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Effect of multi-micronutrient-fortified rice on cognitive performance depends on premix composition and cognitive function tested: results of an effectiveness study in Cambodian schoolchildren

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Abstract

Objective: Even though current policy is strongly focused on the crucial first '1000 days', it might be still possible to enhance cognitive function during the pre-adolescent and adolescent years by improving micronutrient status. In Cambodia, nutritional status is poor. Provision of rice fortified with micronutrients through a school meal programme (SMP) could be a cost-effective strategy to help improve health and school performance. The present study aimed to evaluate the effect of three different micronutrient-fortified rice formulations on cognitive function in Cambodian children.

Setting: Sixteen Cambodian schools receiving SMP.

Design: The FORISCA-UltraRice® + NutriRice® study was a randomized, double-blind, placebo-controlled trial. Four groups of four schools were randomly allocated to receive normal rice, UltraRice®Original, UltraRice®New or NutriRice®. Within each school, 132 children were randomly selected. Data on cognitive performance (picture completion, block design and Raven's coloured progressive matrices (RCPM)), anthropometry, parasite infestation and micronutrient status were collected before the intervention and after 6 months.

Subjects: Cognitive data were available for 1796 children aged 6–16 years.

Results: All cognitive scores improved after 6 months ($P < 0{\cdot}001$). Block design score improvement was significantly higher in children consuming UltraRice® Original ($P = 0{\cdot}03$) compared with the other fortified rice groups and placebo. No difference among groups was found on RCPM or picture completion scores. Stunting, parasite infestation and inflammation negatively affected the impact of the intervention.

Conclusions: Combined with other interventions, using SMP to distribute fortified rice to schoolchildren may be a cost-effective way to increase cognitive performance and thereby improve school performance and educational achievements.

Keywords
Cognition
Micronutrients
Schoolchildren
Cambodia

In South-East Asia, micronutrient deficiencies remain highly prevalent[1]. In addition to the most vulnerable groups (i.e. pregnant and lactating women, young children), the prevalence of micronutrient deficiencies is also high among school-aged children. Over 30 % of school-aged children in South-East Asia are affected by Zn deficiency, while 20 % of school-aged children are deficient in Fe or vitamin A[2]. During the primary school years, anaemia and deficiencies of Fe, Zn, iodine, vitamin A, vitamin B_{12}, vitamin B_6 or folic acid can impair concentration and cognitive function[2–5] and reduce school attendance by increasing morbidity[6]. Therefore, micronutrient deficiencies are detrimental to

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optimal schooling, while at the same time education is recognized as a prime opportunity to break the cycle of poverty and undernutrition[7,8].

Although the '1000-day window' concept rightly highlights that micronutrient deficiencies in fetal life or early childhood can permanently damage cognitive function and development[9], it is important to realize that it might still possible to improve cognition among children of primary-school age by improving micronutrient status. Several studies have clearly shown a beneficial impact of micronutrient supplementation or food fortification on morbidity and cognitive performance[5,10–13], with multiple micronutrients appearing to be more efficient than single or double micronutrient interventions[14]. However, the overall evidence remains equivocal[15,16]. In South-East Asia, a region where refined rice typically provides 70 % of the dietary energy intake, rice fortification could be a cost-effective strategy to reduce micronutrient deficiencies and anaemia[17–22] and therefore possibly improve cognitive performance in school-aged children.

The school meal programme (SMP) of the UN World Food Programme (WFP) reached almost 26 million children worldwide in 2011[23]. The primary objective of the WFP SMP is to increase school enrolment and attendance; however, it can also serve to improve child nutritional status at the same time[23]. Fortification of the school meal with micronutrients could help reduce the prevalence of micronutrient deficiency at very little additional cost. For example, depending on technologies, to fortify rice in school meals would add only $US 0·50–2·00 per child per year[24].

In Cambodia, malnutrition among children under 5 years of age is highly prevalent, with 40 % stunting and 11 % wasting prevalence[25]. Unfortunately, data on the nutritional status of primary-school-aged children are scarce in Cambodia. The WFP distributes school meals to more than 500 000 school-aged children in rural Cambodia[23]. The standard school meal, eaten as breakfast, consists of rice cooked with chick peas, served with canned tomato sauce with fish. Rice is the main component of the programme and is also the main vehicle in many other food-based social safety net programmes in Cambodia as well as in Asia. Therefore, evidence for the effectiveness of rice fortification would be of immediate importance to these programmes as well as for the development of national fortification guidelines in the whole region. The FORISCA project (Fortified Rice for School children in Cambodia) aimed to assess the effect of fortifying the rice in the WFP SMP with multiple micronutrients on micronutrient status and functional outcomes. The present paper reports on the effects of consuming rice fortified with multiple micronutrients, using three different fortification formulations, on cognitive performance based on specific cognitive functions in Cambodian children of primary-school age.

Participants and methods

Study site

The study was conducted in Cambodia, in sixteen primary schools from Kampong Speu province, around 60 km south-west of the capital Phnom Penh. Kampong Speu province can be characterized as rural, with most families involved in rice farming. In 2010, prevalence of stunting and underweight in pre-school children in Kampong Speu province was 44 and 9 %, respectively[25] and in 2005 more than 60 % of pre-school children in the province were anaemic[26]. Representative data on schoolchildren were not available.

Study design and selection of participants

The study period was November 2012 to June 2013, with a rolling recruitment of 1 month and a 6-month intervention period. The study was a double-blind, cluster-randomized, placebo-controlled feeding trial, conducted in sixteen schools selected from all primary schools participating in the SMP of WFP (n 18) in Kampong Speu province that gave daily breakfast at school, but did not give take-home rations. The sixteen schools were randomly allocated to receive: (i) placebo (normal rice); (ii) UltraRice®Original; (iii) UltraRice®New; or (iv) NutriRice®. To ensure blinding, the intervention groups were coded with letters (A–H) and two schools were allocated to each intervention letter code. Randomization of schools was done by a researcher not involved in the study implementation (M.A.D.) and codes were known only to the researcher and the head of the logistics department of WFP, responsible for the distribution of rice to the schools. Codes were broken only after data collection was completed and all biochemical data analysis had been done. Before the study commenced, all parents of children of the selected schools were invited to attend a meeting at which objectives and procedures of the study were explained, as well as their right to refuse to participate in or to continue the study. Only children for whom written informed parental consent was obtained were eligible for randomization. In each school, 132 children were randomly selected from lists provided by the school director, with stratification by grade and gender, giving a total number of 2112 eligible children. Age of each child was calculated using the birth date provided by the registration book at school, itself based on the birth certificate. Exclusion criteria were age <6 years and severe anaemia (Hb concentration <70 g/l[27]). Severely anaemic children received appropriate treatment.

Intervention

The meal was composed of rice (115 g uncooked) and yellow split peas (15 g uncooked) cooked together with a sauce made from canned fish (15 g), vegetable oil fortified with vitamin A and vitamin D (5 g), and iodized salt (3 g).

Table 1 Micronutrient composition of uncooked rice, per 100 g of blended rice

	Fe (mg)	Zn (mg)	Vitamin A (μg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Thiamin (mg)	Folic acid (mg)	Niacin (mg)
URO	10·7	3·0	0·0	0·0	0·0	1·1	0·2	0·0
URN	7·6	2·0	2140·0	0·0	3·8	1·4	0·3	12·6
NR	7·5	3·7	960·0	0·9	1·3	0·7	0·1	8·0

URO, UltraRice®Original; URN, UltraRice®New; NR, NutriRice®.

Fortified rice kernels were produced from fortified rice flour by extrusion. NutriRice® (NR) was produced by hot extrusion by DSM and Buhler Food (Wuxi, China). Both types of UltraRice® were custom-made for the project, with UltraRice®Original (URO) produced using cold extrusion techniques by Maple Grove Gluten Free Foods, Ltd (Chino, CA, USA) and UltraRice®New (URN) using warm extrusion techniques by the Food Technology department of Kansas State University (Manhattan, KS, USA). Kernels were blended at a ratio of 1:99 with local normal rice to produce fortified rice. The same rice was used for the placebo intervention. The micronutrient composition of the URO, URN and NR is provided in Table 1. The meal was cooked in the school kitchen and served as breakfast at the beginning of the school day. The school meal was distributed for at least 6 months during school days (6 d/week, except during national holidays). An earlier study had shown that fortified rice (URO and NR) were highly accepted by Cambodian school-aged children[28]. Children were dewormed using mebendazole after baseline and endline sample collection. Cognitive performance, anthropometry, parasite infestation and micronutrient status were evaluated at baseline and after 6 months of intervention.

Data collection

Blood samples and anthropometric measurements were collected and cognitive tests performed at schools, after breakfast between 07.30 and 10.00 hours.

Cognitive tests

Cognitive tools generally suffer from intercultural variability[15,29]; to avoid bias linked to translation into Khmer language, tests not involving oral items and with predominantly pictorial content were chosen for the present study[30]. Three tests were performed to assess cognitive performance: (i) Raven's coloured progressive matrices (RCPM), a common non-verbal test of overall intellectual ability in children aged ≥5 years[31–33]; and two standardized tests from the Wechsler Intelligence Scale for Children (WISC III) widely used to evaluate the intelligence of children aged 6–16 years[34], namely (ii) block design and (iii) picture completion. RCPM and WISC III tests were previously used in Vietnamese schoolchildren in nutritional intervention trials[35]. RCPM consists of selecting the correct piece (choice of six different patterns) that completes an illustrated pattern. The block design test requires constructing a design with blocks to match a given design on a picture. The picture completion test consists of identifying the missing detail in a picture. Higher scores indicate better performance. Fluid intelligence underlies reasoning functions, whereas crystallized intelligence is related to experience, education and culture as it requires acquired skills and knowledge[13]. Block design and RCPM tests are associated with fluid intelligence[36–38] while picture completion is related to crystallized intelligence[39].

Each child was tested individually. The cognitive tests were administered by a team of twenty-five students from the Psychology department of Royal University of Phnom Penh. The team was specifically trained during a one-week workshop prior to the start of the intervention to ensure standardization of procedures and scoring.

Anthropometric measurements

Weight and height were measured without footwear and wearing minimal clothing, using standardized procedures[40]. Weight was measured once to the nearest 100 g (model 881 U scale; Seca, Hamburg, Germany). The accuracy of the scales was checked every day using a set of two calibration weights. Height was measured twice to the nearest 0·1 cm on a wooden stadiometer and mean values were used. When differences between two measures of height for the same child exceeded 0·5 cm, measurements were repeated. Height-for-age *Z*-score (HAZ) and BMI-for-age *Z*-score (BAZ) were calculated according to the WHO 2006 reference[41]. Stunting and severe stunting were defined as HAZ < −2 and HAZ < −3, respectively. Thinness and severe thinness were defined as BAZ < −2 and BAZ < −3, respectively. Overweight was defined as 1 < BAZ ≤ 2 and obesity as BAZ > 2. Anthropometric measurements were performed by three teams who were trained and standardized before baseline and again before endline[40].

Blood and urine sample collection and Hb concentration measurements

Five millilitres of venous blood were collected by experienced phlebotomists into a Vacuette® (Greiner Bio-One, Kremsmünster, Austria) trace-element-free vacutainer without anticoagulant. Urine was taken in a sterile container. Urine and blood samples were stored immediately at 4 °C in an icebox containing ice-packs and transported to the laboratory within a maximum of 5 h after the first sample was obtained. Blood samples were centrifuged at 2700 ***g*** for

10 min at room temperature. Serum and urine were then aliquoted into Eppendorf tubes and stored at −30 °C. Hb concentration was measured on-site in whole blood immediately after blood taking, using a HemoCue® R 301+ System and HemoCue controls (Hemotrol low, medium and high; HemoCue AB, Ängelholm, Sweden).

Parasite infestation

Plastic containers and instructions for stool sample collection were given to the child on the day of data collection and requested to be returned to the school the following day. Stool samples were then stored in a cool box, transferred to the National Malaria Center (CNM, Phnom Penh, Cambodia) and stored at 4 °C until analysis. Quantitative parasite eggs counts were performed by CNM using the Kato–Katz method[42].

Laboratory analyses

Ferritin, soluble transferrin receptor, retinol-binding protein, C-reactive protein and α_1-acid glycoprotein serum concentrations

Serum samples were sent on dry ice to the VitMin laboratory (Willstaett, Germany) for determination of ferritin (FER), soluble transferrin receptor (TfR), retinol-binding protein (RBP), C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) concentrations. All of these proteins were measured by a sandwich ELISA technique[43]. Inflammation was defined as an elevated CRP (>5 mg/l) and/or elevated AGP (>1 g/l), allowing differentiation between incubation phase (high CRP), convalescence phase (both AGP and CRP elevated) and late convalescence phase (elevated AGP only)[44]. Anaemia was defined as Hb below cut-offs depending on age and gender: 115 g/l for participants aged <12 years, 120 g/l for adolescents aged 12–15 years and girls aged ≥15 years, and 130 g/l for boys aged ≥15 years; severe anaemia was defined as Hb < 70 g/l; depleted Fe stores were defined as inflammation-corrected FER <15 μg/l[27]. Correction factors for FER were 0·77, 0·53 and 0·75 for participants in incubation, early convalescence and late convalescence phases, respectively, after Thurnham *et al.*[44]. Tissue Fe deficiency was defined as TfR > 8·3 mg/l[43]. Low FER and high TfR concentrations are both considered indicators of Fe deficiency[27], so Fe deficiency was defined as depleted Fe stores (low FER) and/or tissue Fe deficiency (high TfR). Body Fe was calculated according to the formula of Cook *et al.*[45]: body Fe (mg/kg) = [2(log (TfR/FER) × 22·8229)]/0·1207, using FER corrected for inflammation. Body Fe was considered low when < 4 mg/kg[46]. Vitamin A status was measured using RBP concentration, which reflects serum retinol concentration as RBP occurs in a 1:1:1 complex with retinol and transthyretin[47]. RBP concentrations were corrected in participants with inflammation by the factor of 1·15, 1·32 and 1·12, respectively, for incubation, early convalescence and late convalescence[48]. Vitamin A deficiency was defined as corrected RBP < 0·7 μmol/l[47] and marginal vitamin A deficiency as corrected RBP value ≥0·7 and <1·05 μmol/l[49].

Serum zinc concentration

Serum samples were sent on dry ice to the National Institute of Nutrition (Hanoi, Vietnam). Serum Zn was measured by flame atomic absorption spectrophotometry, using trace-element-free procedures. Considering that none of the children were fasting due to the school breakfast, Zn deficiency was defined as serum Zn concentration <0·65 mg/l for participants aged <10 years; for participants aged ≥10 years, the cut-off used was 0·66 mg/l for girls and 0·70 mg/l for boys[50]. Severe Zn deficiency was defined as serum Zn concentration <0·5 mg/l[51].

Urinary iodine concentration

Urine samples were sent on dry ice to the National Institute of Nutrition (Hanoi, Vietnam). Urinary iodine was measured using an ammonium persulfate method at the Thai Nguyen Provincial Hospital, Vietnam, which is one of the three reference laboratories for iodine determination in Vietnam[52]. Iodine deficiency and iodine status above requirements were defined as urinary iodine concentration <50 μg/l and >200 μg/l, respectively[53].

Rice sample analysis

Analysis of the fortified kernels for Fe, Zn, vitamin A, vitamin B_6, vitamin B_{12}, folic acid and niacin was conducted after production by Silliker Laboratories (Chicago, IL, USA) using standard methods. The final composition of blended fortified rice was calculated taking account of a mixing ratio of 1:100. The composition of the fortified rice kernels is given in Table 1.

Data management and statistical analysis

Data entry, including quality checks and validation by double entry of questionnaires, was performed with EpiData version 3·1 (EpiData Association, Odense, Denmark). Data management and analyses were performed using the statistical software package IBM SPSS Statistics version 20.0. Normality of data was checked before analysis with the Kolmogorov–Smirnoff test. In addition, absolute values of skewness and kurtosis of the distribution curves of <2 and <7, respectively, were used to indicate that the distribution of continuous variables was close to normal[54]. Baseline characteristics were compared between intervention groups using ANOVA for continuous variables and the Pearson χ^2 test for categorical variables.

Primary analysis

Generalized linear mixed models including intervention group, visit (baseline, endline), intervention group × visit, age, gender and clustering (school within group) as fixed factors were used to assess the impact of intervention on the raw scores of the cognitive tests compared

with the placebo group. A P value of <0·05 was considered significant and a P value between 0·1 and 0·05 as a tendency.

Secondary analysis

Low Fe status, helminth infection and stunting were identified earlier as risk factors for low cognitive scores in children participating in the FORISCA study[55]. Inflammation was shown to significantly affect the effect of the FORISCA intervention on micronutrient status[56]. To assess if these covariates had an impact on the effect of the intervention on cognitive scores in the present study, interactions of body Fe <4 mg/kg, helminth infection, stunting and inflammation were included as fixed factors in the previous generalized mixed models.

Results

Baseline characteristics

In total, cognition data were available for 1933 children at baseline and 1796 children at endline (Fig. 1). Biochemical, physiological and anthropometric characteristics of the 1933 children at baseline are presented in Table 2.

Mean age at enrolment was 9·7 years old, with participants' age ranging from 6 years to 16 years. Due to late school enrolment and repeating of class, 44 % of the participants were aged ≥10 years, which is adolescent according to the WHO definition[57], but in the present paper all participants are referred to as children. Stunting was highly prevalent in the children participating in the FORISCA study (43 %), whereas 26 % were classified as 'thin'. Fe stores were adequate (FER > 15 μg/l) in most

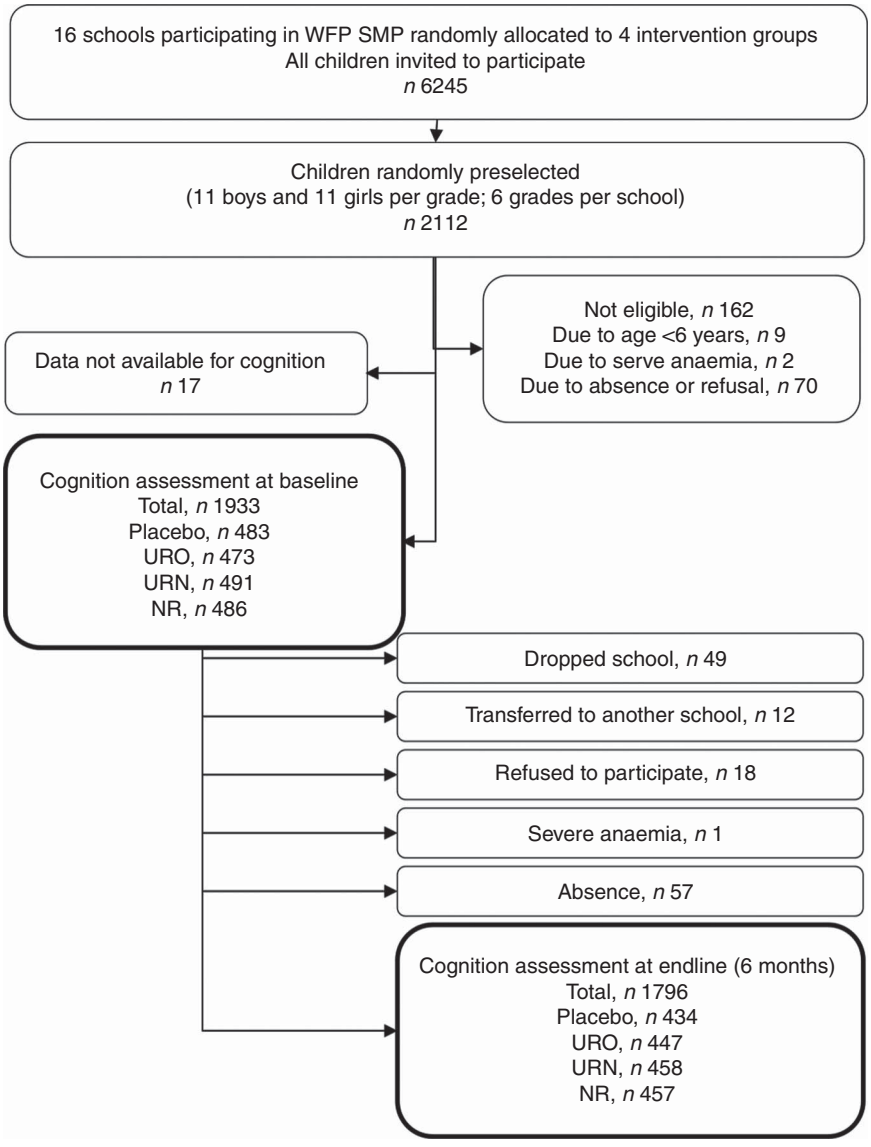

Fig. 1 Design of the FORISCA-UltraRice® + NutriRice® study conducted among Cambodian schoolchildren aged 6–16 years, November 2012–June 2013 (FORISCA, Fortified Rice for School children in Cambodia; WFP, World Food Programme; SMP, school meal programme; URO, UltraRice®Original; URN, UltraRice®New; NR, NutriRice®)

Table 2 Baseline characteristics of Cambodian schoolchildren aged 6–16 years (*n* 1933), FORISCA-UltraRice® + NutriRice® study, November 2012–June 2013

	n	All	Placebo	URO	URN	NR	*P* value
% Girls	1933	50·3	50·7	50·1	50·7	49·8	NS
Age (years), mean		9·7	9·6	9·6	9·7	9·8	NS
SD		2·2	2·3	2·2	2·2	2·4	
% Stunted (HAZ < –2)	1927	42·6	42·9	39·3	45·0	45·1	NS
% Thin (BAZ < – 2)		25·8	24·9	26·7	29·8	21·9	0·040
% Infected by helminths	1442	20·3	23·0	24·9	19·8	12·9	0·000
% Lightly infected by hookworms		17·8	20·0	22·2	17·4	11·1	0·001
% With inflammation (CRP > 5 mg/l or AGP > 1 g/l)	1882	37·8	43·2	42·8	32·8	32·7	0·000
Hb (g/dl), mean	1903	12·4	12·4	12·4	12·4	12·4	NS
SD		1·0	1·0	1·0	0·9	1·1	
% Anaemic*	1903	17·1	18·4	15·4	16·9	17·6	NS
FER† (mg/dl), mean	1882	74·8	77·5	79·8	70·1	71·9	0·000
SD		37·3	38·0	36·6	35·2	37·3	
% With low FER (FER corrected for inflammation <15 µg/l)		1·7	0·4	0·6	3·7	1·9	0·001
TfR (mg/l), mean		8·9	9·0	9·1	8·5	8·9	0·001
SD		2·7	2·6	2·6	2·5	3·1	
% With high TfR (TfR > 8·3 mg/l)		47·8	43·7	43·5	52·4	51·4	0··003
% With Fe-deficiency anaemia (anaemia with high TfR and/or low FER)		7·7	6·5	5·6	9·5	9·0	0·096
Total body Fe (mg/kg), mean		5·9	6·0	6·0	5·8	5·8	0·061
SD		2·3	2·0	2·2	2·4	2·5	
% With low Fe status (total body Fe < 4 mg/kg)		15·1	12·9	13·5	15·9	18·1	NS
Zinc (µmol/l), mean	1546	7·6	7·2	7·8	7·5	7·8	0·000
SD		1·7	1·6	1·7	1·7	1·7	
% Zn deficient‡		93·2	98·1	91·2	93·7	91·9	0·000
% Severely Zn deficient§		53·7	51·1	48·9	55·5	51·1	0·000
UIC (µg/l)	1824	32·8	22·4	25·3	49·0	33·6	0·000
% With UIC < 50 µg/l		17·2	6·4	9·0	35·0	17·4	0·000
% With UIC >200 µg/l		0·3	0·0	0·0	1·3	0·0	0·001
RBP (mmol/l), mean	1882	1·6	1·6	1·7	1·5	1·5	0·000
SD		0·4	0·4	0·4	0·4	0·4	
% Vitamin A deficient (RBP < 0·7 µmol/l)		0·9	0·6	0·2	1·2	1·5	NS
% With marginal vitamin A status (RBP < 1·05 µmol/l)		9·6	7·3	3·6	14·2	12·9	0·000

FORISCA, Fortified Rice for School children in Cambodia; HAZ, height-for-age *Z*-score; BAZ, BMI-for-age *Z*-score; CRP, C-reactive protein; AGP, α_1-acid glycoprotein; FER, ferritin; TfR, soluble transferrin receptor; UIC, urinary iodine concentration; RBP, retinol-binding protein.
*Hb < 115 g/l for participants aged <12 years; 120 g/l for teenagers aged 12–15 years and girls aged ≥15 years; 130 g/l for boys aged ≥15 years.
†FER corrected for inflammation <15 mg/l.
‡Serum Zn < 0·65 mg/l for participants aged <10 years; for participants aged ≥10 years, cut-off is 0·66 mg/l for girls and 0·70 mg/l for boys.
§Serum Zn < 0·5 mg/l.

children (99 %), whereas, paradoxically, tissue Fe deficiency was highly prevalent (48 % of participants with TfR > 8·3 mg/l). Accordingly, half of the children were classified as Fe deficient, but this was almost exclusively related to high TfR. Almost all children were Zn deficient (>90 %) while less than 1 % of the children were vitamin A deficient. Prevalence of anaemia was 17 %.

Primary analysis

All cognitive scores improved over the 6-month intervention (visit (baseline, endline) effect: $P < 0{\cdot}001$; Table 3). On average, the scores increased by 7 (54 %), 3 (20 %) and 3 (38 %) points for the block design, RCPM and picture completion test, respectively (results not shown). The intervention had a significant overall impact on block design score increase (effect of interaction between intervention group and visit, $P = 0{\cdot}003$; Table 3). Improvement in block design scores was significantly higher compared with the placebo group only in children consuming URO ($\beta = 1{\cdot}17$; 95 % CI 0·12, 2·22; $P = 0{\cdot}03$). No significant difference in RCPM scores or picture completion scores was found between the intervention groups.

Secondary analysis

For block design scores, only stunting had an influence on the impact of the intervention ($P = 0{\cdot}006$ for overall interaction; Table 4), while intestinal parasite infection, inflammation and low body Fe did not. The increase in block design scores over time was higher in non-stunted children receiving NR compared with stunted children receiving NR (difference in scores = – 0·69, $P = 0{\cdot}05$).

For RCPM scores, parasite infection had a negative effect on the intervention ($P = 0{\cdot}045$ for overall interaction). Among children receiving URO, RCPM scores increased more in children without parasites than in children with parasites (difference in scores = – 0·08, $P = 0{\cdot}010$). Low body Fe, stunting and inflammation did not affect the impact of the intervention regarding RCPM scores.

For picture completion scores, both stunting and low body Fe had a strong effect on the impact of the

Table 3 Cognition outcomes and effect sizes after 6 months of intervention for Cambodian schoolchildren aged 6–16 years (*n* 1796), FORISCA-UltraRice®+NutriRice® study, November 2012–June 2013

	Effect of variables and interactions on score variation*		Effect on score variation of each intervention group *v.* placebo group*			Score at baseline		Score at endine	
	P value	Group	*β* coefficient	95 % CI	*P* value	Mean score	SE	Mean score	SE
Block design test									
Visit (baseline, endline)	0·000	URO	1·17	0·12, 2·22	0·029	13·6	0·4	22·0	0·5
Intervention group × visit†	0·003	URN	−0·66	−1·71, 0·38	0·214	14·1	0·4	20·5	0·5
Age	0·000	NR	−0·45	−1·50, 0·60	0·396	13·6	0·4	20·4	0·5
Gender	0·000	Placebo	–		–	13·1	0·4	20·5	0·5
RCPM test									
Visit (baseline, endline)	0·000	URO	0·02	−0·52, 0·57	0·93	17·5	0·2	21·0	0·2
Intervention group × visit†	0·153	URN	−0·34	−0·87, 0·20	0·221	17·1	0·2	20·1	0·2
Age	0·000	NR	0·29	−0·25, 0·82	0·3	16·4	0·2	19·9	0·2
Gender	0·000	Placebo	–		–	16·6	0·2	20·1	0·2
Picture completion test									
Visit (baseline, endline)	0·000	URO	0·09	−0·47, 0·64	0·76	7·9	1·9	11·0	1·9
Intervention group × visit†	0·094	URN	0·34	−0·21, 0·89	0·23	7·5	1·8	10·9	1·9
Age	0·000	NR	−0·36	−0·91, 0·19	0·204	7·8	1·8	10·4	1·9
Gender	0·078	Placebo	–		–	7·6	1·9	10·7	1·9

FORISCA, Fortified Rice for School children in Cambodia; RCPM, Raven's coloured progressive matrices; URO, UltraRice®Original; URN, UltraRice®New; NR, NutriRice®.
*Results from generalized mixed models including intervention group, visit (baseline, endline), intervention group × visit, age, gender and clustering (school within group) as fixed factors.
†Effect of intervention group on the difference in scores between baseline and endline.

intervention ($P<0·001$ and $P=0·001$, respectively). In children receiving URN, those with low body Fe increased less in their picture completion scores compared with children with body Fe ≥4 mg/kg (difference in scores = 0·51, $P=0·001$). Similarly, the scores of stunted children increased less compared with non-stunted children receiving URO (difference in scores = −0·51, $P=0·015$).

Discussion

To our knowledge, the present paper is the first reporting modest but significant effects of a micronutrient-fortified rice provided in an SMP on cognitive function in primary-school children. Beneficial effects of URO on micronutrient status and anaemia were reported in small populations (*n* 210) of Mexican women[20], Indian school-aged children[18] and Thai school-aged children[58]. No effect of NR was found on Indian children's cognitive function, but the sample size was considerably smaller than in the present study[19]. Duration of these studies was comparable to the FORISCA study, with meals containing URO or NR being served 5–6 d/week for 5 to 8 months.

Perhaps surprisingly, the present study found an effect of only URO on cognitive performance, and not of the two other types of fortified rice.

URO was fortified with Zn, which is involved in neurotransmission pathways[59]. Moreover, Zn, as a component of Zn-dependent enzymes, plays an important role in neuronal genesis and migration, making adolescents particularly sensitive to Zn deficiency due to rapid brain growth, similar to early childhood[5,59,60]. Even though data are lacking about long-term effects of Zn treatment on cognitive performance, there is some evidence that Zn supplementation may improve neuropsychological functions[5] especially reasoning[61]. For example, Zn supplementation had beneficial effects on cognitive scores of Indian adolescent girls, by reducing reaction time, improving memory and RCPM scores[62]. However, a recent meta-analysis found no effect of Zn on cognitive functioning, but reported that there is a lack of high-quality trials[63]. Zn composition was close between the different formulas of fortified rice and the FORISCA intervention improved Zn status and reduced Zn deficiency and severe Zn deficiency in all intervention groups (K Kuong, P Tor, M Fiorentino *et al.*, unpublished results). Plus, negative cases of interactions between Zn and Fe in fortification or supplementation trials were reported[64]. Therefore, we may not attribute cognitive improvement to Zn in URO.

URO contained the highest concentration of Fe, which is known to be important in brain development also during pre-adolescence and adolescence. Fe plays a role in neurotransmission, especially in the dopamine pathway, which serves in memory, learning, attention, motor control and emotional affect modulation[65,66]. Increasing Fe intake in Fe-deficient and/or anaemic children using fortification or supplementation has been shown to improve schooling or cognitive outcomes like learning, memory, concentration or school achievement[11,12,67–71]. URO did not improve Fe stores and was not fortified with vitamin A, but it was the only fortified rice that improved cognitive performance, which is tempting to link to the improved tissue Fe status observed only in children receiving URO (decrease of TfR concentrations, $P=0·088$, as reported elsewhere[56]).

Table 4 Effects of risk factors for low cognitive scores on the 6-month intervention among Cambodian schoolchildren aged 6–16 years (*n* 1796), FORISCA-UltraRice® + NutriRice® study, November 2012–June 2013

	Overall effect of interaction with the intervention on score variation*	URO			URN			NR		
	P value	*β* coefficient*	Difference in scores' increase†	*P* value‡	*β* coefficient*	Difference in scores' increase†	*P* value‡	*β* coefficient*	Difference in scores' increase†	*P* value‡
Stunting										
Block design	0·006	−1·411	0·42	0·234	−1·805	0·93	0·144	−2·687	−0·69	0·05
Picture completion	0·000	−1·146	−0·51	0·015	−0·434	0·68	0·374	−0·243	0·91	0·654
RCPM	0·352	−0·774	0·14	0·147	−1·131	−0·29	0·042	−0·566	−0·21	0·358
Parasite infection										
Block design	0·197	3·152	−1·18	0·009	−0·352	−0·18	0·785	0·935	−0·31	0·524
Picture completion	0·129	−0·389	−0·29	0·411	0·407	−0·82	0·428	−0·072	−0·32	0·902
RCPM	0·045	−1·389	−0·08	0·01	−0·121	−0·66	0·835	−0·532	0·03	0·421
Inflammation										
Block design	0·141	−1·130	−0·80	0·314	0·471	−0·67	0·706	−0·136	2·08	0·919
Picture completion	0·873	0·275	0·35	0·534	−0·436	0·00	0·379	−0·01	0·18	0·985
RCPM	0·058	0·388	0·38	0·442	−0·283	0·04	0·615	0·09	1·30	0·881
Low body Fe										
Block design	0·157	1·079	0·06	0·453	2·328	−2·91	0·103	−1·582	0·67	0·308
Picture completion	0·001	−1·011	0·50	0·076	−1·853	−0·51	0·001	0·607	−0·63	0·323
RCPM	0·211	−1·144	−0·44	0·077	−0·971	−0·66	0·13	−0·102	−0·29	0·884

FORISCA, Fortified Rice for School children in Cambodia; URO, UltraRice®Original; URN, UltraRice®New; NR, NutriRice®; RCPM, Raven's coloured progressive matrices.
Results from generalized mixed models including age, gender, clustering (school within group), intervention group, visit (baseline, endline), intervention group × visit, and each risk factor (stunting, parasite infection, inflammation or low body Fe) × intervention group × visit, as fixed factors.
*β coefficient of the interaction intervention group × visit × risk factor (stunting, parasite infection, inflammation or low body Fe).
†Difference in improvement of scores between baseline and endline in stunted children compared with non-stunted children (or in children with parasites compared with children without parasites, or in children with inflammation compared with children without inflammation, or in children with low body Fe compared with children with normal body Fe).
‡Significance of the difference of improvement in scores.

At baseline in all groups, Fe deficiency was uncommon, which could possibly have limited the impact of the fortified rice formulas on cognitive performance. The high prevalence of high TfR concentrations, suggesting functional Fe deficiency with the absence of depleted Fe stores, may appear surprising, as the Fe depletion process usually consists of depletion of Fe stores before tissue Fe decrease and erythropoiesis impairment. However, such discrepancy between adequate Fe stores and tissue Fe deficiency has been reported in several other studies, and attributed among other factors to malaria or haemoglobinopathies[56,72–75]. Therefore, we may hypothesize that external Fe provided to children with replete Fe stores but functional Fe deficiency was first used for filling tissues and for functions, such as cognitive functions, before building Fe stores in the URO group. A slight decrease of Hb was also observed at midline in this group ($P = 0{\cdot}068$)[56], thus these physiological functions were probably non-erythropoietic functions. Although URO contained the highest Fe content, the difference compared with the two other types of fortified rice was relatively small, and other micronutrients might have contributed to the observed difference in impact on cognition.

No cognitive improvement, but a significant increase of FER and TfR, respectively indicating improvement of Fe stores and decline of Fe status, was observed between baseline and endline only in children receiving URN and NR ($P < 0{\cdot}001$ for all). The additional vitamin A in these two groups improved vitamin A status of the children ($P < 0{\cdot}001$)[56] and may have helped to build Fe stores, as was already observed in adolescents supplemented with multiple micronutrients[76] or children consuming vitamin A-fortified food[77]. At the same time, tissue Fe decreased in children receiving URN and NR, an effect that could perhaps be attributed to the vitamin A in the rice premix in these two groups also. Vitamin A might have led to higher erythropoietin concentrations, pushing erythropoiesis as reported elsewhere[78]. Vitamin A is known to help in mobilizing Fe for physiological functions, especially erythropoiesis, as was observed in anaemic children receiving vitamin A supplementation[78,79] and in animal models[80]. Interestingly, a slight increase of Hb concentrations was observed in children receiving URN between baseline and midline ($P = 0{\cdot}048$). Oppositely to what was observed in the URO group, external Fe was apparently first used for building stores and erythropoiesis before physiological functions. One may assume that vitamin A provided in URN and NR groups has increased Fe storage to the detriment of tissue uptake of Fe for other functions such as cognitive improvement on one hand, and stimulated erythropoiesis using Fe from tissues rather than from stores, on the other hand.

Moreover, it is likely that interactions with other micronutrients present in the URN and NR, but not in the URO, also played a role in modifying the effect on cognitive outcomes. URN had the lowest concentrations of Zn and Fe but the highest of vitamins (A, B_6, B_{12}, thiamin, folic acid and niacin). Even though vitamins B_{12}, B_6 and folic acid are known to play a role in cognitive performance by being involved in methylation in the central nervous system[81,82], URN and NR, fortified with these vitamins, did not improve cognition performance in the present study, while URO, not fortified with these vitamins, did improve cognition performance. Therefore, we could hypothesize that improvement in cognition performance in the present study is more likely to be related to the additional Fe and Zn provided through URO, and not to additional B-vitamins. However, these results should be interpreted with caution. Another hypothesis to explain our discrepant results about Fe status and cognitive performance between fortified rice formulas may be that vitamins A, B_{12} and niacin in URN and NR helped to build Fe stores and pushed erythropoiesis[83] to the detriment of tissue Fe uptake for cognitive development. Little is known about the role micronutrients on the physiological pathways of Fe distribution between Fe storage, erythropoiesis and other physiological functions. No data about vitamin B_{12} and niacin deficiencies were collected during the study, hence the prevalence and potential impact of vitamin B_{12} and niacin deficiencies on the intervention are not known. Apart from Zn and Fe negative interactions, other contradictory benefits of different micronutrients on cognitive improvement are not well documented to our knowledge.

Therefore, the possible effect of Fe alone and the interaction between Fe and other micronutrients remain unclear. The results also raise questions on which indicator should be used for assessing functional Fe deficiency.

In an overview of randomized controlled trials of multiple micronutrient supplementation in school-aged children, Eilander *et al.* noticed a potential beneficial effect on fluid intelligence and school performance, but not on crystallized intelligence[13], which is consistent with fortified rice having an impact on block design scores but not on picture completion scores in children participating in the FORISCA study. The block design test measures perceptual reasoning and executive functions, both of which are associated with frontal lobe functions[39,84]. The development of the full range of executive functions is suggested to occur in late childhood and adolescence as myelination of the frontal lobes proceeds[31], with peaks at 7, 9 and 12 years of age[66,85]. There is some evidence that Fe is a key nutrient in the development of these executive functions[66].

Although the impact of URO on RCPM scores did not reach significance, it was significantly lower in children with intestinal parasite infection than in those without parasites. Hence, while being a risk factor for poor cognitive scores in itself, parasite infestation also limited the impact of fortified rice on RCPM scores. Earlier we reported that fortified rice also increased parasite infestation in children participating in the FORISCA study[86], highlighting the complex interaction between nutrition and infection.

Albeit no significant impact of fortified rice on RCPM and picture completion scores was observed in the general sample, low body Fe diminished the increase of these scores over the intervention period regardless of intervention type, indicating that children participating in the FORISCA study with low Fe status benefited less from fortified rice with respect to cognition scores, in comparison to children with adequate Fe status. Even if increased Fe intake during the intervention had an overall beneficial effect on block design scores, this suggests that the amount of bioavailable Fe in the fortified rice may not be sufficient to reverse the negative effect of Fe deficiency on cognition scores.

Inflammation also had a negative effect on RCPM score improvement. Interestingly, this is in parallel with the observed effects of fortified rice on Fe status of children participating in the FORISCA study, which emerged stronger after removing children with inflammation from the analysis[56]. This suggests that the acute-phase response may disturb the response to fortified foods and therefore the impact on functional outcomes such as cognitive performance.

Strengths and limitations of the study

One of the strengths of the present study was its design as an effectiveness trial. We assessed the impact of the introduction of fortified rice within an existing programme, with minimal interference in the programme's daily logistics. Hence, the results obtained provide a realistic indication of what can be expected from the introduction of multi-micronutrient-fortified rice on cognitive performance in Cambodian school-aged children. Another strength of the study is the sample size, with >2000 children being followed over the study period.

One limitation of the study was the intervention's short duration of only 6 months; this amount of time may not have been enough to improve cognitive performance to its full potential. An intervention exceeding 6 months may have shown a larger impact on cognitive outcomes. Also, the present study was an effectiveness study, not an efficacy study. Hence, quantities of rice consumed every day were not controlled. Moreover, it was not possible to ensure that micronutrient status at baseline would be the same between randomized groups. The prevalence of low Fe stores was very low, and larger impact on cognitive function may have been observed in a population with a higher prevalence. Although the schools were randomly allocated to the different intervention groups, groups still differed with respect to thinness, Fe status, iodine status, micronutrient deficiencies and parasite infestation. Groups

did not differ with respect to gender, age and stunting. As the sample size is large, and the statistical modelling robust, no attempts were made to correct or amend for this; instead the validity of each analysis was carefully checked. In addition, the pre–post comparison model of the study design further aids the clear interpretation of the data. Another limitation of the FORISCA study is that levels of Fe and Zn used in the present study are below the current recommended levels for fortified rice, which might have reduced the effectiveness[87].

Conclusion

The WFP SMP is known to be an incentive for school attendance and enrolment, and could enhance learning by reducing hunger and increasing concentration[23]. However, only selected cognitive functions may still be improved with nutritional interventions at school age, which stresses the importance to pursue efforts for prevention of malnutrition over the life cycle, especially before and during pregnancy and early childhood, together with school interventions in order to prevent irreversible cognitive damages. The current study illustrates that fortification of school meals with multiple micronutrients can improve cognitive performance and thus promote schooling further. However, the improvements in cognitive performance were modest and impact was modified by several external factors such as stunting, intestinal parasite infestation, low body Fe and inflammation. This strengthens the case for combining health interventions, such as deworming combined with provision of micronutrients. However, micronutrients used in rice fortification should be carefully selected and appropriately dosed, taking the initial nutritional and infection status of the target population also into account.

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