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RESEARCH ARTICLE

Micronutrient-fortified rice improves haemoglobin, anaemia prevalence and cognitive performance among schoolchildren in Gujarat, India: a case-control study

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ABSTRACT

Anaemia is a public health problem in India. A case-control, quasi-experimental study was conducted to evaluate the effect of a multiple micronutrient fortified rice intervention among school children (6–12 years) through the midday meal programme in Gujarat, India, over 8 months. The fortified rice provided approximately 10% Recommended Dietary Allowance of iron; 25–33% of vitamin A, thiamine, niacin and vitamin B6; and 100% of folic acid and vitamin B12. Outcomes of interest included haemoglobin concentration, anaemia prevalence, and cognitive performance. Cognitive performance was evaluated using J-PAL-validated Pratham reading and mathematics testing tools. 973 children completed the study (cases $n = 484$; controls $n = 489$). The intervention significantly increased mean haemoglobin by 0.4 g/dL ($p = 0.001$), reduced anaemia prevalence by 10% ($p < 0.00001$), and improved average cognitive scores by 11.3 points ($p < 0.001$). Rice fortification can help address anaemia in settings where rice is a staple food.

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Rice fortification; iron; haemoglobin; anaemia; micronutrients; cognition

Introduction

Current food systems are increasingly stressed by climatic extremes, and school feeding programs have the opportunity to provide preventive public health action by nourishing vulnerable children and supporting future generations (Myers et al. 2017; Development Initiatives Poverty Research Ltd 2020). Globally, micronutrient deficiencies present a widespread public health concern in many low- and middle-income countries (Bailey et al. 2015). Deficiencies of essential micronutrients such as iron, vitamin A, iodine, B vitamins and zinc are of particular importance because they can lead to impaired cognition, immune status, growth, and development, with significant and lasting effects on the socio-economic development of both individuals and nations (Black 2003; Hoddinott et al. 2012; Prado and Dewey 2014).

Despite India's economic growth, micronutrient malnutrition remains one of the country's most significant human development challenges (Harding et al. 2018; Swaminathan et al. 2019). In particular, India's high burden of anaemia spans across all age groups (Gonmei and Toteja 2018; Nguyen et al. 2018). The most recent Comprehensive National Nutrition Survey conducted between 2016 and 2018 found that 24% of children aged 5–9 years and 28% of adolescents aged 10–19 years are anaemic (Ministry of Health and Family Welfare, Government of India, UNICEF, and Population Council 2019). The prevalence of anaemia varies according to many factors, including age, wealth quintile, and schooling status (National Family Health Survey-4 2016; Ministry of Health and Family Welfare, Government of India, UNICEF, and Population Council 2019).

Anaemia can result from multiple pathways, including dietary deficiencies of micronutrients, infection, inflammation, and haemoglobinopathies (Kassebaum 2016; Wieringa et al. 2016). As a protective measure, children must consume diets rich in a variety of micronutrients, specifically iron, vitamin A, and B vitamins (e.g. B12, folate) but such deficiencies persist in India (National Family Health Survey-4 2016; Gonmei and Toteja 2018). Among school-aged children (5–9 years) and adolescents (10–19 years), vitamin A deficiency (serum retinol concentration <20 µg/dL) affects 22 and 16%, respectively; vitamin

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B12 deficiency (serum vitamin B12 < 203 pg/mL) affects 17 and 31%, respectively; and folate deficiency (serum erythrocyte folate <151 ng/mL) affects 28 and 37%, respectively (Ministry of Health and Family Welfare, Government of India, UNICEF, and Population Council 2019).

India has a long history of political commitment and programmatic engagement aimed at addressing anaemia. In 1970, the National Nutritional Anaemia Prophylaxis Programme was launched to prevent anaemia primarily through the provision of iron and folic acid (IFA) supplements to women and children (Anand et al. 2014; National Institute of Health and Family Welfare 2014; Kapil et al. 2019). This program was later revised and expanded under the National Iron Plus Initiative for Anaemia Control in 2013. Anaemia reduction has also been highlighted within India's 2017 National Nutrition Strategy key objectives, and within *Poshan Abhiyaan (Nutrition Campaign)*, India's multisectoral nutrition initiative in 2018. In 2019, the country launched *Anaemia Mukt Bharat (Anaemia Free India Campaign)*, a large-scale initiative to reduce anaemia prevalence among target populations – including children, adolescents, pregnant women, and women of reproductive age – to 9% by 2022 (Ministry of Health and Family Welfare 2020). These recent initiatives adopt a more integrated approach to prevention and treatment by combining several strategies such as IFA supplementation, deworming, malaria screening, dietary diversification, and food fortification in schools (Nguyen et al. 2018; Ministry of Health and Family Welfare 2020). Yet, despite India's political commitment and programming, anaemia persists as a challenging public health issue due to its severity and multifactorial nature.

Not with standing national efforts, progress has been slow to date. Between 2000 and 2010, there was no significant change in the prevalence of child (under 5 years) anaemia, only a modest annual decrease of 1.8 percentage prevalence points between 2010 and 2017 (Swaminathan et al. 2019). Addressing the challenge of micronutrient deficiencies both in India and globally will require a multi-pronged approach that includes investment in evidence-based, nutrition-specific programs such as supplementation and fortification tailored to the needs of high-risk populations, coupled with food system interventions targeting the many complex root causes of malnutrition.

India is the second-largest producer of rice worldwide (112 million metric tons) and rice is a key staple for approximately 70% of the Indian population (Singh 2019). Fortification of staples with vitamins and minerals is a proven, cost-effective strategy to increase micronutrient consumption (Hunt 2002; Horton et al. 2008; World Health Organization 2018). In 2016, the Food Safety and Standards Authority of India (FSSAI) operationalized standards on rice fortification for local manufacturers and government programs (Food Safety and Standards Authority of India 2016). In recent years, fortified rice has continued to gain recognition as a public health intervention on India's national agenda.

Multiple studies have evaluated the impact of micronutrient fortified rice on health outcomes globally (Pinkaew et al. 2013; Perignon et al. 2016; Peña-Rosas et al. 2019), including several that have previously investigated its effect on the health, growth, and development of school children in India specifically (Moretti et al. 2006; Zimmerman et al. 2006; Radhika et al. 2011; Thankachan et al. 2012; Hussain et al. 2014). Though these studies tested the efficacy and effectiveness of fortified rice, there has not yet been a large-scale Indian effectiveness study examining the impact of fortified rice on nutrition, cognition, and physical development outcomes.

The objective of the present study was to evaluate a rice fortification intervention in Gujarat State, India. Specifically, this study aimed to evaluate the effect of the regular consumption of fortified rice provided through India's midday meal program – one of the largest government-led food security programs (Ramachandran 2019) – on the health of school children aged 6–12 years. This paper examines the impact of regular consumption of fortified rice on the primary outcome of haemoglobin concentration, and accordingly, the change in anaemia prevalence. The effect of fortified rice on cognition and morbidity was also measured as secondary and tertiary outcomes.

Methods

Study site

Gujarat State is located on India's western coast. The staple diet relies heavily on cereals, milk and milk products and less on pulses, legumes, green leafy vegetables and iron-rich, animal source foods (e.g. egg, meat, fish) (Polasa and Rao 2013). Among Gujarati children (5–9 years) and adolescents (10–19 years), about one-third are anaemic (29%, 33%) and micronutrient deficiencies are widespread: vitamin A (26%, 17%), vitamin B-12 (28%, 48%), folate (55%, 59%) (Ministry of Health and Family Welfare, Government of India, UNICEF, and Population Council 2019).

Intervention description

Through India's government-subsidized midday meal program, cooked rice (100 g dry weight) is served daily to school-children in Gujarat State, with vegetables, legumes, and occasionally curd. For this intervention, PATH partnered with the Akshaya Patra Foundation, a local non-governmental organisation, to provide fortified rice in place of regular rice in two districts of Gujarat State. The Akshaya Patra Foundation has centralised kitchens in Ahmedabad, (serving Ahmedabad and Gandhinagar) and Vadodara. For this intervention, fortified rice was served at 666 schools in the Ahmedabad and Gandhinagar districts of Gujarat for 8 months of the school year between June 2018 and February 2019. Each kilogram of fortified rice contained 20 mg of iron (ferric pyrophosphate), 1300 µg of folic acid, 10 µg of vitamin B12, 1500 µg retinol equivalents, 3.5 mg of thiamine, 42 mg of niacin, and 5 mg of vitamin B6. This level of fortification provided the approximate% recommended dietary allowance (RDA) for children aged 7–9 years: iron (13% RDA), vitamin A (25% RDA), thiamine (33% RDA), niacin (33% RDA), and B6 (33% RDA), folic acid (100% RDA), and B12 (100% RDA), as per FSSAI standards and guidelines (Food Safety and Standards Authority of India 2016). Boys and girls aged 10 to 12-years-old received: iron (7 or 10% RDA), vitamin A (25% RDA), thiamine (33% RDA), niacin (33% RDA), and B6 (33% RDA), folic acid (100% RDA), and B12 (100% RDA), respectively, as per FSSAI standards and guidelines (Food Safety and Standards Authority of India 2016). PATH provided training, quality management, and technical support to Akshaya Patra throughout the implementation process. Fortified rice kernels were procured from a vendor and a blending system was installed at the Akshaya Patra facility in Ahmedabad with support from PATH. Fortified rice kernels were manufactured through a hot extrusion process and blended with non-fortified kernels at a ratio of 1:100.

Study design and sampling

A case-control, quasi-experimental study was conducted to evaluate the intervention. The aim of the study was to evaluate the effect of the regular consumption of fortified rice provided through the intervention to school children aged 6–12 years over a period of 8 months, between June 2018 and February 2019. During this period, regular, unfortified rice was served at 616 schools in the Vadodara district, which functioned as a control district for the evaluation of the intervention. Accordingly, the study had two arms: (1) intervention arm: children receiving fortified rice within their school lunch (Ahmedabad and Gandhinagar districts), (2) control arm: children receiving regular rice within their school lunch (Vadodara district). "Case" children were selected from schools receiving fortified rice in the Ahmedabad and Gandhinagar districts of Gujarat; "control" children were selected from the schools receiving unfortified rice in the Vadodara district of Gujarat. Outcome indicators were measured at baseline (between February and April 2018) and again 12 months later at endline (between February and March 2019).

A required sample size of 525 children per group was calculated based on an expected change in haemoglobin of 0.40 g/dL or greater (standard deviation of 1.4 g/dL) and to account for a 9% attrition rate based on previous studies conducted on the effect of rice fortification in children (Ekvall et al. 2000; Desai et al. 2004; Schümann et al. 2005; Perignon et al. 2016). The total sample size of 1050 was calculated to afford 80% power and a two-tailed outcome.

Multi-stage random sampling was used to select participants for the study (Figure 1). From the 666 schools receiving fortified rice in the intervention districts (Ahmedabad and Gandhinagar) and 616 schools in the control district (Vadodara), 15 schools from each group were randomly selected for a total of 30 participating schools. The schools in the intervention area (Ahmedabad and Gandhinagar districts) were served with fortified rice from the Ahmedabad Akshaya Patra Foundation kitchen while the schools in the control area (Vadodara) were served with regular, un-fortified rice from the Vadodara Akshaya Patra Foundation kitchen. All schools with fewer than 200 students were excluded from the sampling frame, and schools were sampled to allow for an equal proportion of urban and rural schools to ensure adequate representation. From each selected school, 35 children 6–12 years of age were randomly sampled from class rosters and evenly stratified by grade level, for a total of 1050 children. Children were eligible for inclusion in the study if they had plans to continue attending the same school for the entire academic year and consume the school meal; did not have plans to take additional IFA supplements for mild or moderate anaemia in addition to the weekly IFA supplements; gave verbal assent to participate; and their parents provided signed, informed consent for all study components. Children with severe anaemia; children who were taking IFA supplements for mild, moderate, or

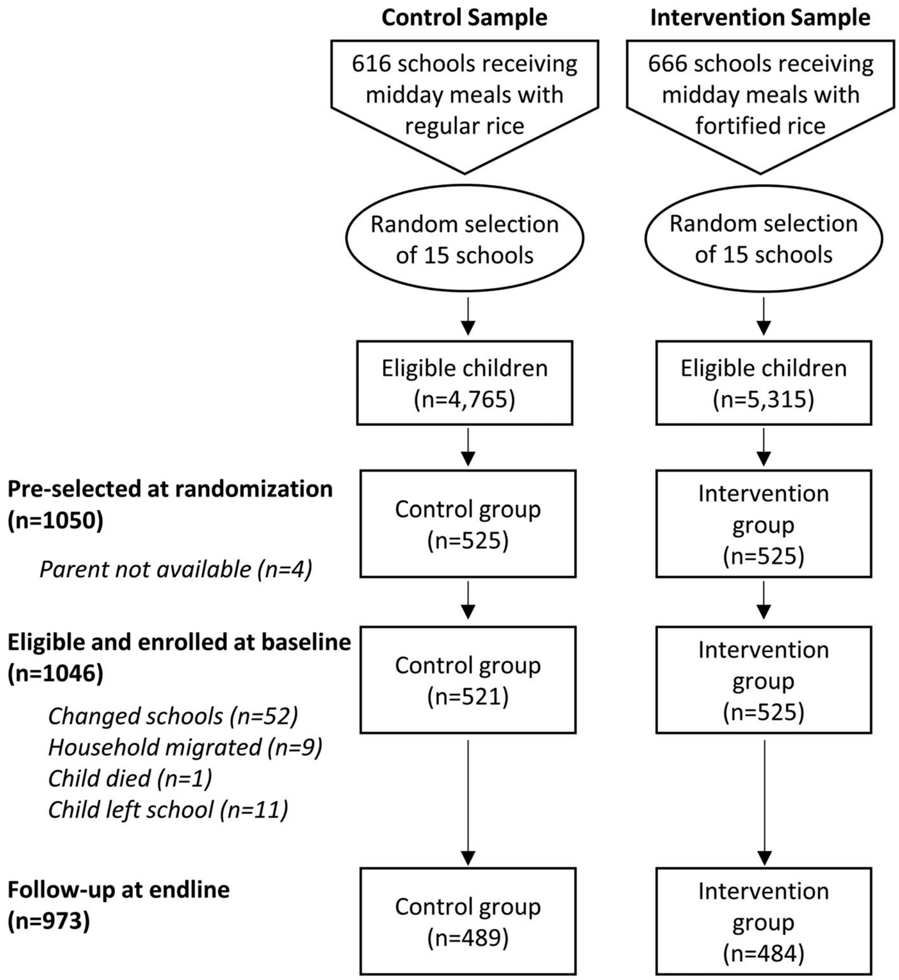

Figure 1. Study sample selection schematic.

severe anaemia in addition to weekly IFA supplements; children who were unable to attend baseline and endline visits or comply with study procedures; and children with symptoms of or confirmation of a chronic/acute illness, metabolic disorder, or other serious medical ailment were excluded from the study. Children who refused assent to participate or whose parents refused consent were also excluded. A total of 1046 children were enrolled in the study at baseline (intervention group $n = 525$; control group $n = 521$), and 973 completed the endline survey (intervention group $n = 484$; control group $n = 489$).

Outcome measurement

Study outcome measures were assessed at two time points (baseline and endline), both by a third-party evaluation firm (Nielsen India Pvt. Ltd.). At baseline, a structured household survey was administered to parents of enrolled children in the local language (Gujarati) to collect socioeconomic and demographic data, as well as information on school absenteeism, illnesses, and nutrition indicators. Socio-economic class (SEC) categorisation was calculated from the education of chief earner and the number of consumer durables adopted from the model used by Market Research Society of India (2011). At both baseline and endline, data was collected on child height, weight, morbidity incidence (e.g. fever, diarrhoea) within the past 2 weeks, daily WASH practices, and receipt of deworming medicine within the past 9 months from parents as part of the structured household survey. Child weight was measured using a battery-operated, portable, digital scale to the nearest 0.1 kg and the child was barefoot and wearing light clothing. Body height was measured on barefoot children using a portable stadiometer attached to the wall. The stadiometer was calibrated and used to measure child height to the nearest 0.1 cm. Both height and weight were measured three times, and the two closest measurements were averaged. BMI-for-age *Z*-scores (BAZ) were calculated and used to construct categories for severe thinness (BAZ < -3 *Z*-scores), thinness (-3 *Z*-scores $\leq$ BAZ < -2 *Z*-scores), normal (-2

Z-scores $\leq$ BAZ $<$ 1 Z-score), overweight (1 Z-score $\leq$ BAZ $<$ 2 Z-scores), and obese (2 Z-scores $\leq$ BAZ) status according to the WHO BMI-for-age growth reference standards for children 5–19 years of age (World Health Organization 2007).

School attendance records were reviewed by the study team at the end of each month to calculate the number of absences for each enrolled child during the intervention period. School records were also used to track the distribution of IFA tablets to participants as part of the National Iron Plus Initiative.

Additionally, haemoglobin measurements were obtained from enrolled children and cognitive tests were administered at both baseline and endline. Anaemia was defined according to WHO guidelines (haemoglobin concentration of <12.0 g/dL among children aged 12–14 years of age; <11.5 g/dL among children aged 5–11 years of age) (World Health Organization 2011). Children were grouped into categories of healthy/normal, mildly anaemic, and moderately anaemic, depending upon age (World Health Organization 2011). The mild anaemia category contained children aged 6–11 years with haemoglobin concentrations of 11.0–11.4 g/dL and children 12–14 years with haemoglobin concentrations of 11.0–11.9 g/dL. Children between 6 and 14 years of age with haemoglobin concentrations of 8–10.9 g/dL are considered to be moderately anaemic; Haemoglobin concentrations of less than 8 g/dL are categorised as severely anaemic. Haemoglobin measurements were taken non-invasively at baseline and endline using the Masimo Rad 67™ Spot-check pulse CO-Oximeter (Masimo Corp, Irvine, CA). This device uses a simple finger probe and wavelengths – similar to a conventional pulse oximeter – to estimate haemoglobin concentration based on principles of infra-red spectrophotometry (Hiscock et al. 2015; Levy et al. 2017; Parker et al. 2018).

To assess cognitive development, measures of a child's reading and mathematical abilities were evaluated with Pratham Resource Center's Annual Status of Education Report (ASER) cognitive testing tools using a 30–38 point scale (dependent on grade-specific tests) and calculated into a percentage. These tools have been validated in the Indian context by the Abdul Latif Jameel Poverty Action Lab (J-PAL) and are routinely used in nation-wide educational surveys (Vagh 2012).

Ethics

Ethical approval for the study was obtained from the PATH Research Ethics Committee, as well as Sigma-IRB in India (Ethical board clearance number: 10064/IRB/D/18-19). Written permission for the intervention and for carrying out the study was also obtained from the government of Gujarat. All participation in the study was voluntary. Informed, written consent was obtained from parents of all children participating in the study, and verbal assent was also obtained from all participating children.

Data management and analysis

Data was captured electronically on tablets using CSPro, and analysis was conducted using SPSS 22.0 and STATA version 12 (StataCorp LLC, College Station, TX). 10% of the interviews were observed and 20% were back-checked by the study team to ensure data accuracy. Data were uploaded daily to a secured server and simple frequency tables were generated to assess the quality of the data prior to statistical analysis. Pearson's Chi-square test was used to compare baseline characteristics between intervention and control samples. Descriptive statistics were calculated for haemoglobin concentrations and anaemia prevalence at baseline and endline; one-way ANOVAs examined differences between baseline measures, difference in difference (D-I-D) regression models examined the impact of the intervention on haemoglobin over time while adjusting for age, caste, gender, grade, and religion, and a test of two proportions examined the difference in anaemia prevalence rates at endline (Lechner 2010). Similar D-I-D models were also used to examine the impact of the intervention over time on cognitive test scores. Endline supplementation, sanitation, and hygiene practices were examined using Pearson's Chi-square test.

D-I-D is a quasi-experimental design that makes use of longitudinal data from treatment and control groups to obtain an appropriate counterfactual to estimate a causal effect. It is used to estimate the effect of a specific intervention or treatment (e.g. implementation of rice fortification) by comparing the changes in outcomes over time between a population that is enrolled in an intervention (school children in Ahmedabad and Gandhinagar) and a population that is not (school children in Vadodara). D-I-D is implemented as an interaction term between time and treatment group dummy variables in a regression model using the following formula:

$$Y = \beta 0 + \beta 1 \mathrm{Post}t + \beta 2 Tv + \delta 1 \mathrm{Post}t Tv + \varepsilon$$

In this formula, Y is a binary variable indicating whether the selected child has a positive value for the selected indicator, $\beta 0$ is the average value at baseline,

$\beta 1$ is a time trend in the control group, Post = 1 if the measurement is taken at the endline survey and zero otherwise, $\beta 2$ is difference between two groups pre-intervention, Tv is a dummy variable indicating treatment status, $\delta 1$ is the difference in changes over time, and ε is the unobserved error term. The same model was used for all outcomes of interest unless otherwise specified as the findings were reported. The unit of analysis is always the selected child. The mentioned formula was estimated in the least-squares models, reflecting an intent to treat at the child level. The parameter of interest is $\delta 1$, the D-I-D estimate of the intervention effect at endline relative to baseline in the intervention as compared to the control. For all analyses, two-tailed tests were used and an $\alpha < 0.05$ was considered to be significant.

Results

Table 1 presents the demographic characteristics of study participants in the control ($n = 489$) and intervention ($n = 484$) groups. The intervention group sample was selected from Ahmedabad (14%) and Gandhinagar (86%) districts, and the control group sample was selected only from the Vadodara district. Among households participating in the study, a majority had a head of household with 5–9 years of schooling, and less than one-sixth had a head of household with secondary school education. The majority of study participants lived in rural areas within both the control (69%) and intervention (73%) districts, respectively. The intervention group had more boys (63%) than the control sample (53%). Participants were recruited equally across primary school grades 1–5 (aged 6–12 years).

Table 1. Demographic characteristics of the child sample population[+].

Variable	Control group ($n = 489$)	Intervention group ($n = 484$)	p-Value
Education of household head			
Illiterate	80 (16%)	87 (18%)	0.012
Literate, no formal schooling, or 0–4 years of schooling	37 (8%)	55 (11%)	
5–9 years of schooling	263 (54%)	269 (56%)	
Completed secondary school	109 (22%)	73 (15%)	
Urban/rural status			
Rural	335 (69%)	353 (73%)	0.129
Urban	154 (31%)	131 (27%)	
Gender			
Boys	259 (53%)	305 (63%)	0.000
Girls	229 (47%)	179 (37%)	
Age in years			
Mean (SD)	8.3 (1.6)	8.0 (1.4)	0.002
Primary school grade			
1	97 (20%)	97 (20%)	0.999
2	102 (21%)	100 (21%)	
3	98 (20%)	98 (20%)	
4	99 (20%)	100 (21%)	
5	93 (19%)	89 (18%)	
Anthropometry[a]			
Mean BMI (SD)	14.3 (2.0)	14.3 (1.7)	0.485
Severe thinness	40 (8%)	50 (10%)	
Thinness	134 (27%)	105 (22%)	
Normal	299 (61%)	318 (66%)	
Overweight	12 (2%)	9 (2%)	
Obese	4 (1%)	2 (0%)	
Religion			
Hindu	394 (81%)	476 (98%)	0.000
Muslim	94 (19%)	8 (2%)	
Christian	1 (0%)	0 (0%)	
Household socioeconomic status			
Below poverty[b]	166 (34%)	227 (47%)	0.000
Socio-economic class (SEC)[c]			
Class A (A1-A2-A3)	28 (6%)	29 (6%)	0.146
Class B (B1-B2)	171 (35%)	146 (30%)	
Class C (C1-C2)	170 (35%)	168 (35%)	
Class D (D1-D2)	94 (19%)	99 (21%)	
Class E (E1-E2-E3)	26 (5%)	42 (9%)	

[+]Each cell lists the number of children in that category and their percent contribution to that variable's category.
[a]Severe thinness is defined as having a BAZ < -3, thinness is defined as -3 Z-score $\leq$ BAZ < -2, normal is defined as -2 Z-score $\leq$ BAZ < 1 Z-score; Overweight is defined as 1 Z-score $\leq$ BAZ < 2 Z-score, obese is defined as BAZ ≥ 2 Z-score. [b]Defined as household possession of a Below Poverty Line (BPL) ration card. [c]SEC calculated from the education of chief earner and number of consumer durables adopted from the model used by Market Research Society of India (2011).

Haemoglobin concentration and anaemia prevalence

Table 2 presents the mean haemoglobin concentrations and anaemia prevalence rates at baseline and endline. Haemoglobin concentrations between the two samples did not differ at baseline ($p = 0.15$), but by the end of the school feeding program, the intervention group exhibited a 0.4 g/dL improvement in haemoglobin concentration over the control sample when controlling for age, caste, gender, grade, and religion ($p = 0.001$). At baseline, the prevalence of anaemia (combined mild and moderate) was similar ($p = 0.937$) between the two groups (44.4% control; 44.6% intervention) but declined by 10.1% within the intervention sample and increased by 4.3% within the control sample over the course of the school year. The intervention showed significant attribution reducing anaemia by 10% amongst the children consuming fortified rice ($Z = -4.49$; $p < 0.00001$). D-I-D analysis also confirmed the attributional effect of 0.144 points ($p = 0.001$) after controlling for time constant effects. Mild and moderate anaemia declined within the intervention sample by 5.2 and 5.0%, respectively, but within the control sample, mild anaemia declined by only 0.8%, and moderate anaemia increased by 5.2%. Further analysis by age group shows that the impact of the intervention is more visible among children aged 6–9 years (Figure 2).

Figure 3 presents the change in anaemia categories over time within the intervention and control samples. Within the intervention group, 30.9% of children improved their anaemia status from moderate to mild anaemia, mild anaemia to healthy, or moderate anaemia to a healthy status. Within the control group, 24.3% of children improved their anaemia status from moderate to mild anaemia, mild anaemia to healthy, or moderate anaemia to a healthy status.

Table 2. Effect of the intervention on haemoglobin levels and anaemia prevalence.

Variable	Control group ($n = 489$)	Intervention group ($n = 484$)	p-Value
Haemoglobin[a]			
Baseline	11.8 g/dL [11.7, 11.9]	11.7 g/dL [11.6, 11.8]	
Endline	11.6 g/dL [11.5, 11.8]	11.9 g/dL [11.8, 12.0]	
DID adjusted β	0.4 g/dL [0.2, 0.7]		0.001
Anaemia			
Baseline	44.4%	44.6%	
Endline	48.7%	34.5%	<0.00001
DID adjusted β	0.144		0.001

[a]Haemoglobin is represented as Mean [95% Confidence Interval].
DID: Difference in difference.

Cognition

As described above, surveyed children completed mathematics and language tests at both baseline and endline to measure cognitive performance. Scores were converted into percentages and averages were calculated for mathematics, language, and overall. Table 3 shows the impact of the intervention on cognitive test scores, highlighting that the improvements observed within the intervention group were significantly greater than the improvements observed within the control group by 11 points for maths ($\beta = 10.6$; p-value $= 0.000$), 12 points for language ($\beta = 11.8$;

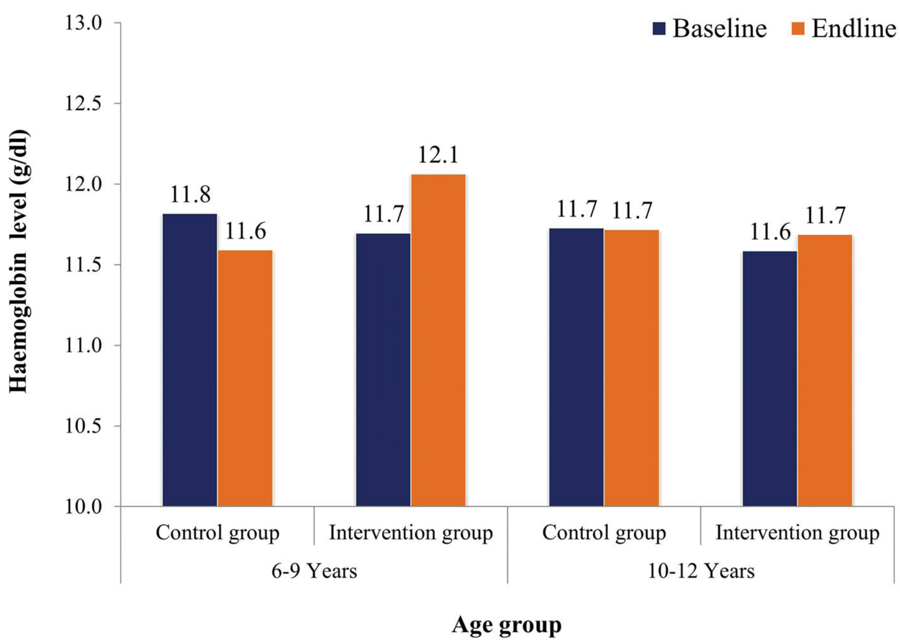

Figure 2. Changes in haemoglobin level from baseline to endline, by age group.

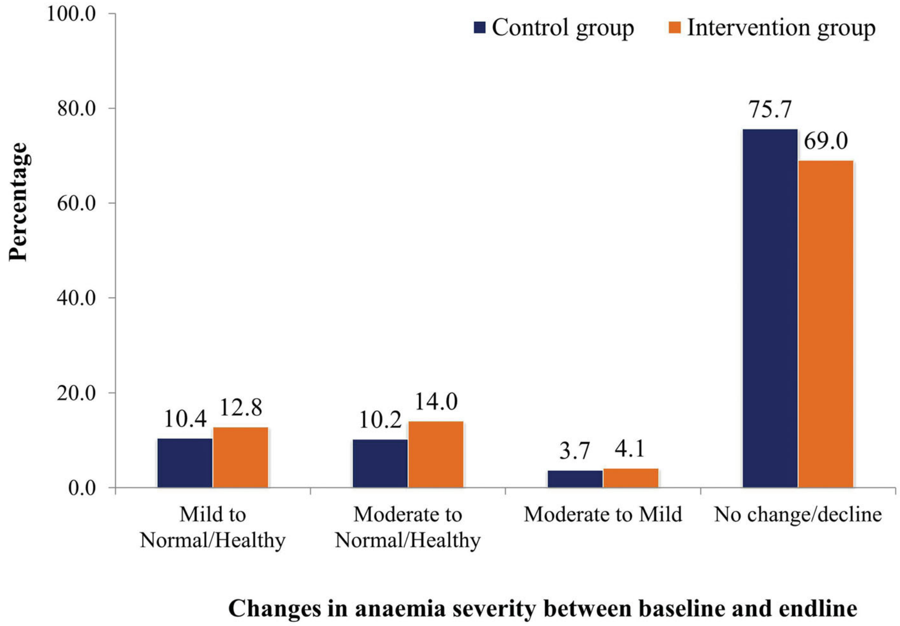

Figure 3. Changes in anaemia severity between baseline and endline.

Table 3. Impact of the intervention on cognitive test scores.

	Control group		Intervention group		D-I-D	
Cognitive Score	Baseline	Endline	Baseline	Endline	Coeff.	*p*-Value
Total (*n*)	489	489	484	484		
Average cognition score[a]	60.3	69.6	49.8	70.4	11.3	0.000
Maths[a]	52.6	64.6	42.7	65.6	10.6 [5.5, 15.7]	0.000
Language[a]	68.0	74.6	56.9	75.1	11.8 [6.7, 16.9]	0.000

[a]Maths and language scores are continuous variables; Results indicate adjusted improvement in percentage points.

p-value $=$ 0.000), and 11 points overall (p-value $=$ 0.000) (Table 3).

The average cognition score was also analysed further in terms of the percentage of children who scored more than 80% in both maths and language (Table 3). Within the intervention sample, almost one-third (27%) of children obtained scores higher than 80% in both mathematics and language tests at endline, an improvement from only 8% at baseline. Improvements were seen within each gender. The majority of children (82%) within the intervention sample obtained higher cognitive test scores at endline versus baseline; however, only about two-thirds (68%) of children within the control sample improved.

Morbidity

Morbidity within the past 2 weeks was measured at baseline and endline to collect information on the incidence of fever, diarrhoea, malaria, or any other illness that resulted in missing school. None of these outcomes differed at baseline or over the course of the study except for diarrhoea which increased amongst the intervention sample from 3 to 5% and reduced within the control sample from 8 to 5% ($\beta = 3.31$; p-value $=$ 0.008). At endline, the percentage of control and intervention sample children reporting morbidities was low. Among the intervention and control groups, fever was reported among 17 and 24% of children, respectively. Similarly, malaria was reported at 2% for both intervention and control groups, and school absenteeism due to illness was 12 and 21%, respectively.

Supplementation, deworming and health behaviours

Table 4 presents the consumption of micronutrient supplements among study participants within the past 30 d. Almost all children (>87%) received and consumed iron supplements in accordance with India's weekly IFA supplementation program adopted by

Table 4. Iron supplementation, deworming and child health behaviours at endline.

Variable	Control group ($n = 489$)	Intervention group ($n = 484$)	p-Value
Micronutrient supplementation within the past 30 d			
Iron pills	87% (425)[a]	90% (436)	0.258
Multiple micronutrient powders	10% (459)	8% (460)	
Iron syrup	8% (457)	12% (458)	<0.05
Iodised salt	99%	99%	
Deworming within the past 9 months			
Deworming tablets	77% (441)	71% (446)	<0.01
Handwashing practices			
Before eating	99% (488)	98% (481)	
After defaecating	100% (488)	99% (479)	
After returning home	95% (485)	92% (478)	
After touching dust or dirt	96% (485)	94% (478)	
Hygiene behaviours			
Wears shoes consistently	96% (489)	92% (479)	<0.05
Takes clean drinking water to school	75% (489)	45% (482)	<0.001
Bathes everyday	99% (489)	98% (481)	
Uses toilet at home	87% (489)	80% (484)	<0.02
Cuts fingernails	99% (489)	99% (480)	
Receives health education at school	98% (469)	99% (471)	

[a]Values in the parenthesis indicate the sample size from which the percentage is estimated. For example, 87% (425) indicates 87% of 425.
The sample size is variable as there was missing data due to vacation.

schools in Gujarat State. Consumption of multiple micronutrient powders and iron syrups was less prevalent. The majority of children in both the control and intervention samples had received deworming tablets within the past 9 months, but significantly more children in the control group received this medicine. The children in both control and intervention groups excelled at practicing handwashing before eating, after defaecating, after returning home from school, and after touching dirt. While hygiene practices were good in both groups, the control group wore shoes more consistently, were more likely to take clean drinking water with them to school, and reported higher access to a home toilet.

Discussion

This case-control study investigated the effect of the regular consumption of fortified rice among school children aged 6–12 years over an 8-month period within a large-scale school feeding program in Gujarat, India. Results indicate that the regular consumption of multiple micronutrient-fortified rice can provide multiple health benefits to schoolchildren. More specifically, among the intervention group, there was a 0.4 g/dL improvement in mean haemoglobin ($p = 0.001$), a reduction in mean anaemia prevalence by 10% ($p < 0.00001$), and improvements in average cognitive test scores by 11% ($p < 0.001$). This study did not find lower rates of morbidity or improved school attendance due to fortified rice alone.

A recent systematic review by Peña-Rosas et al. (2019) found that rice fortified with iron alone or in combination with other micronutrients may reduce the risk of iron deficiency (risk ratio [RR] 0.66, 95% CI 0.51–0.84) and increase mean haemoglobin (mean difference [MD] 1.83, 95% CI 0.66–3.00) but make little to no difference in the risk of having anaemia (RR 0.72, 95% CI 0.54–0.97) compared with unfortified rice (Peña-Rosas et al. 2019). In contrast, the present feeding trial reported improvements in haemoglobin with the additional benefit of significantly lowering anaemia prevalence. This improvement may be attributable to additional factors taken in combination with daily consumption of multiple micronutrient fortified rice, including standard weekly IFA supplementation, biannual deworming, optimal handwashing practices, and good hygiene practices.

Previous school feeding trials involving fortified rice have demonstrated substantial variability in the intervention's impact on the outcomes of haemoglobin and anaemia. This variability may result from multiple factors, including differences in the micronutrient formulations provided (e.g. Fe/Zn ratio; %RDA), the presence of enhancing agents, the baseline health status of the sample (e.g. haemoglobinopathies, anaemia prevalence, micronutrient deficiencies, infection, inflammation), dietary diversity, consumption of antinutrients (e.g. tea), sanitation and hygiene behaviours, and access to high-quality health care. Of the 10 studies (Moretti et al. 2006; Angeles-Agdeppa et al. 2008; Radhika et al. 2011; Thankachan et al. 2012; Pinkaew et al. 2013; Hussain et al. 2014; Parker et al. 2015; Hardinsyah et al. 2016; Perignon et al. 2016; Dutta et al. 2019) that fed schoolchildren fortified rice containing micronized ferric pyrophosphate (mFePP) iron alone (Moretti et al. 2006; Angeles-Agdeppa et al. 2008; Radhika et al. 2011), or in combination with other micronutrients, five studies reported significant improvements in haemoglobin concentration within the intervention group (Angeles-Agdeppa et al. 2008; Thankachan et al. 2012; Hussain et al. 2014; Hardinsyah et al. 2016; Dutta et al. 2019), and a sixth study found improved haemoglobin only among children without inflammation (CRP < 5 mg/L and AGP < 1 g/L) (Perignon et al. 2016). Half of the studies also reported improvements in anaemia prevalence at endline (Angeles-Agdeppa et al. 2008; Thankachan et al. 2012; Hussain et al. 2014; Hardinsyah et al. 2016; Dutta et al. 2019).

Five of the existing ten fortified rice school feeding program studies were conducted in India. Only three of these studies used multiple micronutrient formulations (e.g. iron, zinc, vitamin A, vitamin B), instead of iron-only formulations, and all three reported improvements in haemoglobin and anaemia (Moretti et al. 2006; Radhika et al. 2011; Thankachan et al. 2012; Hussain et al. 2014; Dutta et al. 2019); the two iron-only formulations did not report significant improvements in haemoglobin or anaemia, but did significantly decrease iron deficiency. In order to comprehensively combat anaemia, children likely require more than just iron in fortified rice. Our study results from Gujarat add to the growing body of literature on multiple micronutrients fortified rice studies that have produced positive health benefits among Indian children. To our knowledge, this is the first study to date to evaluate the effect of fortified rice consumption among school children in Gujarat State.

The prevalence of haemoglobinopathies may also play a role in the findings of our study. Haemoglobinopathies, dependent on the specific type, impact haemoglobin synthesis and the lifespan of red blood cells (Wieringa et al. 2016). A previous school feeding program conducted in Cambodia provided multiple micronutrient fortified rice to children for the duration of their school year but did not find significant improvements in haemoglobin or anaemia, only minor improvements among a sub-group of children without inflammation (0.08 g/dL) (Perignon et al. 2016). One of the proposed reasons for the lack of impact on this study sample was the high rate of blood disorders among Cambodians (Wieringa et al. 2016). Studies indicate lower rates of haemoglobinopathies in Gujarat state and India overall (<10%) in comparison to Cambodia (>50%) (Mohanty et al. 2013; Wieringa et al. 2016) and this may be why the present study population was able to experience further improvements in haemoglobin and anaemia prevalence.

Improvements in cognition

Iron supplementation in older children has been found to improve attention and concentration, regardless of baseline iron status, and intelligence quotient scores within anaemic populations (Falkingham et al. 2010). Our study found significant increases in cognitive test scores among the intervention group. This impact may have been due to improvements in attention span and concentration levels within children receiving iron-fortified rice. The biological mechanism by which iron improves cognitive function may be related to iron's essential role in energy production within the mitochondria of neurons. The brain requires large amounts of oxygen and glucose for optimal function yet neither can be metabolised by the mitochondria without adequate amounts of iron (Singh et al. 2014). Few studies have investigated the relationship between fortified rice consumption and cognitive outcomes and inconsistent findings have been reported (Thankachan et al. 2012; Fiorentino et al. 2018). A systematic review on the impact of multi-micronutrient food fortification also reported mixed results on the ability of interventions to improve cognitive outcomes (Best et al. 2011). This variability in the literature may be explained by a number of factors, including differences in the intervention (e.g. formulation), study populations, contextual factors, and, notably, different methods used to assess cognitive performance.

Deworming

The absorption of iron from the diet is influenced by the intestinal parasitic load (Kozat et al. 2007; Ngui et al. 2012; Glinz et al. 2015; Lazarte et al. 2015). Ngui et al. (2012) found high prevalence rates of soil-transmitted helminths (*Trichuris trichiura*, *Ascaris lumbricoides*) among rural children in West Malaysia was significantly associated with the odds of iron deficiency anaemia (Ngui et al. 2012). At endline, the majority of our study's intervention group (71%) reported receipt of deworming tablets over the past 9 months, which likely contributed to improved iron absorption from fortified rice during the school year.

Of note, the intervention group had a higher prevalence of diarrhoea in the 2 weeks preceding endline. Morbidity incidence is not only dependent on improved nutrition, but requires health behaviour change activities including water, sanitation, and hygiene (WASH). Though the intervention and control samples both maintained a high standard of hygiene and sanitation, increased diarrhoea within in the intervention group may be attributed to the lower rate of drinking clean water (45 versus 75%). This gives a vivid picture that although this population was provided with nutritious food, the water used for drinking and other hygiene measures plays an important role in converting the consumed nutrients into better health.

Limitations

There are certain limitations associated with this study. First, our analysis did not account for several factors which may have influenced our findings, such as dietary diversity, food intake, iron deficiency (as measured by ferritin), or physical activity among the study population. Given the central role of diets in iron absorption, nutrition outcomes, and overall health, this presents a significant limitation to our analysis. Additionally, we did not analyse faecal samples for parasitic load or blood samples for infection and inflammation markers among the study sample at baseline and endline. Given the observed increase in diarrhoea at endline, this would have been valuable data to inform our findings. Further, our study also employed a non-invasive diagnostic developed by Masimo to measure haemoglobin concentration among children. Multiple research teams, including this team, have evaluated the Masimo Pronto and Radical devices within active paediatric populations alongside HemoCue devices, and against automated haematology analysers. These efforts have found accurate precision and predictability (Levy et al. 2017; Gamal et al. 2018), and similar predictability, accuracy, and bias as the HemoCue against the gold standard (Levy et al. 2017; Parker et al. 2018). This tool was appropriate for the context of this study, as it was better received by local authorities, communities, and the child study population than an invasive method for measurement of haemoglobin concentration, and also did not produce any biological waste. Further, the study team understood that any potential presence of a slight downward bias in haemoglobin concentration would affect the results of both the control and intervention samples and thus would not invalidate our study findings (Amano and Murakami 2013; Levy et al. 2017; Parker et al. 2018; Whitehead et al. 2019). However, despite these limitations, this study provides a robust assessment of the impact of fortified rice on key indicators of health among a large sample size of school-aged children in Gujarat State, India.

Conclusions

In summary, this study found that regular consumption of fortified rice among school children aged 6–12 years through India's midday meal program in Gujarat State was effective in improving haemoglobin concentrations and cognitive test scores, and in reducing anaemia. These findings demonstrate that the integration of fortified rice into large-scale school feeding programs could lead to improvements in health outcomes for vulnerable school-aged children. The results of this study also further suggest that combining rice fortification alongside other nutrition-specific and sensitive public health interventions (e.g. WASH, deworming, IFA supplementation) could further amplify improvements in nutrition outcomes. Creating proper awareness amongst populations about the need to consume nutritious foods, as well as the importance of clean drinking water, hygiene, and sanitary measures will play a synergistic role in improving nutrition.

This study provides the essential evidence required by the state of Gujarat that fortified rice is an effective vehicle through which micronutrient deficiencies among school children can be addressed. The precedent set by the success of salt iodisation programs in India (Yadav and Pandav 2018) indicates the potential of rice fortification. India's midday meal program has a wide reach serving 95.2 million children in 1.1 million schools across the country between 2017 and 2018 (Ministry of Human Resource Development 2019). The government's leadership on policy guidelines has created an important opportunity for India to scale up rice fortification through the country's social safety net program which reaches 800 million vulnerable people including women and children (Banik 2016). This paper aims to contribute relevant local evidence that will inform this scale up by supporting Indian state authorities to integrate fortified rice into their programs. India can also serve as a positive example for other countries where rice is a staple food.

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