[3]	22.0 (16.0–27.0)	20.0 (17.8–25.0)	20.0 (16.0–25.4)
Anthropometric indexes, n (%)				
Stunting (HAZ <−2 SDs)	10 (9.1)	13 (11.6)	11 (9.5)	9 (7.7)
Underweight (WAZ <−2 SDs)	10 (9.1)	11 (9.8)	5 (4.3)	7 (6.0)
Overweight (BAZ >1 SD and <2 SDs)	12 (10.9)	8 (7.1)	14 (12.1)	12 (10.3)
Obese (BAZ ≥2 SDs)	6 (5.5)	2 (1.8)	3 (2.6)	1 (0.9)
Inflammation status, n (%)				
No inflammation	68 (62)	67 (60)	67 (58)	68 (58)
Inflammation[4]	42 (38)	44 (40)	49 (42)	51 (42)

[1] BAZ, BMI-for-age z score; $FeSO_4$, ferrous sulfate; HAZ, height-for-age z score; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA; WAZ, weight-for-age z score.
[2] Mean ± SD (all such values).
[3] Median; IQR in parentheses (all such values).
[4] Inflammation defined as CRP ≥5 mg/L or AGP ≤1 g/L.

TABLE 2
Effects of providing children with fortified biscuits containing iron and EDTA, alone and in combination, for 28 wk on blood lead concentrations[1]

					Estimated intervention effect[2]		
	$FeSO_4$ (n = 110)	Na_2EDTA (n = 112)	NaFeEDTA (n = 116)	Placebo (n = 117)	Iron	EDTA	Iron × EDTA, P
Blood lead, μg/dL							
Baseline	4.10 (3.84, 4.40)[3]	4.53 (4.24, 4.84)	4.41 (4.09, 4.74)	4.03 (3.78, 4.31)			
Endpoint	3.30 (3.11, 3.48)	3.29 (3.09, 3.49)	2.87 (2.71, 3.04)	3.66 (3.47, 3.86)	−0.046 (−0.078, −0.013)	−0.047 (−0.080, −0.013)	0.606
Prevalences							
Blood lead >10 μg/dL							
Baseline	1 (0.9)[4]	4 (3.6)	3 (2.6)	1 (0.9)			
Endpoint	1 (0.9)	1 (0.9)	0 (0)	0 (0)	NA	NA	NA
Blood lead >5 μg/dL							
Baseline	30 (27)	39 (35)	37 (32)	29 (25)			
Endpoint	14 (13)	19 (17)	13 (11)	19 (16)	0.695 (0.279, 1.727)	0.777 (0.280, 2.160)	0.969
Blood lead >3 μg/dL							
Baseline	93 (85)	100 (89)	100 (86)	93 (80)			
Endpoint	59 (54)	70 (63)	57 (49)	77 (66)	0.475 (0.250, 0.902)	0.675 (0.355, 1.285)	0.921

[1] $FeSO_4$, ferrous sulfate; NA, not applicable; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA.
[2] Intervention effects [β values (95% CIs)] were estimated for continuous outcome variables by 2-factor ANCOVA adjusting for respective baseline values, age, sex, grade, and site. Values were log-transformed to perform ANCOVA, and the reported intervention effects [βs (95% CIs)] are reported as log-transformed data. ORs (95% CIs) were estimated for dichotomous outcome variables by using 2-factor binary logistic regression analysis adjusting for respective baseline values, sex, age, grade, and site.
[3] Covariate-adjusted geometric means (95% CIs), calculated from the estimated marginal means obtained by running separate 1-factor (treatment group as factor) ANCOVA on all outcome variables (baseline and endpoint), including respective baseline values (only for endpoint variables), age, sex, site, and grade as covariates (all such values). Data were log-transformed to perform ANCOVA.
[4] n; percentage in parentheses (all such values).

BPb compared with placebo; BPb was determined by atomic absorption spectrophotometry (15). In a controlled trial in Indian schoolchildren (n = 186), subjects who received an iron-fortified rice meal (15 mg Fe/d as ferric pyrophosphate) for 16 wk had lower BPb concentrations at endpoint than did the control group, but baseline BPb was not measured; BPb was measured by anodic stripping voltammetry (38). Differences in study design, such as subject age, iron dose and duration, and analytic method for the determination of BPb, make comparisons between studies difficult.

Our findings on the effects of iron and EDTA fortification on BPb concentrations are generalizable to other populations of lead-exposed children. First, we provided an iron dose (~8 mg/d) comparable to that typically delivered to children by iron-fortification programs (47). Second, an advantage to our study population was that our subjects had only modest elevations in BPb; these are concentrations that are common in children in many low- and high-income countries (3). Finally, we included both preschool- and school-aged children (3–14 y); this may be relevant, because current studies suggest that BPb concentrations at 5–9 y of age are strongly associated with IQ (1, 5, 48), possibly even more so than in earlier childhood (49).

Although the observed decrease in BPb concentrations was modest (35% reduction in the NaFeEDTA group of 1.54 μg/dL from a baseline concentration of ~4.4 μg/dL), cross-sectional data suggest that this change in BPb might result in a measurable increment in child IQ and school performance (1, 5). A current pooled analysis concluded that the increment in IQ points was greatest when decreasing BPb from 10.0 to 2.4 μg/dL (3.9 μg/dL; 95% CI: 2.4, 5.3 μg/dL) than with comparable decreases at higher BPb concentrations (1). Our observed reductions in BPb by the provision of iron (−0.8 μg/dL) and EDTA (−0.8 μg/dL) are within the benchmark dose of 0.1–1.0 μg/dL estimated to lead to a loss of 1 IQ point. This is in agreement with a recent large study (n = 58,650) in US children, which observed a nonlinear negative effect of BPb on reading and math skills, with a steeper failure rate for reading at BPb concentrations <5 μg/dL (5). For children with BPb concentrations <10 μg/dL, a 1-μg/dL difference in BPb was associated with an increased RR for both reading and math failure of 1.06 (95% CI: 1.05, 1.07) (5). However, we did not find an effect of iron or EDTA on KABC-II and HVLT test scores. This may have been due to the short duration of the intervention, the choice of cognitive tests, and/or a β error due to small sample size. Some experts have argued that the cognitive deficits of lead poisoning are irreversible (50), and it is unclear whether an improvement in BPb with iron and/or EDTA fortification can reverse early deficits induced by lead exposure. To our knowledge, this is the first randomized controlled intervention trial evaluating the cognitive effects of improved low-lead exposure in children. Furthermore, it is not known whether the neurodevelopmental effects of lead may be more severe when ID is also present (51), but most of the evidence suggests that their effects are independent of one another (52). Ruff et al. (43) provided parenteral EDTA treatment and oral iron supplements, a combination of both, or neither to 1- to 7-y-old children (n = 154) and measured cognitive outcomes after 6 mo of treatment. A reduction in BPb was associated with higher cognitive scores, but there was no interaction with an increase in iron status. Our study period was only 28 wk; a sustained NaFeEDTA fortification program throughout childhood would likely have a greater impact.

TABLE 3
Effects of providing children with fortified biscuits containing iron and EDTA, alone and in combination, for 28 wk on biochemical indicators of iron and inflammatory status[1]

					Estimated intervention effects[2]		
	$FeSO_4$ (n = 110)	Na_2EDTA (n = 112)	NaFeEDTA (n = 116)	Placebo (n = 117)	Iron	EDTA	Iron × EDTA, P
Hemoglobin, g/dL							0.309
Baseline	11.9 (11.7, 12.2)[3]	11.8 (11.5, 12.1)	12.0 (11.7, 12.2)	12.1 (11.8, 12.3)			
Endpoint	12.3 (12.2, 12.5)	12.0 (11.8, 12.2)	12.2 (12.1, 12.4)	11.9 (11.7, 12.1)	0.02 (0.01, 0.02)	0.00 (−0.01, 0.01)	
SF, μg/L							0.156
Baseline	16.8 (14.7, 19.2)	16.2 (14.2, 18.5)	17.2 (15.1, 19.6)	17.3 (15.1, 19.7)			
Endpoint	34.5 (31.8, 37.4)	20.4 (18.3, 22.6)	32.6 (29.9, 35.6)	19.2 (17.5, 21.0)	0.25 (0.20, 0.31)	0.03 (−0.02, 0.07)	
Serum TfR, mg/L							0.505
Baseline	5.3 (5.0, 5.7)	5.3 (5.0, 5.7)	5.3 (5.0, 5.7)	5.1 (4.8, 5.4)			
Endpoint	2.9 (2.8, 3.1)	3.1 (3.0, 3.3)	2.8 (2.7, 2.9)	3.1 (3.0, 3.3)	−0.03 (−0.06, −0.01)	−0.01 (−0.03, 0.02)	
Body iron, mg/kg							0.164
Baseline	2.0 (1.1, 3.0)	1.7 (0.8, 2.7)	2.2 (1.3, 3.1)	2.4 (1.5, 3.4)			
Endpoint	7.2 (6.8, 7.7)	5.0 (4.6, 5.5)	7.1 (6.7, 7.6)	4.7 (4.3, 5.0)	0.06 (0.05, 0.07)	0.01 (−0.00, 0.02)	
ZnPP, μmol/mol heme							0.984
Baseline	66.5 (61.1, 72.4)	69.0 (63.4, 75.2)	67.6 (62.1, 73.6)	66.1 (60.7, 71.9)			
Endpoint	75.2 (71.8, 78.5)	87.5 (83.6, 91.8)	75.2 (71.8, 78.7)	87.7 (83.4, 92.0)	−0.07 (−0.10, −0.04)	0.00 (−0.03, 0.03)	
Mean corpuscular volume, fL							0.734
Baseline	78.5 (77.4, 79.6)	78.7 (77.6, 79.8)	78.2 (77.1, 79.3)	79.1 (78.0, 80.4)			
Endpoint	81.1 (80.5, 81.7)	79.8 (79.3, 80.4)	81.1 (80.5, 81.7)	79.6 (79.1, 80.2)	0.01 (0.00, 0.11)	0.00 (−0.00, 0.01)	
CRP, mg/L							0.056
Baseline	0.63 (0.45, 0.82)	0.65 (0.47, 0.85)	0.66 (0.49, 0.86)	0.67 (0.50, 0.87)			
Endpoint	0.93 (0.67, 1.22)	1.23 (0.94, 1.58)	0.80 (0.52, 1.12)	0.81 (0.56, 1.10)	0.03 (−0.05, 0.11)	0.09 (0.01, 0.18)	
AGP, g/L							0.044
Baseline	0.81 (0.78, 0.85)	0.80 (0.76, 0.83)	0.82 (0.79, 0.86)	0.80 (0.76, 0.83)			
Endpoint	0.80 (0.76, 0.84)	0.84 (0.79, 0.89)	0.78 (0.74, 0.83)	0.77 (0.73, 0.81)	0.02 (−0.02, 0.05)	0.04 (0.01, 0.07)	
Anemia[4]							0.236
Baseline	23 (21)[5]	26 (23)	25 (22)	22 (19)	0.21 (0.08, 0.57)	0.69 (0.30, 1.60)	
Endpoint	9 (8)	39 (35)	14 (12)	28 (24)			
ID[6]							0.982
Baseline	38 (35)	32 (29)	42 (36)	35 (30)	0.07 (0.02, 0.24)	1.05 (0.46, 2.41)	
Endpoint	5 (5)	30 (27)	6 (5)	31 (27)			
IDA[7]							0.592
Baseline	14 (13)	15 (13)	18 (16)	12 (10)	0.06 (0.01, 0.38)	0.83 (0.26, 2.60)	
Endpoint	3 (3)	16 (14)	2 (2)	15 (13)			
Elevated CRP (>5 mg/L)							0.064
Baseline	40 (36)	42 (38)	45 (39)	47 (40)	1.36 (0.47, 3.90)	2.11 (0.79, 5.64)	
Endpoint	10 (9)	15 (13)	5 (4)	9 (8)			
Elevated AGP (>1 g/L)							0.356
Baseline	16 (15)	17 (15)	23 (20)	19 (16%)	0.98 (0.43, 2.20)	2.43 (1.16, 5.10)	
Endpoint	17 (16)	34 (30)	26 (22)	19 (16%)			

[1] AGP, α_1-acid glycoprotein; CRP, C-reactive protein; $FeSO_4$, ferrous sulfate; ID, iron deficiency; IDA, iron deficiency anemia; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA; SF, serum ferritin; TfR, transferrin receptor; ZnPP, zinc protoporphyrin.

[2] Intervention effects [β values (95% CIs)] were estimated for continuous outcome variables by 2-factor ANCOVA adjusting for respective baseline values, age, sex, grade, and site. Values were log-transformed to perform ANCOVA, and the reported intervention effects [βs (95% CIs)] are reported as log-transformed data. ORs (95% CIs) were estimated for dichotomous outcome variables by using 2-factor binary logistic regression analysis adjusting for respective baseline values, sex, age, grade, and site.

[3] Covariate-adjusted geometric means (95% CIs), calculated from the estimated marginal means obtained by running separate 1-factor (treatment group as factor) ANCOVA on all outcome variables (baseline and endpoint), including respective baseline values (only for endpoint variables), age, sex, site, and grade as covariates (all such values). Data were log-transformed to perform ANCOVA.

[4] Anemia was defined according to the age specific cutoffs for hemoglobin of <11.0, <115, and <120 g/dL for children aged <5 y, 5–11, and ≥12 y of age, respectively.

[5] n; percentage in parentheses (all such values).

[6] ID was defined as SF <12 μg/L for children <5 y of age, SF <15 μg/L for children ≥5 y (28), or TfR >8.3 mg/L. We adjusted SF for inflammation by using the correction factors suggested by Thurnham et al. (29, 30).

[7] IDA was defined as being iron-deficient and anemic.

TABLE 4
Effects of providing children with fortified biscuits containing iron and EDTA, alone and in combination, for 28 wk on cognition[1]

					Estimated intervention effects[2]		
	$FeSO_4$ (n = 110)	Na_2EDTA (n = 112)	NaFeEDTA (n = 116)	Placebo (n = 117)	Iron	EDTA	Iron × EDTA, P
KABC-II							
Atlantis (working memory)							0.618
Baseline	31.5 (28.6, 34.7)[3]	30.9 (28.1, 34.0)	28.4 (25.4, 31.7)	28.5 (25.8, 31.4)			
Endpoint	35.2 (32.1, 38.6)	37.1 (34.1, 40.4)	36.4 (33.5, 39.6)	37.5 (34.1, 41.2)	−0.03 (−0.09, 0.03)	−0.01 (−0.06, 0.05)	
Atlantis delayed (long-term memory)							0.912
Baseline	5.4 (4.6, 6.2)	5.5 (4.8, 6.2)	5.5 (4.8, 6.2)	4.9 (4.2, 5.6)			
Endpoint	7.6 (6.9, 8.4)	7.9 (7.1, 8.7)	7.5 (6.7, 8.3)	7.9 (7.1, 8.8)	−0.02 (−0.08, 0.04)	−0.002 (−0.06, 0.06)	
Hand movement (short-term memory)							0.479
Baseline	5.3 (4.6, 6.0)	5.2 (4.6, 5.8)	5.5 (4.8, 6.2)	5.0 (4.3, 5.7)			
Endpoint	5.5 (5.1, 6.1)	5.8 (5.2, 6.3)	5.8 (5.3, 6.4)	5.9 (5.3, 6.5)	−0.02 (−0.07, 0.03)	−0.01 (−0.06, 0.05)	
Triangles test (visuospatial cognition)							0.372
Baseline	9.9 (8.8, 11.1)	9.6 (8.5, 10.8)	9.0 (7.7, 10.4)	9.9 (8.3, 11.8)			
Endpoint	11.0 (10.3, 11.8)	11.8 (11.0, 12.6)	11.0 (10.3, 11.8)	10.8 (10.1, 11.5)	−0.00 (−0.04, 0.03)	0.04 (−0.002, 0.07)	
HVLT							
Recall 1							0.666
Baseline	3.6 (3.1, 4.1)	3.2 (2.8, 3.6)	3.3 (2.9, 3.7)	3.6 (3.2, 4.0)			
Endpoint	4.2 (3.8, 4.6)	3.9 (3.5, 4.4)	4.0 (3.6, 4.4)	4.0 (3.5, 4.5)	0.02 (−0.04, 0.08)	−0.002 (−0.06, 0.05)	
Recall 2							0.578
Baseline	5.1 (4.5, 5.7)	4.7 (4.2, 5.1)	4.5 (4.0, 5.0)	5.4 (4.9, 6.0)			
Endpoint	5.6 (5.2, 6.1)	5.7 (5.2, 6.2)	5.9 (5.4, 6.4)	5.2 (4.7, 5.8)	0.03 (−0.02, 0.08)	0.03 (−0.01, 0.08)	
Recall 3							0.590
Baseline	5.2 (4.6, 5.8)	4.8 (4.2, 5.3)	5.2 (4.6. 5.8)	6.0 (5.2, 6.8)			
Endpoint	6.1 (5.6, 6.6)	6.3 (5.8, 6.9)	6.4 (5.8, 7.0)	6.1 (5.6, 6.7)	0.00 (−0.05, 0.05)	−0.01 (−0.03, 0.06)	
Total scores of recalls							0.650
Baseline	10.7 (9.2, 12.5)	11.2 (9.6, 13.1)	11.5 (9.8, 13.4)	11.9 (10.2, 13.9)			
Endpoint	12.3 (10.3, 14.6)	11.3 (9.5, 13.5)	12.1 (10.2, 14.3)	10.6 (8.9, 12.6)	0.06 (−0.04, 0.16)	−0.03 (−0.07, 0.03)	
Recognition							0.723
Baseline	10.2 (9.6, 10.8)	10.5 (9.8, 11.1)	10.1 (9.6, 10.7)	10.1 (9.5, 10.7)			
Endpoint	10.4 (9.9, 10.9)	10.6 (10.1, 11.2)	10.4 (9.9, 10.8)	10.8 (10.3, 11.3)	−0.02 (−0.04, 0.01)	−0.01 (−0.03, 0.02)	
Discrimination index							0.333
Baseline	6.5 (5.7, 7.4)	5.8 (5.1, 6.7)	5.7 (4.9, 6.5)	6.1 (5.3, 6.9)			
Endpoint	6.1 (5.3, 7.0)	6.1 (5.3, 7.0)	6.6 (5.8, 7.5)	6.5 (5.7, 7.4)	−0.02 (−0.07, 0.04)	−0.02 (−0.07, −0.04)	

[1] $FeSO_4$, ferrous sulfate; HVLT, Hopkins Verbal Learning Test; KABC-II, Kaufman Assessment Battery for Children, Second Edition; Na_2EDTA, sodium EDTA; NaFeEDTA, ferric sodium EDTA.

[2] Intervention effects [β values (95% CIs)] were estimated for continuous outcome variables by 2-factor ANCOVA adjusting for respective baseline values, age, sex, grade, and site. Values were log-transformed to perform ANCOVA, and the reported intervention effects [βs (95% CIs)] are reported as log-transformed data.

[3] Covariate-adjusted geometric means (95% CIs), calculated from the estimated marginal means obtained by running separate 1-factor (treatment group as factor) ANCOVA on all outcome variables (baseline and endpoint), including respective baseline values (only for endpoint variables), age, sex, site, and grade as covariates (all such values). Data were log-transformed to perform ANCOVA.

Our study has limitations. We did not measure fecal or renal excretion of EDTA-chelated lead because of the difficulties of obtaining multiple fecal and 24-h urine collections from children in our study setting. The fortificants were provided in a noninhibitory food matrix; the effects of iron fortification of inhibitory foods on BPb may differ because of the presence of native chelators, such as phytic acid and polyphenols. The actual change in BPb was lower than that used for the power calculation; however, we were able to discriminate this smaller change because the variance in BPb was much lower than in the Indian study used to estimate the original sample size; we also enrolled ~20% more subjects than required from the sample size calculation. Although ZnPP was significantly lower in the iron groups at endpoint than in the Na_2EDTA or placebo groups, ZnPP remained elevated in all 4 groups. This suggests the persistence of iron-deficient erythropoiesis despite a clear reduction in ID on the basis of SF and TfR in the 2 groups who received iron fortification. Lead inhibits the activity of the ferrochelatase that catalyzes insertion of iron into the protoporphyrin ring to form heme; in severe lead poisoning (BPb $\geq$30 μg/dL), this can increase ZnPP (53), but the BPb elevations in our subjects were much lower, suggesting that the persisting elevations of ZnPP in our study were unlikely due to lead exposure.

In conclusion, our findings suggest that, in lead-exposed populations, NaFeEDTA should be the iron fortificant of choice, because it reduces both ID and BPb. Because the cognitive deficits of lead poisoning may be irreversible (50), primary prevention is critical. Iron-fortification programs are rapidly expanding worldwide, and the urban poor of Latin America, Africa, and Asia are major target groups. An advantage of iron fortification of staple foods as a BPb mitigation strategy is that it may reach even the poorest and most disadvantaged groups (i.e., those most likely to be lead-exposed). In addition, iron fortification can reduce the risk of IDA in women of reproductive age and pregnant women, and thereby might reduce fetal lead exposure.

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