UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE EFFECT OF IRON FORTIFICATION OF LENTILS ON BLOOD AND COGNITIVE STATUS AMONG ADOLESCENT GIRLS IN BANGLADESH

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for

the degree of

MASTER OF SCIENCE

By

AMY L. BARNETT

Norman, Oklahoma

THE EFFECT OF IRON FORTIFICATION OF LENTILS ON BLOOD AND COGNITIVE STATUS AMONG ADOLESCENT GIRLS IN BANGLADESH

A THESIS APPROVED FOR THE DEPARTMENT OF PSYCHOLOGY

BY THE COMMITTEE CONSISTING OF

Dr. Michael J. Wenger, Chair

Dr. Lauren Ethridge

Dr. Eric Day

© Copyright by AMY L. BARNETT 2022

All Rights Reserved.

Table of Contents

Table of Contents	
Prevalence of Iron Defieciency	1
Iron Biomarkers	1
Iron Status in Bangladesh	2
Iron Supplementation	3
Feasibility Study	4
Methods	5
Results	10
Discussion	19
References	35

List of Tables

Table 1, Participant Characteristics	
Table 2, Baseline Biomarkers	
Table 3, Endline Biomarkers	
Table 4, Baseline Cognitive Measures	
Table 5, Endline Cognitive Measures	
Table 6, Plausibility Analysis	

List of Figures

Figure 1, CONSORT Diagram	

Abstract

Iron deficiency is the world's most common nutrient deficiency according to the World Health Organization. Iron deficiency is especially prevalent in undeveloped countries and rural areas. A double-blind, cluster-randomized design was used among 359 10-17 year old Bangladeshi girls in a three arm trial. Iron-fortified lentils, non-fortified lentils, and no lentil (usual intake) were served to the girls over 4 months with a 100% compliance rate. Blood biomarkers and cognition were assessed using ANCOVAs with group as a factor and age as a covariate. Participants in the fortified lentil condition showed better blood iron status at endline (Hb, sFt, sFtR, TBI) than the other groups. The fortified lentil group also performed better on cognitive measures. Implementing iron-fortified lentils into rural areas may be an effective way to protect against cognitive deficits associated with iron deficiency.

Keywords: iron deficiency, anemia, cognition, biomarkers, Bangladesh, adolescent

Introduction

Approximately 22.8% of adults worldwide are iron deficient making it the most common nutrient deficiency in the world (Gardner & Kassebaum, 2020). Iron deficiency (ID) is the leading cause of anemia, contributing about 50% of all anemia cases (World Health Organization; WHO, 2001). According to the World Health Organization, the global prevalence of anemia is even higher in women of reproductive age with rates of 29.9% (WHO, 2019). Children aged 5 to 59 months have a prevalence of anemia at 39.8% (WHO, 2019). ID and iron deficiency anemia (IDA) in south Asia affects 600 million people (WHO, 2000). In southeast Asia, including Bangladesh, Bhutan, India, and others, 60% of women, 36% of men, and 66% of children were iron deficient anemic resulting in 32,400 deaths in 2000 (Stoltzfus, 2003).

Iron is the component of hemoglobin (Hb) that binds oxygen and carries it to the tissues of the body (Pittman, 2011). Iron is present in every cell of the body, including the brain (Mills, Dong, Wang, & Xu, 2010). In addition to aiding in oxygen transport, iron affects the brain in other ways (Ward et al., 2014). Iron is a cofactor for the production of myelin, which is the insulation of the axon of a neuron that allows neurons to communicate quickly and efficiently. Iron is important in the process of neurogenesis and synaptogenesis, and iron is also required for neurotransmitter synthesis and reuptake (Beard, 2003).

There are a variety of biomarkers for iron status. Serum ferritin (sFt) is a protein that binds stored iron. The amount of ferritin in the blood usually gives us an idea of how much iron the body has in storage overall, with higher amounts meaning better iron stores. The amount of ferritin in the blood can be inflated by factors such as inflammation or infection. Soluble transferrin receptor (sTfR) is a transporter protein for iron. Generally, the amount of sTfR in the blood increases with iron deficiency, so lower numbers indicate a more positive iron status. Total

iron binding capacity measures the blood's capacity to bind iron with transferrin, reflecting the total transferrin concentration. Total body iron (TBI) is calculated as: body iron (mg/kg) = - [log(R/F ratio) - 2.8229] / 0.1207, where R/F is the ratio of sTfR to sFt (Cook et al., 2003). Other biomarkers include red blood cell distribution width, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

The various iron biomarkers change over time at different rates as iron stores change. For example, in the case of iron deficiency non-anemia (IDNA), stored iron and sFt both decline relatively rapidly, while Hb levels decline at a slower rate. Once Hb levels have declined to a predefined value (usually 12 g/dL), the diagnosis changes from IDNA to IDA. Meanwhile, sTfR levels increase as there is less iron to be transported (Guthrie & Picciano, 1995; Northrop-Clewes & Thurnham, 2013). Over the course of development, there are periods during which the demand for iron is high. For example, children and adolescents who had chronic iron deficiency show poorer affect, motor, and cognitive skills when compared to peers who were not iron deficient early in life (Lukowski et al., 2010). Adolescence is another period during which the demand for iron is high, particularly for female adolescents, because iron needs double during this period due to the onset of menses and other factors (Beard, 2000). The present study is focused on female adolescents in Bangladesh.

Iron status in Bangladesh and the rationale for fortification

Bangladesh is a South Asian country consisting of eight divisions, 64 districts, and 495 upazilas, or "sub-districts" (*Upazila List*, 2022). The Dhaka upazila within the Dhaka Division, containing the country's capital, is the most populous upazila in Bangladesh (*Upazila List*, 2022). Bangladesh has a population of over 167 million people, making it the 10th most densely populated country in the world (United Nations, 2019).

Rates of anemia in Bangladesh are high, especially when compared to other countries around the world. Specifically in Bangladesh, 36.7% of women of childbearing age (15-49 years) are anemic, and 43.1% of children aged 6-59 months are anemic (WHO, 2021). For comparison, the global prevalence of anemia for women of childbearing age is 29.9% (WHO, 2021). This is considered as a severe public health problem by the World Health Organization. Rural areas in Bangladesh have even higher rates of anemia. Among school aged children (SAC) 12-14 years old living in rural areas, 18.1% have anemia compared to 13.2% of SAC living in urban areas (Rahman et al., 2016).

Lack of compliance with iron supplementation has been shown to be a crucial factor for the still existing high rates of iron deficiency in developing countries. Other causes of poor compliance include incorrect storage and imprecise methods of supplementation intake (Rabindrakumar et al., 2021). Additionally, contaminated water, poor hygiene, and lack of bioavailability may lead to poor compliance with iron supplementation (Northrop-Clewes & Thurnham, 2013).

An alternative to supplementation is iron fortification, and there are many benefits of fortifying staple foods such as lentils with iron. For example, fortification helps improve the availability of iron-rich food sources in locations which previously have had little access. It also increases the bioavailability of iron to be absorbed (DellaValle et al., 2013). Previous research has indicated that location may be important when choosing a correct method of growing iron fortified lentils, and DellaValle et al. found that an increase in bioavailability of iron is more important than the iron concentration when adding iron fortified lentils into a diet (2013). Using crops that are regionally available to the target population is beneficial when implementing iron fortification. Many studies have used beans, pearl millets, and lentils depending on the location

of the study. By doing so, the residents of the area are more likely to effectively integrate the iron fortified crops into their diet. All of this suggests that provision of an iron fortified lentil in Bangladesh may be an effective way to address the iron needs of female adolescents

Iron fortified lentils and a feasibility study

Small red lentils grown in Saskatchewan were fortified with a solution of sodium ferric ethylenediaminetetraacetate (NaFeEDTA), resulting in approximately 13-14 mg of iron per 100 kg of lentils (Yunus, 2018). A feasibility study was conducted to determine the correct amount and preparation of the lentils, regionally called daal, for the adolescent girls in Bangladesh. In this feasibility study, the researchers had 100 10–17-year-old adolescent females eat lentils for 12 weeks in order to determine the best amount and way to prepare the lentils. The researchers provided both thick and thin lentils. One hundred grams of uncooked lentils were prepared for both the thick and thin conditions. The differences in the recipes for the lentils are that the thick lentils are prepared with 5 grams of turmeric compared to 3 grams, 700 mL of water compared to 1.5L, and the cooking time was 18 minutes compared to 53 minutes (Yunus, 2018). Based on measures of palpability, hunger, satiety, etc., the researchers found that there was a higher palpability and preference for the thick lentils, meaning that the girls preferred the thick over thin lentils. Therefore, the thick lentils were chosen for the study.

To determine the correct amount of lentils to prepare, the researchers analyzed how many lentils were not eaten during observational feeding for each of three test amounts, 25 grams, 37.5 grams, and 50 grams. During this observational period, the researchers found the best amount of lentils to prepare is 37.5 grams, which provided 86% of the recommended dietary allowance (RDA) for the younger girls (9-13 years old) and 46% for the older girls (14-18 years old) (Yunus, 2018; Institute of Medicine, 2003). The 25-gram lentil preparation only provided only

32.6% of the RDA in the older girls, so this quantity was rejected. On the other hand, the 50gram lentil exceeded the RDA for the younger girls (Yunus, 2018; Institute of Medicine, 2003). In essence, the 37.5 gram serving of thick lentils was the most effective way to add the iron fortified lentils into the diet of Bangladesh adolescent girls with a 100% compliance rate. Therefore, the current study hypothesized that iron fortified lentils would improve their iron status as measured by iron biomarkers and that this improvement in iron status will be accompanied by improvements in cognitive performance.

Methods

Subjects

Participants included 359 adolescent girls aged 10-17 years from four upazilas in Bangladesh including Muktagacha, Mymensingh Sadar, Bhaluka, and Gaffargaon in the Mymensingh district. The study was conducted at Bangladesh Rural Advancement Committee (BRAC) clubs within the four upazilas. Adolescent girls in the BRAC adolescent development program were chosen for the study. This program provides scholarly education, health education, social education, poverty education, etc. among both young women and men regardless of socioeconomic status and education. Participants were generally healthy and were excluded if they were either pregnant or breastfeeding.

Study design

A double-blind, cluster-randomized design was used from the community sample from the BRAC clubs. Forty-eight BRAC clubs (clusters) were randomly selected from the four subregions. This was further randomly divided into 16 blocks with 3 clusters in each block for a total sample size of 1260 adolescent girls with 420 girls in each block. A total of 118 girls were included in the final analysis in the no lentil condition, 124 in the non-fortified lentil condition,

and 117 in the fortified lentil condition for a final sample size of 359 adolescent girls with both blood and cognitive data. (Fig. 1).

Three intervention groups were used in this study. In the first group the adolescent girls were served iron-fortified lentils, in the second group the girls were served non-iron-fortified lentils, and the third group was not served any lentils (control). The iron-fortified lentils were fortified with 1600 ppm of iron compared to approximately 75-90 ppm of iron in the non-fortified lentils. In the iron-fortified and non-iron-fortified conditions, 37.5 grams of raw lentils (~200 grams cooked) were served 5 days a week for a total of 85 feeding days. All lentils were served with one cup of cooked rice.

At baseline and endline, self-reported demographic factors were measured, along with a Food Frequency Questionnaire (FFQ), anthropometrics, and 6mL sample of venous blood taken to assess the iron biomarkers. The intervention occurred during the 6th-23rd weeks of the total 28 weeks. Blood measures included (a) complete blood count (CBC), which included the erythrocyte sedimentation rate (ESR); hemoglobin (Hb); hematocrit (HCT); packed cell volume (PCV); mean corpuscular hemoglobin (MCH); mean corpuscular volume (MCV); mean corpuscular hemoglobin concentration (MCHC); red blood cell (RBC) and white blood cell (WBC) total count; (b) serum ferritin (sFt); (c) soluble transferrin receptor (sTfR); (d) total body iron (TBI); and (e) C-reactive protein (CRP).

Cognitive tasks

A total of five tasks were used to measure cognition, including the Simple Reaction Time Task (SRT), Go/No-Go Task (GNG), Attentional Network Task (ANT), Cued Recognition Task (CRT), and the Sternberg Memory Search Task (SMS).

The SRT is a simple measure of reaction time which requires no decision-making or discrimination (Wickens et al, 2004; Wenger et al., 2021). The participants were instructed to press a keyboard key as soon a visual stimulus was presented on the computer monitor. Five practice trials were completed where feedback was provided. After the practice trials, there were 20 test trials and feedback was not provided. The interstimulus interval varied between presentations with a range of 750-1500 ms. Lower reaction times indicate better cognitive status on the SRT.

The GNG explores sustained attention and inhibitory control. Participants were presented two stimuli in random order: one that they were supposed to respond to (Go) and one to which they were supposed to inhibit their response (No-Go) (Wickens et al., 2004; Wenger et al., 2021). In 20% of the trials, the stimuli required a 'Go' response and 80% required a 'No-Go' response. On trials where the Go stimulus was presented, participants were instructed to press the / key with the index finger of their dominant hand. Participants were instructed to withhold their response on a no-go stimulus. Ten practice trials were completed prior to testing and participants were provided feedback on the accuracy of their responses. A total of 30 go trials and 120 no-go trials were completed without feedback after practicing. A centrally fixated cross was presented on the screen for 400-700 ms and the stimulus was presented for 300 ms. The participants had up to 1700 ms to respond, otherwise the trials continued, and the next fixation cross was presented. Improvement on the GNG is indicated by a decrease in reaction time from baseline to endline.

The ANT examines three levels of attention: low level attentional capture, mid-level spatial selective attention, and high-level control (Fan et al., 2002; Wenger et al., 2021). The participants were presented with a fixation cross on the center of the screen for 300-600 ms. A cue (asterisk) then appeared on the screen in the one of five locations (absent, center, above

fixation, below fixation, above and below fixation) for 200 ms. A second fixation cross was then presented for 200 ms followed by the test stimulus for 1500 ms, a central arrow pointing left or right along with a set of flanker stimuli---a pair of arrows on either side of the central arrow pointing either the same way or opposite way relative to the central arrow. The arrows could be displayed either above or below the fixation point. Participants were instructed to determine the direction of the central arrow (ignoring the flanker arrows) using the z or / keys. An alerting score was calculated by subtracting the median reaction time of correct responses on the trials with 2 cues from the trials with 0 cues. An orienting score was calculated by subtracting the median reaction time of correct responses on the trials with spatial cues from the trials with center cues. Finally, a conflict score was calculated by subtracting the median reaction time of correct responses on the trials with consistent flankers from the trials with inconsistent flankers. Improvement on the ANT is indicated by a decrease in reaction time from baseline to endline on the items that varied in cue and flanker type. Higher reaction times on the alerting, orienting, and conflict scores indicate improvement on the ANT.

The CRT is a recognition memory paradigm where the participant is presented with pictures of common, nameable objects and then is later tested on how well they remember those items when later presented along with an equal number of "new" items (Wenger et al., 2010; Wenger et al., 2021). The participant judged whether the presented item at the time of testing is "old" or "new." In the testing phase, each test image (old or new) was presented with two, three, or four quadrants (two, three, or four cues) of the image visible for 3 seconds and the participants were instructed to judge each image by pressing the z key for a "new" stimulus and the / key for an "old" stimulus. No feedback was provided. Improvement on the CRT is indicated by a

decrease in reaction time from baseline to endline. Higher reaction times on the percent change in capacity reflect improvement on the CRT.

The SMS tests the participant's ability to search memory (Sternberg, 1966; Wenger et al., 2021). On each trial, participants were presented with either 1, 3, or 6 items to be remembered. The participants are presented both old and new items at the time of testing. Participants are shown a fixation cross for a random duration from 400-1000 ms. This was followed by the sequential presentation of 1, 3, or 6 graphical symbols. There was then a 2 sec blank interval followed by the test item. The test item could be either one of the symbols presented on that trial (old) or a novel symbol (new). Decreased reaction times from baseline to endline indicate an improvement on the SMS for: intercept, new items; intercept, old items; slope, new items; and slope, old items.

Ethics

Ethical approvals were received from the University of Saskatchewan, Canada (Bio#17– 177), Marywood University, USA (IRB#1139116–2), and the Bangladesh Medical Research Council (BMRC/NREC/2016–2019/455) as per their respective protocols. Informed written consent and assent were taken from each participant and their respective parents, and a copy of the signed assent and consent form was given to the participants and parents.

Statistical analyses

One-way analyses of covariance (ANCOVA) with post-hoc Tukey's tests were used to compare the baseline blood measures between the three intervention groups using group as a factor and age as a covariate. Endline blood and behavioral variables were analyzed with oneway ANCOVAs with group as a factor and the baseline value of the variable and age as covariates.

The plausibility of the change in the iron biomarkers as the source of the change in the behavioral measures was assessed by regressing the change scores from the cognitive tasks onto the change scores for each of the iron biomarkers. Change scores were calculated by subtracting the endline value from the baseline variable. A final model for each regression was determined using a stepwise model selection procedure. For all analyses, both the value of sFt and its log transformed value were used, given the non-normal distribution of sFt.

Results

No participants were inflamed based on CRP levels at either baseline or endline (> 10.0mg/l) (Table 1). Anemia (Hb < 12g/dL) increased approximately 16% and iron deficiency (sFt < 15 μ g/L) increased about 10% from baseline to endline. Iron deficiency anemia (Hb < 12.0g/dL and sFt < 15.0 μ g/L) increased approximately 10%, and iron deficiency non-anemia (Hb < 12.0g/dL and sFt > 15.0 μ g/L) remained the same throughout the entire timeline. *Blood biomarkers* (Table 2, Table 3)

All three conditions had equivalent blood markers at baseline except for sFt, where the group consuming the iron-fortified lentils (FL) had a higher sFt (M = 54.32, SE = 2.8) than the group consuming the control lentils (M = 45.03, SE = 2.72) and the no lentil condition (M = 50.96, SE = 2.77) (Table 2). Age was a significant covariate (F = 5.05, p < .05) at baseline for ln sFt, RBC, and MCV.

At endline, the fortified lentil condition (M = 12.32, SE = 0.05) had a higher Hb level than both the non-fortified lentil (M = 12.17, SE = 0.05) and the control groups (M = 12.12, SE = 0.05) (Table 3). From baseline to endline, Hb levels decreased in all three groups, with the largest decrease seen in the no-lentil group (mean change = 0.36). Note that even though Hb levels decreased overall, IDA decreased and IDNA remained the same. At endline, the fortified lentil group (M = 47.88, SE = 1.57) had higher sFt levels than both the non-fortified lentil (M = 37.11, SE = 1.56) and no lentil (M = 38.13, SE = 1.58) groups. Ferritin levels decreased in all three groups from baseline to endline. The highest level of decrease was found in the no lentil control group (mean change = 12.83) and the least amount of decrease was found in the fortified-lentil group (mean change = 6.47). Note that even with these decreases, sFt levels were on average well above the criteria for IDNA and that the overall prevalence of IDNA did not change.

At endline, the non-fortified lentil group (M = 4.24, SE = .11) had a higher sTfR value than the fortified lentil group (M = 3.68, SE = .11) but there was no significant difference between the non-fortified lentils and no-lentil groups or between the fortified lentils and no lentil groups (M = 3.97, SE = .12). From baseline to endline, the fortified lentil and non-fortified lentil groups increased in sFtR (mean change = .1), with the non-fortified lentil group showing the most increase (mean change = .32). The no lentil group (M = 3.98, SE = .32) showed a decrease in sTfR from baseline to endline (mean change = .01).

At endline, the fortified lentil group (M = 7.37, SE = .15) had a higher TBI value than both the non-fortified lentil group (M = 6.17, SE = .15). and the no lentil group (M = 6.56, SE = .16), although there was no significant difference between the non-fortified and no lentil groups. From baseline to endline, TBI values decreased for all groups with the least decrease in the fortified-lentil group (mean change = .63).

At the conclusion of the study, there were no differences among the three groups for RBC, Hct, MCV, CRP, or WBC and the baseline blood values were significant covariates for all variables except for CRP (Table 2). From baseline to endline, Hb, sFt, RBC, Hct, CRP, WBC, TBI, and ln_sFt values decreased in all groups. It should be noted that although the mean values for these variables decreased, the endline values were above the threshold for ID and anemia, and the percentage of participants who were ID or anemic decreased or stayed unchanged. *Cognitive Tasks* (Table 4, Table 5)

SRT

Group was a significant predictor, and age was a significant covariate for BL RTs in each of the three conditions separately, but post-hoc comparisons revealed no significant differences in overall reaction time between the three groups at baseline. At endline, the fortified lentil group performed faster (M = 257 ms, SE = 3) than both the non-fortified lentil (M = 277 ms, SE = 3) and no lentil (M = 268 ms, SE = 3) groups, although there was no difference between the non-fortified and no lentil groups. Reaction times from baseline to endline decreased for all three groups with the largest decrease seen in the fortified lentil group (mean change = 10 ms).

GNG

Group was a significant predictor and age was a significant covariate in each of the three groups separately, but post-hoc comparisons revealed no significant differences in RT between the three groups at baseline or endline. Age and baseline RT were significant factors at endline. RTs decreased for all three groups, with the least decrease in the no lentil group (mean change = 12 ms).

ANT

Age was a significant covariate at baseline while age and baseline reaction time were significant covariates for the 0-cue condition at endline. Group was a significant predictor at endline, although post-hoc comparisons revealed no significant differences in RT between the three groups at baseline or endline. RTs in the 0-cue condition decreased in all groups from

baseline to endline, with the fortified lentil group showing the most decrease (mean change = 43 ms).

There were no significant differences in RTs in the 2-cue condition at baseline. At endline, the non-fortified lentils had a longer RT (M = 544 ms, SE = 3) than both the fortified (M= 525 ms, SE = 3) and no lentil (M = 531 ms, SE = 3) groups, although there was no significant difference between the fortified and no lentil groups. Group was a significant predictor and baseline reaction time was a significant covariate at endline. Reaction time in the 2-cue condition decreased in all groups from baseline to endline with the fortified lentils group (mean change = 51 ms) showing the most decrease and the non-fortified lentil group (mean change = 40 ms) showing the least decrease.

There were no significant differences in alerting scores at baseline. At endline, the nonfortified lentil group (M = 31 ms, SE = 3) had a lower alerting score than the fortified lentils group (M = 46 ms, SE = 3). There was no difference between the non-fortified lentil group or between the no lentil (M = 41 ms, SE = 3) and fortified lentil group. Age was a significant covariate at endline. Alerting scores from baseline to endline increased in the fortified (mean change = 12 ms) and no lentil group (mean change = 7 ms) with the fortified lentils showing the most increase in alerting scores.

There were no significant differences in RTs in the center cue condition between the three groups at baseline. At endline, group was a significant predictor and age, and baseline RT were significant covariates on the ANT center cue variable. At endline, the fortified lentil group (M = 565 ms, SE = 3) had a higher reaction time than both the non-fortified (M = 549 ms, SE = 3) and no lentil (M = 552 ms, SE = 3) groups, although there was no difference between the non-

fortified and no lentil groups. From baseline to endline all three groups decreased in reaction time, with the non-fortified lentil (mean change = 62 ms) showing the most decrease.

There were no significant differences in RTs between the three groups at baseline or endline for the spatial cue condition. Age was a significant covariate at baseline. Baseline reaction time and age were significant covariates at endline. From baseline to endline all reaction times in the spatial cue condition decreased with the most decrease seen for the fortified lentil group (mean change = 53 ms).

There were no significant differences in the orienting score at baseline. Group was a significant predictor, and age and baseline orienting score were significant covariates at endline. At endline the fortified lentil group (M = 57 ms, SE = 3) had a higher orienting score than both the non-fortified (M = 32 ms, SE = 3) and no lentil (M = 35 ms, SE = 3) groups. There was no significant difference between the non-fortified and no lentil groups. From baseline to endline, orienting score in the fortified lentil increased (mean change = 18 ms), while orienting score in the non-fortified lentil (mean change = 15 ms) and no lentil (mean change = 2 ms) groups decreased.

For the incongruent flanker condition, the fortified lentil group (M = 770 ms, SE = 7) had a longer RT at baseline than the non-fortified lentils (M = 738 ms, SE = 7), which had a longer RT than the no lentil group (M = 715 ms, SE = 7). Group and age were significant factors at baseline. Group and baseline reaction time were significant factors at endline, however post-hoc analyses revealed no significant differences in RT at endline. From baseline to endline all RTs in in the incongruent flanker condition for all three groups decreased with the fortified lentil group (mean change = 118 ms) showing the most decrease and the no lentil group (mean change = 64 ms) showing the least decrease. Although group was a significant predictor and age was a significant covariate, post-hoc analyses revealed no significant difference in RTs for the congruent flankers between the three groups at baseline. At endline, the fortified lentil group (M = 572 ms, SE = 4) had a shorter RT than both the non-fortified (M = 586 ms, SE = 3) and no lentil (M = 585 ms, SE = 4) groups. There was no difference between the non-fortified and no lentil groups. Group was a significant predictor, and age and baseline reaction time were significant covariates at endline. From baseline to endline reaction times decreased in all groups, with the most decrease seen in the fortified lentil group (mean change = 78 ms) and the least decrease seen in the no lentil group (mean change = 49 ms).

There was a significant difference in conflict score at baseline, with the fortified lentil group (M = 120 ms, SE = 7) showing the highest conflict score. There was no difference between the non-fortified (M = 89 ms, SE = 6) and no lentil (M = 81 ms, SE = 7) groups. Group was a significant factor at baseline. There was no significant difference in conflict scores between the three groups at endline, although age and baseline conflict score were significant covariates. All three groups decreased in conflict scores from baseline to endline with the fortified lentil group (mean change = 46 ms) showing the most decrease and the no lentil group (mean change = 15 ms) showing the least decrease.

CRT

For the new items given four cues, there was no significant difference in reaction time between the three groups at baseline or endline. Age was a significant covariate at baseline. Age and baseline reaction time were significant covariates at endline. From baseline to endline reaction times for all groups decreased, with the most decrease seen in the fortified lentil group

(mean change = 77 ms) and the least decrease seen in the non-fortified lentil group (mean change = 32 ms).

For the old items given four cues, there was no significant difference in reaction time between the three groups at baseline or endline. Age was a significant covariate at baseline. Age and baseline reaction time were significant factors at endline. From baseline to endline reaction times for all groups decreased, with the most decrease seen in the fortified lentil group (mean change = 69 ms) and the least decrease seen in the non-fortified lentil group (mean change = 14 ms).

With respect to percent change in capacity, at baseline the non-fortified lentil group (M = 40%, SE = 3) had a larger percent change in capacity than the fortified lentil (M = 26%, SE = 3). The no lentil group (M = 38%, SE = 3) had a larger percent change in capacity than the fortified lentil group (M = 26%, SE = 3). There was no significant difference between the non-fortified and no lentil groups at baseline. Group was a significant predictor at baseline. There were no significant differences in percent change in capacity among the three groups at endline. Neither group nor age nor baseline percent change in capacity affected endline percent change in capacity. From baseline to endline percent change in capacity in all three groups increased, with the fortified lentil group (M = 45%, SE = 4) showing the most increase in percent change in capacity.

SMS

The no lentil group (M = 1060 ms, SE = 26) had a significantly lower intercept for the search function for new items at baseline than the non-fortified (M = 958 ms, SE = 25). There was no significant difference between the non-fortified group and the fortified lentil (M = 977 ms, SE = 26) group at baseline. Group was a significant predictor at baseline. There was no

significant difference in reaction times among the three groups at endline, although baseline intercept was a significant covariate at endline. From baseline to endline all three groups decreased the value of the intercept, with the most decrease in the no lentil group (mean change = 123 ms) and the least decrease in the non-fortified lentil group (mean change = 5 ms).

There was no significant difference in the slope of the search function for new items between the three groups at baseline or endline. Neither age nor group nor baseline slope affected endline slope for any group. From baseline to endline slopes for all groups increased, with the most increase seen in the non-fortified lentil condition (mean change = 35 ms).

There were no significant differences in intercepts for the search function for old items among the three groups at baseline or endline. Neither age nor group nor baseline intercept affected endline reaction time for any group. From baseline to endline intercept values for all groups decreased, with the least increase seen in the control group (mean change = 24 ms) and the greatest increase seen in the fortified lentil group (mean change = 50 ms).

The endline slope for the search function for old items for the fortified lentil group (M = 32 ms, SE = 2) was smaller than both the non-fortified (M = 42 ms, SE = 2) and no lentil (M = 38 ms, SE = 2) groups, showing that group was a significant predictor at endline. There was no significant difference between the non-fortified and no lentil groups. From baseline to endline all three groups increased the value of the slope, with the least increase seen in the fortified lentil group (mean change = 3 ms).

SRT

A change in sFt and ln sFt predicted a change in reaction time on the SRT. The more that ln sFt increased from baseline to endline, the more RT decreased. Additionally, the more that sTfR increased, the more RT increased.

GNG

A change in TBI predicted a change in reaction time on the GNG. The more that TBI increased, the more RT decreased.

ANT

None of the changes in biomarkers predicted a change in reaction time in the 0-cue condition, center cue condition, or conflict score. A change in ln sFt predicted a change in reaction time in the 2-cue condition. The more ln sFt increased, the more RT decreased. A change in ln sFt predicted a change in reaction time on the ANT Alerting score. The more the ln sFt increased, the more the alerting score increased.

A change in TBI predicted a change in reaction time in the spatial cue condition. The more TBI increased, the more RT decreased on the spatial cue condition. A change in TBI predicted a change in the orienting score. The more TBI increased, the more the orienting score increased.

A change in ln sFt predicted a change in reaction time in the incongruent flanker condition. The more ln sFt increased, the more RT decreased. A change in sFt predicted a change in reaction time in the congruent flanker condition. The more sFt increased, the more RT decreased.

CRT

None of the changes in the biomarkers predicted a change in RT for new items with 4 cues. A change in sFt predicted a change in the RT for old items with 4 cues. The more sFt increased, the more RT decreased. None of the changes in the biomarkers predicted change in percent change in capacity.

SMS

None of the changes in the biomarkers predicted a change in the intercept for the search function for new items. A change in sTfR predicted a change in the slope of the search function for new items. The more that sTfR increased, the more the slope increased. A change in ln sFt predicted a change in the intercept of the search function for old items. The more ln sFt increased, the more the slope decreased. A change in ln sFt predicted a change in the slope of the search function for old items. The more ln sFt increased, the more the slope decreased. A change in ln sFt predicted a change in the slope of the search function for old items. The more ln sFt increased, the more the slope decreased.

Discussion

In contrast to prior work, this study did not obtain uniform improvements in the iron biomarkers as a function of consuming the fortified lentils (van Thuy et al., 2005; Sun et al., 2007; Andang'o et al., 2007). Instead, this study found that the adolescents who consumed the iron fortified lentils were protected against decreasing iron levels compared to the other two conditions. This is seen specifically in the decrease of sFt and TBI from baseline to endline, with the iron fortified lentil condition showing the least decrease of the three groups.

The participants in the iron-fortified group had the highest levels of Hb, sFt, sFt, and TBI at endline. They also showed the lowest level of sTfR at endline indicating that iron was actively being used in the body. These levels indicate that the iron-fortified lentils were protective against the larger decrease seen in the control lentil or no lentil conditions. The participants in the iron-

fortified lentils group had the least amount of decrease in Hb, sFt, and TBI from baseline to endline compared to the other groups. Although the iron-fortified group showed a decrease in sTfR from baseline to endline, this decrease was minor. The decreases in Hb, sFt, sFtR, and TBI from baseline to endline were modest considering that these levels were above what is considered to be ID or IDA.

Similar protective effects of the iron-fortification lentil were seen in the cognitive tasks. At endline, the iron-fortified lentil condition had the lowest reaction times in the SRT, GNG, CRT, and all aspects of the SMS except for the search function of new items (slope new). The iron-fortified lentil group had the highest alerting and orienting scores on the ANT. On the other hand, the non-fortified lentil group had the lowest reaction times at endline in the ANT center cue condition.

The iron-fortified lentil group had the highest increase in percent change capacity on the CRT. Regarding the SMS, the slope old variable increased in all groups, although the iron-fortified lentil group showed the least increase in slope. In sum, although the iron biomarkers did not improve overall from baseline to endline, smaller declines were seen in the iron-fortified lentil condition, which then corresponded to improvements on the cognitive measures.

Results from this study, including cognitive improvements after intervention, are consistent with previous work. In 2018, Scott et al. found that consumption of a biofortified pearl millet improved cognition over 6 months on the SRT, GNG, ANT, and CRT in adolescents in India. In a study by Murray-Kolb et al., female students in Rwanda had better cognitive scores and iron levels after consuming iron-fortified beans (2017).

In the United States, up to 25% of adolescent girls are iron deficient (Bruner et al., 1996). In a study conducted in Baltimore, MD, adolescent girls aged 13-18 improved on cognitive tasks

regarding memory and attention while taking iron supplements when compared to the control group (Bruner et al., 1996). This study showed that even in an urban population, iron supplementation is still effective.

Family and social factors could have affected the individual differences. Home life, friendships, and school involvement play a role in the adolescents' lives. In addition, socioeconomic status might influence these results, as approximately 24.3% of people in Bangladesh lived under the poverty line in 2016 (Chowdhury et al., 2018). The urban rate of poverty was 29.6% compared to the rural estimate of 33.3% (Chowdhury et al., 2018).

Individual factors of the adolescent girls could have affected the results as well. For example, the target age was 10-17, and this is a time of normal puberty in adolescents. In a study by Malitha et al., researchers found that 51.55% of 10–12-year-olds in the Rajshahi Division in Bangladesh had not yet reached menarche (2020). Since menstruation can lead to iron loss, and thus iron deficiency, menstrual status is important although not measured in this study. Strengths

The BRAC clubs are also a strength in this study. The maintenance of iron status that was seen in this study could positively impact children and adolescents in the BRAC clubs. The BRAC clubs are public clubs not funded by the government, which makes them accessible to a wider population of attendees. Both male and female attendees could benefit from the implementation of iron-fortified lentils in the BRAC clubs.

This study showed the ability to predict changes in cognition through changes in blood biomarkers. The biofortified lentils showed a maintenance effect of iron status as well as improved cognition (through decreased reaction times) during the time of testing. The adolescents had a 0% attrition rate as 100% of the girls enrolled completed the feeding for the

entire 85 days. This shows that the girls enjoyed eating the lentils and the intervention was not difficult to implement into their diets. This is an improvement of taking nutritional supplements, as those have a higher attrition rate (Schultink, 1996).

Future directions

Based on the findings from this study, future studies should investigate the feasibility of implementing iron into the Bangladeshi diet, especially by utilizing non-profit organizations such as the BRAC clubs. Accessibility to iron-fortified lentils through a BRAC club is not only convenient, but necessary, for these adolescent girls. BRAC clubs in Bangladesh should consider incorporating iron-fortified lentils into the diets of the adolescents the club serves. The BRAC clubs would be a positive exemplar for other non-profit organizations in Bangladesh and surrounding countries.

Although this study did not find improvement in all blood and cognitive measures, a theory for maintenance is still plausible. From baseline to endline, the adolescent girls who consumed the iron-fortified lentils had higher levels of sFt than the non-fortified lentil or no lentil conditions at baseline and endline, suggesting a smaller decrease than the other two groups. All groups had a lower risk for anemia, iron deficiency, and iron deficiency non-anemia at baseline (Table 1). The only condition that did not change was iron deficiency non-anemia, suggesting that although iron levels improved, perhaps hemoglobin levels could not compensate (Table 1).

The results of this study suggest that adding biofortified foods into a daily diet could be beneficial for iron status and could help improve cognition. BRAC clubs and other non-profit organizations are especially important in providing nutritious meals to adolescents who would not otherwise have access to iron-rich foods. More work is needed to determine the feasibility of

implementing more programs like the adolescent development programs and the effectiveness on iron-fortified foods to both increase compliance with iron supplementation and improve overall blood status and cognition.

Figure 1: CONSORT Diagram

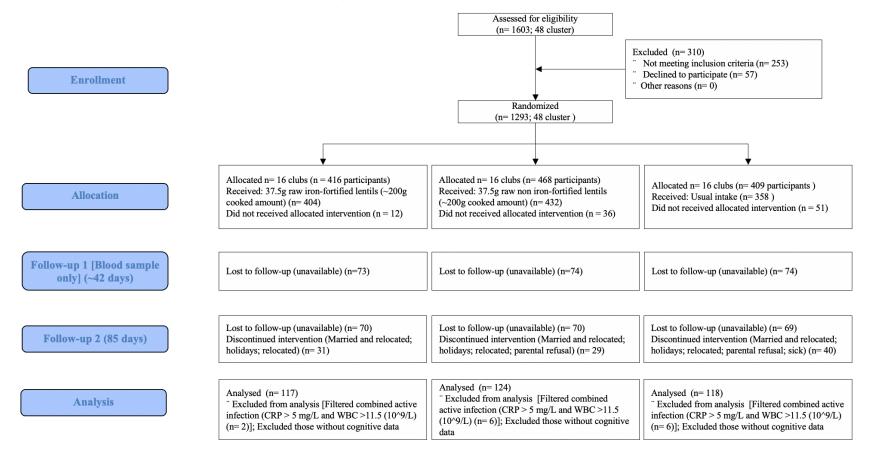


Table 1: Participant Characteristics									
	Group (%)								
Measure	FL	CL	NL						
Anemia BL	29 (24.79%)	35 (28.23%)	33 (27.97%)						
Anemia EL	41 (35.04%)	55 (44.35%)	60 (50.85%)						
ID BL	9 (7.69%)	13 (10.48%)	15 (12.71%)						
ID EL	18 (15.38%)	28 (22.58%)	27 (22.88%)						
IDA BL	5 (4.27%)	9 (7.26%)	11 (9.32%)						
IDA EL	15 (12.82%)	21 (16.94%)	26 (22.03%)						
IDNA BL	4 (3.42%)	4 (3.23%)	4 (3.39%)						
IDNA EL	3 (2.56%)	7 (5.65%)	1 (0.85%)						
Inflammation BL	1 (0.85%)	3 (2.42%)	0						
Inflammation EL	0	0	0						

n = 359, ID, iron deficiency; IDA, iron deficiency anemia; IDNA, iron deficiency non anemia; FL, fortified lentils; CL, control lentils; NL, no lentils

Table 2: B	aseline Bi	omarkers						
				Partial	L	S Means (S	SE)	
Variable	Factor	F	MSE	eta^2	FL	CL	NL	Ordering ¹
						12.38		
Hb	Group	0.60	0.71	0.004	12.50 (0.08)	(0.08)	12.48 (0.08)	FL = CL = NL
	Age	0.25		0.001				
_						45.03		
sFt	Group	2.34	879.13	0.013	54.35 (2.8)	(2.72)	50.96 (2.77)	FL > CL = NL
	Age	5.05*		0.015				
					/	3.56	/	
ln_sFt	Group	1.93	0.56	0.011	3.78 (0.07)	(0.07)	3.72 (0.07)	FL = CL = NL
	Age	7.72**		0.022				
	~					7.11		
TBI	Group	1.31	14.95	0.007	8.00 (0.36)	(0.35)	7.48 (0.36)	FL = CL = NL
	Age	2.14		0.006				
DDC	C	0.10	0.10	0.001		4.61		
RBC	Group	0.13	0.12	0.001	4.62 (0.03)	(0.03)	4.60 (0.03)	FL = CL = NL
	Age	11.86***		0.032		2 0.00		
TT /	C	0.14	0.27	0.010	20(7(0,27))	38.88	20.42 (0.27)	
Hct	Group	2.14	8.37	0.012	39.67 (0.27)	(0.26)	39.43 (0.27)	FL = CL = NL
	Age	0.95		0.003		04 70		
MCV	Cassa	2.22	15 50	0.012	9(12)(0)(2)	84.72	P(0)(0)	$\mathbf{E}\mathbf{I} = \mathbf{C}\mathbf{I} = \mathbf{N}\mathbf{I}$
MCV	Group	2.32	45.50	0.013	86.13 (0.63)	(0.61)	80.00 (0.02)	FL = CL = NL
	Age	6.09*		0.017		26.64		
MCH	Group	0.85	6.64	0.005	26.91 (0.24)	26.64 (0.23)	27.00(0.24)	FL = CL = NL
MCT	Group		0.04		20.91 (0.24)	(0.23)	27.00 (0.24)	$\Gamma L = CL = NL$
	Age	2.12		0.006		31.4		
MCHC	Group	1.24	1.41	0.007	31.20 (0.11)	(0.11)	31 35 (0 11)	FL = CL = NL
WICHC			1.41	0.007	51.20 (0.11)	(0.11)	51.55 (0.11)	$\Gamma L = CL = INL$
	Age	2.5		0.007		3.92		
sTfR	Group	0.09	11.72	0.001	3.78 (0.32)	(0.31)	3.98 (0.32)	FL = CL = NL
5111	Group	0.07	11./2	0.001	5.70 (0.52)	(0.31)	5.70 (0.52)	$\mathbf{L} = \mathbf{C}\mathbf{L} = \mathbf{M}\mathbf{L}$

	Age	0.35		0.001				
						0.99		
CRP	Group	0.24	5.67	0.001	0.78 (0.22)	(0.21)	0.91 (0.22)	FL = CL = NL
	Age	0		0				
	e					10.12		
WBC	Group	2.53	5.06	0.014	9.70 (0.21)	(0.2)	9.60 (0.21)	FL = CL = NL
	Age	0.15		0.001				

n = 359, * p < .05, ** p < .01, *** p < .001; LS, least squares MSE, mean squared error; SE, standard error FL, fortified lentils; CL, control lentils; NL, no lentils; ¹ordering determined using Tukey HSD

				Partial		LS Means (SE)	1	
Variable	Factor	F	MSE	eta^2	FL	CL	NL	Ordering ¹
Hb	Group	5.36**	0.25	0.034	12.32 (0.05)	12.17 (0.05)	12.12 (0.05)	FL > CL = NL
	Age	5.42*		0.018				
	Hb BL	332.81***		0.522				
sFt	Group	35.99***	249.96	0.189	47.88 (1.57)	37.11 (1.56)	38.13 (1.58)	FL > CL = NL
	Age	1.47		0.005				
	sFt BL	515.82***		0.626				
ln_sFt	Group	35.22***	0.14	0.186	3.65 (0.04)	3.39 (0.04)	3.43 (0.04)	FL > CL = NL
	Age	1.47		0.005				
	ln_sFt	764.34***		0.712				
ТВІ	BL Group	47.47***	2.49	0.229	7.37 (0.15)	6.17 (0.15)	6.56 (0.16)	FL > CL = NL
	Age	0.69		0.002	、 ,	~ /	~ /	
	TBI BL	1424.86** *		0.817				
RBC	Group	3.48*	0.04	0.021	4.52 (0.02)	4.50 (0.02)	4.46 (0.02)	FL = CL = NL
	Age	0.68		0.002				
	RBC BL	498.73***		0.611				
Hct	Group	3.74*	3.01	0.023	38.17 (0.16)	38.09 (0.16)	37.7 (0.17)	FL = CL = NL
	Age	5.07*		0.016				
	Hct BL	455.50***		0.589				
MCV	Group	9.32***	4.74	0.056	84.74 (0.21)	85.00 (0.2)	84.73 (0.22)	FL = CL = NL
	Age	0.38		0.001				
МСН	MCV BL Group	2567.17** * 28.77***	0.46	0.890 0.153	27.17 (0.07)	26.74 (0.06)	26.88 (0.07)	FL > CL = NL
	Age	0.02		0.000				
	MCH	4172.05**		0.929				
мснс	BL Group	* 12.84***	0.37	0.075	32.04 (0.06)	31.43 (0.06)	31.68 (0.06)	FL > NL > CL
	Age	1.34	0.07	0.004	52.0 . (0.00)	21.12 (0.00)	21.00 (0.00)	
	MCHC	888.63***		0.737				
sTfR	BL Group	8.23***	1.35	0.049	3.68 (0.11)	4.24 (0.11)	3.97 (0.12)	CL > FL = CL
	Age	0.62		0.002				
	sTfR BL	2357.7***		0.881				
CRP	Group	1.90	2.68	0.011	0.32 (0.16)	0.76 (0.15)	0.37 (0.16)	FL = CL = NL
	Age	3.61		0.011				
	CRP	0.03		0				
WBC	BL Group	2.26	2.37	0.014	9.10 (0.15)	9.31 (0.14)	9.33 (0.15)	FL = CL = NL

Table 3: Endline Biomarkers

Age	0.90	0.003
WBC BL	194.29***	0.379

n = 359, BL, baseline; * p < .05, ** p < .01, *** p < .001, MSE, mean squared error; SE, standard error FL, fortified lentils; CL, control lentils; NL, no lentils; ¹ordering determined using Tukey HSD

Measures					Partial	LSM	error)		
Task	Variable	Factor	F	MSE	eta^2	FL	eans (std CL	NL	Ordering ¹
SRT	RT	Group	3.45*	1655.00	0.019	267 (4)	278 (4)	271 (4)	FL = CL = NL
		Age	14.27***		0.039	(.)	_/ (/)	_/ _ (/)	
GNG	RT	Group	3.09*	3892.67	0.017	387 (6)	395 (6)	379 (6)	FL = CL = NL
		Age	24.08***		0.064				
ANT 0 cues	RT	Group	2.11	11981.90	0.003	609 (5)	611 (5)	605 (5)	FL = CL = NL
		Age	124.08***		0.008				
ANT 2 cues	RT	Group	2.24	9076.01	0.003	576 (4)	577 (4)	571 (4)	FL = CL = NL
		Age	137.61***		0.088				
ANT Alerting	RT	Group	0.05	8172.83	0.0001	34 (4)	35 (4)	34 (4)	FL = CL = NL
		Age	1.27		0.001				
ANT Center	RT	Group	3.62*	11886.50	0.005	607 (5)	611 (5)	598 (5)	FL = CL = NL
		Age	80.19***		0.053				
ANT Spatial	RT	Group	0.95	10241.20	0.001	568 (5)	564 (5)	561 (5)	FL = CL = NL
		Age	115.04***		0.075				
ANT Orienting	RT	Group	1.44	8432.54	0.002	39 (4)	47 (4)	37 (4)	FL = CL = NL
		Age	1.41		0.001				
ANT Incongruent	RT	Group	14.48***	21844.60	0.020	770 (7)	738 (7)	715 (7)	FL > CL > NL
		Age	99.03***		0.065				
ANT Congruent	RT	Group	4.24*	14543.80	0.006	650 (6)	650 (5)	634 (6)	FL = CL = NL
		Age	99.31***		0.065				
ANT Conflict	RT	Group	9.23**	20029.00	0.013	120 (7)	89 (6)	81 (7)	FL > CL = NL
		Age	3.58		0.059		0.50	001	
CDT Neve 4	рт	Carrier	0.79	21654.00	0.004	000 (14)	853	881	
CRT New, 4 cues	RT	Group	0.78 27.44***	21654.90	0.004	890 (14)	(13)	(14)	FL = CL = NL
		Age	37.44***		0.095		699	707	
CRT Old, 4 cues	RT	Group	0.39	14523.70	0.002	720 (11)	(11)	(11)	FL = CL = NL
	1/1	Oroup	0.57	17525.70	0.002	/20(11)	(11)	(11)	

Table 4: Baseline CognitiveMeasures

		Age	21.51***		0.058				
CRT Percent									
Change	RT	Group	6.73**	972.30	0.037	26 (3)	40 (3)	38 (3)	FL < CL = NL
in Capacity		Age	0.01		0				
SMS Intercept		C					958	1060	
New	RT	Group	4.29*	78983.90	0.024	977 (26)	(25)	(26)	CL < FL = NL
		Age	0.31		0.001				
SMS Slope new	RT	Group	1.36	1481.77	0.008	47 (4)	42 (3)	50 (4)	FL = CL = NL
•		Age	0.38		0.001				
		C					826	812	
SMS Intercept old	RT	Group	0.68	41141.00	0.002	802 (19)	(18)	(19)	FL = CL = NL
-		Age	4.29*		0.039				
SMS Slope Old	RT	Group	0.59	510.629	0.003	29 (2)	29 (2)	26 (2)	FL = CL = NL
1		Age	0.22		0.001	. ,			
2.50 ** 0.5 *		ale ale ale	A1) (GE	1	AD	. 1 1			11 GT 1

n = 359, * p < .05, ** p < .01, *** p < .001, MSE, mean squared error; SE, standard error FL, fortified lentils; CL, control lentils; NL, no lentils; ¹ ordering determined using Tukey HSD

				Partial	LS N			
Task	Factor	F	MSE	eta^2	FL	CL	NL	Ordering ¹
SRT	Group	17.11***	980.00	0.110	257 (3)	277 (3)	268 (3)	FL < CL = NL
	Age	5.59*		0.020				
	RT BL	67.23***		0.190				
GNG	Group	2.16	1435.21	0.015	364 (4)	368 (4)	367 (4)	FL = CL = NL
	Age	7.49**		0.026				
	RT BL	182.97***		0.391				
ANT 0 Cues	Group	8.52**	4385.51	0.014	566 (3)	573 (3)	568 (3)	FL = CL = NL
	Age	84.57***		0.064				
	RT BL	482.43***		0.279				
ANT 2 Cues	Group	15.41***	3677.17	0.024	525 (3)	544 (3)	531 (3)	CL > FL = NL
	Age	14.74**		0.011				
	RT BL	316.24***		0.204				
ANT Alerting	Group	2.84	4040.50	0.005	46 (3)	31 (3)	41 (3)	FL > CL = NL
	Age	36.41***		0.030				
	RT BL	0.23		0				
ANT Center	Group	4.25*	4256.63	0.007	565 (3)	549 (3)	552 (3)	FL > CL = NL
	Age	64.55***		0.049				
	RT BL	542.65***		0.304				
ANT Spatial	Group	1.77	4318.30	0.003	515 (3)	521 (3)	518 (3)	Fl = CL = NL
	Age	9.84**		0.008				
	RT BL	226.46***		0.156				
ANT Orienting	Group	14.15***	3533.70	0.024	57 (3)	32 (3)	35 (3)	FL > CL = NL
	Age	39.88***		0.033				
	RT BL	6.42*		0.006				
ANT Incongruent	Group	4.25*	7611.21	0.007	638 (4)	652 (4)	651 (4)	FL = CL = NL
-	Age	45.60***		0.035		. /	. /	
	Rt BL	433.49***		0.260				

 Table 5: Endline Cognitive Measures

ANT Congruent	Group Age	6.86** 18.01***	5204.17	0.011 0.014	572 (4)	586 (3)	585 (4)	FL < CL = NL
ANT Conflict	Rt BL Group Age	445.94*** 2.19 9.39**	7478.78	$0.260 \\ 0.004 \\ 0.008$	74 (4)	65 (4)	66 (4)	FL = CL = NL
	RT BL	73.49***		0.057				
CRT New, 4 Cues	Group	1.35	15504.63	0.001	813 (12)	821 (12)	837 (13)	FL = CL = NL
	Age	6.66*		0.023				
	RT BL	58.51***		0.162				
CRT Old, 4 Cues	Group	2.8	16807.65	0.018	651 (13)	685 (13)	678 (13)	FL = CL = NL
	Age	7.37**		0.024				
	RT BL	6.52*		0.021				
CRT Percent Change in Capacity	Group	0.23	1461.34	0.001	45 (4)	44 (4)	41 (4)	FL = CL = NL
	Age	0.01		0.000				
	RT BL	1.81		0.006				
SMS Intercept New	Group	0.09	47640.76	0.001	944 (21)	953 (20)	937 (22)	FL = CL = NL
	Age	0.98		0.003				
	RT BL	6.87**		0.021				
SMS Slope New	Group	1.15	2220.38	0.007	68 (5)	77 (4)	69 (5)	FL = CL = NL
	Age	0		0.000				
	RT BL	2.87		0.009				
SMS Intercept Old	Group	3.36*	23462.63	0.021	752 (15)	802 (14)	770 (15)	FL = CL = NL
	Age	2.11		0.007				
	Rt BL	5.95*		0.018				
SMS Slope Old	Group	7.81***	317.29	0.047	32 (2)	42 (2)	38 (2)	FL < CL = NL
	Age	0.93		0.003				
n = 250 * n < 05 **	RT BL	0.01		0.000				

n = 359, * p < .05, ** p < .01, *** p < .001, MSE, mean squared error; SE, standard error FL, fortified lentils; CL, control lentils; NL, no lentils; ¹ ordering determined using Tukey HSD

	Change	Predictors: Change Variables						
Task	Variable	Intercept	Hb	sFt	ln sFt	sTfR	TBI	R^2
SRT	RT	-8.24			-12.38	10.49		0.043
GNG	RT	-24.21					-3.34	0.012
ANT	RT 0 cues	-28.55						0.000
	RT 2 cues	-43.31			-35.25			0.019
	RT Alerting	15.31			37.98			0.021
	RT Center	-40.38						0.000
	RT Spatial	-39.17					-8.10	0.019
	RT Orienting	-3.54					5.40	0.008
	RT Incongruent	-102.40			-35.49			0.011
	RT Congruent	-60.99			-27.75			0.011
	RT Conflict	-40.37						0.000
CRT	RT New	0.021						0.000
	RT Old	-66.91		-1.03				0.014
	Percent Change	-0.03						0.000
SMS	Intercept New	-38.35						0.000
	Slope New	23.3				9.32		0.009
	Intercept Old	-56.58			-80.46			0.026
	Slope Old	5.81			-9.97			0.021

Table 6: Plausibility Analysis

n = 359, * p < .05, ** p < .01, *** p < .001

References

- Andang'o, P. E., Osendarp, S. J., Ayah, R., West, C. E., Mwaniki, D. L., De Wolf, C. A., ... Verhoef, H. (2007). Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. *Lancet*, 369(9575), 1799–1806.
- Banerjee, A., Barnhardt, S., & Duflo, E. (2013). Nutrition, iron deficiency anemia, and the demand for iron-fortified salt: Evidence from an experiment in rural Bihar. *Discoveries in the Economics of Aging*, 343–384.
- Beard, J. L. (2000). Iron Requirements in Adolescent Females. *The Journal of Nutrition*, *130*(2), 440S442S. https://doi.org/10.1093/jn/130.2.440s
- Beard, John L.; Connor, James R. (2003). Iron status and neural functioning. *Annual Review of Nutrition, 23*(1), 41–58. doi:10.1146/annurev.nutr.23.020102.075739
- Bruner, A. B., Joffe, A., Duggan, A. K., Casella, J. F., & Brandt, J. (1996). Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet (London, England)*, 348(9033), 992–996. https://doi.org/10.1016/S0140-6736(96)02341-0
- Cook, J. D., Flowers, C. H., & Skikne, B. S. (2003). The quantitative assessment of body iron. *Blood*, *101*(9), 3359–3363. https://doi.org/10.1182/blood-2002-10-3071
- Daugherty, A. M., & Raz, N. (2015). Appraising the role of iron in brain aging and cognition: Promises and limitations of MRI methods. *Neuropsychology Review*, *25*(3), 272–287.
- DellaValle, D. M., Thavarajah, D., Thavarajah, P., Vandenberg, A., & Glahn, R. P. (2013). Lentil (Lens culinaris L.) as a candidate crop for iron biofortification: Is there genetic potential for iron bioavailability? *Field Crops Research*, 144, 119–125. https://doi.org/10.1016/j.fcr.2013.01.002

- Du Clos T. W. (2000). Function of C-reactive protein. *Annals of medicine*, *32*(4), 274–278. https://doi.org/10.3109/07853890009011772
- Erikson, K. M., Pinero, D. J., Connor, J. R., & Beard, J. L. (1997). Regional brain iron, ferritin and transferrin concentrations during iron deficiency and iron repletion in developing rats. *The Journal of Nutrition*, 127(10), 2030–2038.
 - Fan, Jin, McCandliss, Bruce D., Sommer, Tobias, Raz, Amir, Posner, Michael I. (2002).
 Testing the Efficiency and Independence of Attentional Networks. *Journal of Cognitive Neuroscience*, 14(3), 340–347. doi:10.1162/089892902317361886
- Gardner, W., & Kassebaum, N. (2020). Global, Regional, and National Prevalence of Anemia and Its Causes in 204 Countries and Territories, 1990–2019. *Current Developments in Nutrition*, 4(Supplement_2), 830–830. https://doi.org/10.1093/cdn/nzaa053_035

Guthrie, H. A., & Picciano, M. F. (1995). Micronutrient minerals. Human nutrition, 334-351.

- Institute of Medicine. (2003). Dietary Reference Intakes. In *Dietary Reference Intakes: Guiding Principles for Nutrition Labeling and Fortification*. National Academies Press (US).
- Lukowski, A. F., Koss, M., Burden, M. J., Jonides, J., Nelson, C. A., Kaciroti, N., Jimenez, E., & Lozoff, B. (2010). Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. *Nutritional neuroscience*, *13*(2), 54–70. https://doi.org/10.1179/147683010X12611460763689
- Mills, E., Dong, X., Wang, F., & Xu, H. (2010). Mechanisms of brain iron transport: Insight into neurodegeneration and CNS disorders. *Future Medicinal Chemistry*, 2(1), 51.
- Murray-Kolb, L. E., & Beard, J. L. (2007). Iron treatment normalizes cognitive functioning in young women. *The American Journal of Clinical Nutrition*, *85*(3), 778–787.

- Murray-Kolb, L. E., Wenger, M. J., Scott, S. P., Rhoten, S. E., Lung'aho, M. G., & Haas, J. D. (2017). 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. *The Journal of nutrition*, *147*(11), 2109–2117. https://doi.org/10.3945/jn.117.255356
- Northrop-Clewes, C. A., & Thurnham, D. I. (2013). Biomarkers for the differentiation of anemia and their clinical usefulness. *Journal of blood medicine*, *4*, 11–22. https://doi.org/10.2147/JBM.S29212
- Piñero, D. J., & Connor, J. R. (2000). Iron in the brain: An important contributor in normal and diseased states. *The Neuroscientist*, 6(6), 435–453.
- Pittman R. N. (2011). Regulation of tissue oxygenation. In: *Colloquium series on integrated systems physiology: from molecule to function*). Morgan & Claypool Life Sciences.
- Rabindrakumar, M., Wickramasinghe, V. P., Arambepola, C., Senanayake, H., Karunaratne, V., & Thoradeniya, T. (2021). Baseline iron and low-grade inflammation modulate the effectiveness of iron supplementation: evidence from follow-up of pregnant Sri Lankan women. *European journal of nutrition*, 60(2), 1101–1109. https://doi.org/10.1007/s00394-020-02320-2
- Rahman, S., Ahmed, T., Rahman, A. S., Alam, N., Ahmed, A. S., Ireen, S., Chowdhury, I. A., Chowdhury, F. P., & Rahman, S. M. (2016). Determinants of iron status and Hb in the Bangladesh population: the role of groundwater iron. *Public health nutrition*, *19*(10), 1862–1874. https://doi.org/10.1017/S1368980015003651
- Schiepers, O. J., van Boxtel, M. P., de Groot, R. H., Jolles, J., de Kort, W. L., Swinkels, D. W., Kok, F. J., Verhoef, P., & Durga, J. (2010). Serum iron parameters, HFE C282Y

genotype, and cognitive performance in older adults: results from the FACIT study. *The journals of gerontology. Series A, Biological sciences and medical sciences*, 65(12), 1312–1321. https://doi.org/10.1093/gerona/glq149

- Schultink, W. (1996). Iron-supplementation programmes: compliance of target groups and frequency of tablet intake. *Food and Nutrition Bulletin*, *17*(1), 1-5.
- Scott, S. P., Murray-Kolb, L. E., Wenger, M. J., Udipi, S. A., Ghugre, P. S., Boy, E., & Haas, J. D. (2018). Cognitive performance in Indian school-going adolescents is positively affected by consumption of iron-biofortified Pearl Millet: A 6-month randomized controlled efficacy trial. *The Journal of Nutrition*, *148*(9), 1462–1471.
- Sternberg S. (1966). High-speed scanning in human memory. *Science (New York, N.Y.)*, *153*(3736), 652–654. https://doi.org/10.1126/science.153.3736.652
- Stevens, G. A., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., Branca, F., ... Ezzati, M. (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: A systematic analysis of population-representative data. *The Lancet Global Health*, *1*(1), e16–e25.

Stoltzfus, R. J. (2003). Iron Deficiency: Global Prevalence and Consequences. Food and Nutrition Bulletin, 24(4_suppl_1), S99–S103. https://doi.org/10.1177/15648265030244s106

Sun, J., Huang, J., Li, W., Wang, L., Wang, A., Huo, J., ... Chen, C. (2007). Effects of wheat flour fortified with different iron fortificants on iron status and anemia prevalence in iron deficient anemic students in Northern China. *Asia Pacific Journal of Clinical Nutrition*, 16(1), 116–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17215188 United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects 2019, Online Edition. Rev. 1.

Upazila List. (2022). People's Republic of Bangladesh.

http://www.bangladesh.gov.bd/site/view/upazila-list

- Van Thuy, P., Berger, J., Nakanishi, Y., Khan, N. C., Lynch, S., & Dixon, P. (2005). The use of NaFeEDTA-fortified fish sauce is an effective tool for controlling iron deficiency in women of childbearing age in rural Vietnam. *Journal of Nutrition*, 135(11), 2596–2601.
- Ward, R. J., Zucca, F. A., Duyn, J. H., Crichton, R. R., & Zecca, L. (2014). The role of iron in brain ageing and neurodegenerative disorders. *The Lancet. Neurology*, *13*(10), 1045– 1060. https://doi.org/10.1016/S1474-4422(14)70117-6
- Wenger, M. J., Negash, S., Petersen, R. C., & Petersen, L. (2010). Modeling and estimating recall processing capacity: Sensitivity and diagnostic utility in application to mild cognitive impairment. *Journal of Mathematical Psychology*, 54(1), 73–89. https://doi.org/10.1016/j.jmp.2009.04.012
- Wenger, M. J., Murray-Kolb, L. E., Nevins, J. E. H., Venkatramanan, S., Reinhart, G. A., Wesley, A., & Haas, J. D. (2017). Consumption of a double-fortified salt affects perceptual, attentional, and mnemonic functioning in women in a randomized controlled trial in India. *Journal of Nutrition*,
- Wenger, M. J., DellaValle, D. M., Todd, L. E., Barnett, A. L., & Haas, J. D. (2021). Limited Shared Variance among Measures of Cognitive Performance Used in Nutrition Research: The Need to Prioritize Construct Validity and Biological Mechanisms in Choice of Measures. *Current developments in nutrition*, 5(5), nzab070. https://doi.org/10.1093/cdn/nzab070

- Wickens CD, Lee J, Liu Y, Becker SG (2004). An introduction to human factors engineering [Internet]. 2nd ed. New Jersey: Pearson Education, Inc.
- World Health Organization (WHO). (2000). Nutrition for health and development: a global agenda for combating malnutrition. World Health Organization. https://apps.who.int/iris/handle/10665/66509
- World Health Organization (WHO). "Anaemia". (2019). Retrieved from WHO website: https://www.who.int/health-topics/anaemia#tab=tab_1
- World Health Organization (WHO). "Anemia in Women and Children". (2021). Retrieved from WHO website:

https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children#:~:t ext=Summary%20findings&text=In%202019%2C%20global%20anaemia%20prevalence ,women%20aged%2015%2D49%20years.

Yunus, F. M. (2018). Feasibility of field implementation of fortified lentils to improve iron (Fe) status of adolescent girls in Bangladesh [University of Saskatchewan]. https://ecommons.usask.ca/bitstream/handle/10388/8363/YUNUS-THESIS-2018.pdf?sequence=1&isAllowed=y