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# Effect of Iron- and Zinc-Biofortified Pearl Millet Consumption on Growth, Immune Competence and Cognitive Function in Children Aged 12-18 Months in India— Study Protocol for a Randomised Controlled Trial

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Complete List of Authors:	Mehta, Saurabh; Cornell University ; Institute for Nutritional Sciences, Global Health, and Technology Finkelstein, Julia; Cornell University , Division of Nutritional Sciences; St John's Research Institute Venkatramanan, Sudha; Cornell University , Division of Nutritional Sciences Huey, Samantha; Cornell University , Division of Nutritional Sciences Udipi, Shobha; Kasturba Health Society Ghugre, Padmini; SNDT Women's University, Food Science and Nutrition Ruth, Caleb; Data Performance LLC Canfield, Richard; Cornell University , Division of Nutritional Sciences Kurpad, Anura; St John's Research Institute, Nutrition Potdar, Ramesh; Centre for the Study of Social Change Haas, Jere; Cornell University , Division of Nutritional Sciences
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Keywords:	Biofortification, zinc, iron, pearl millet, children, growth

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1	Effect of Iron- and Zinc-Biofortified Pearl Millet Consumption on Growth,
2	Immune Competence and Cognitive Function in Children Aged 12-18 Months
3	in India—Study Protocol for a Randomised Controlled Trial
4	
5	Saurabh Mehta <sup>1,2,3</sup> , Julia L. Finkelstein <sup>1,2,3</sup> , Sudha Venkatramanan <sup>1</sup> , Samantha L.
6	Huey <sup>1</sup> , Shobha A. Udipi <sup>4</sup> , Padmini Ghugre <sup>5</sup> , Caleb Ruth <sup>6</sup> , Richard L. Canfield <sup>1</sup> ,
7	Anura V. Kurpad <sup>3</sup> , Ramesh D. Potdar <sup>7</sup> , Jere D. Haas <sup>1</sup>
8	
9	<sup>1</sup> Division of Nutritional Sciences, Cornell University, Ithaca, New York, US
10	<sup>2</sup> Institute for Nutritional Sciences, Global Health, and Technology, Cornell University,
11	Ithaca, New York, US
12	<sup>3</sup> St. John's Research Institute, Bangalore, India (SJRI)
13	<sup>4</sup> Kasturba Health Society Medical Research Centre (KHS-MRC), Mumbai, India
14	<sup>5</sup> Shreemati Nathibai Damodar Thackersey, Women's University (SNDT), Mumbai,
15	India
16	<sup>6</sup> Data Performance LLC, New York City, New York, US
17	<sup>7</sup> Center for the Study of Social Change, Mumbai, India (CSSC)
18	
19	Corresponding Author (Principal Investigator):
20	Saurabh Mehta, MBBS, ScD
21	314 Savage Hall, Ithaca, New York 14853, US
22	Phone: +1-607-255-2640; Fax: +1-607-255-1033
23	E-mail: smehta@cornell.edu
24	
25	Tables and Figures: 1

Keywords: Biofortification, iron, zinc, pearl millet, children, growth ABSTRACT **Introduction:** Biofortified crops represent a sustainable agricultural solution for the widespread micronutrient malnutrition in India and other resource-limited settings. This study aims to investigate the effect of the consumption of iron and zinc-biofortified pearl millet by children on biomarkers of iron and zinc status and growth outcomes. Additionally, we will assess immune and cognitive function in the participants. Methods and Analysis: We will conduct a randomised controlled feeding trial in identified slums of Mumbai, India among 250 children between 12-18 months of age for 9 months. Children will be randomised to receive the biofortified pearl millet (FeZn-PM, ICTP8203-Fe) or non-biofortified pearl millet. Anthropometric and morbidity data will be gathered every month for 9 months. Biological samples will be collected at baseline, midline, and endline, to assess iron and zinc status, including

haemoglobin, serum ferritin, serum transferrin receptor, serum zinc, C-reactive

protein, and alpha-1 acid glycoprotein, and other biomarkers. The midline

measurement will be a random serial sample between baseline and endline. Immune

function and cognitive function will be assessed at each time point by the

measurement of vaccine responses and *in-vitro* functional assays in a subset and cognition tests, respectively.

**Ethics and Dissemination:** This study has obtained clearance from the Health Ministry Screening Committee (HMSC) of the Indian Council of Medical Research (ICMR). Ethical clearance has been obtained from Cornell University's Institutional

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52	Review Board, the Inter System Biomedica Ethics Committee (ISBEC), and St.
53	John's Research Institute Institutional Ethics Review Board. The results of this study
54	will be disseminated at several research conferences and as published articles in
55	peer-reviewed journals.
56	
57	Registration Details: Clinicaltrials.gov registration number: NCT02233764
58	(registered on September 4, 2014). Clinical Trials Registry of India (CTRI), reference
59	number REF/2014/10/007731, CTRI number CTRI/2015/11/006376.
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2 3	61	Strengths and limitations of this study
4 5 6	62	This is the first longitudinal randomised controlled trial to determine the
7 8	63	efficacy of consuming complementary foods prepared using iron-biofortified
9 10	64	pearl millet on both nutritional status and functional outcomes, among children
11 12 13	65	12-18 months of age.
14 15	66	The longitudinal random midline serial sampling strategy both increases
16 17	67	sensitivity and the power of the proposed study, while reducing cost and
18 19 20	68	invasiveness (by decreasing the number of biological samples from each
21 22	69	participant).
23 24	70	Data will be collected on iPads or laptops, using a mobile electronic data
25 26 27	71	capture system framework on the iOS platform. This will decrease the
28 29	72	potential for error in data entry compared to standard written hard copies of
30 31	73	forms, and allow direct uploading of data using a secure server.
32 33	74	<ul> <li>One limitation of this study is that blinding may not be possible due to</li> </ul>
34 35 26	75	potential sensory differences between the crops used in the two arms of the
37 38	76	study.
39 40	77	
41 42	78	
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INTRODUCTION

82	The burden of iron and zinc deficiency
83	Deficiencies in iron and zinc are widely seen as two of the most important public
84	health problems globally <sup>12</sup> . The impacts of iron and zinc deficiency on growth <sup>34</sup> ,
85	immune competence <sup>5</sup> , and cognitive function <sup>16</sup> affect quality of life, particularly in
86	early childhood. The role of iron deficiency in growth and development of children
87	has been demonstrated in several studies <sup>378</sup> . Similarly, it appears that a major
88	fraction of the growth retardation or stunting can be explained by zinc deficiency <sup>4</sup> .
89	Suboptimal iron and zinc status impairs immune functioning through a number of
90	mechanisms, including a reduction in the proportion of circulating T-lymphocytes and
91	lymphocyte proliferative responses <sup>9</sup> . Cognitive deficits are also observed with iron
92	and zinc deficiency, including irreversible impairments in neurological and
93	psychomotor development of children <sup>10</sup> .
94	
95	Iron and zinc biofortification
96	Biofortification has the potential to be a more sustainable and cost-effective
97	approach compared to other strategies such as diet diversification, fortification, and
98	supplementation, to address micronutrient malnutrition among vulnerable
99	populations <sup>11</sup> . Previous research from southern India and preliminary data from our
100	acceptability study indicate that the mean consumption of pearl millet (PM) flour in
101	children is 61-80 g/day <sup>12 13</sup> . The iron and zinc content in biofortified PM is reported to

- 102 be 70-85 and 35-40 parts per million (ppm), respectively. Thus, for children, iron and
- 103 zinc intakes from PM would be 7-8 mg/100g and 3-4 mg/100g, respectively, and
- 104 would help meet 50 to 70% of the recommended dietary allowances (RDA) for
- 105 children between 1-3 years of age. These estimates are based on the RDA recently

106 recommended for Indians by the Indian Council of Medical Research<sup>14</sup>.

107	
108	The primary objectives of this randomised controlled trial are to study the effect of
109	the daily consumption of high iron- and zinc-biofortified pearl millet on biomarkers of
110	iron and zinc status, as defined by haemoglobin, serum ferritin, serum transferrin
111	receptor, serum zinc, and C-reactive protein; and growth, as defined by length-for-
112	age, weight-for-age and weight-for-length, among 12-18-month-old children, as
113	compared to children receiving conventional pearl millet. Additionally, we will assess
114	the effect of high iron- and zinc-biofortified pearl millet by 12-18-month-old children
115	on immune and cognitive outcomes.
116	
117	METHODS AND ANALYSIS
118	Study setting
119	This study will take place in identified slums of urban Mumbai, Maharashtra, India.
120	Mumbai is the commercial capital of India, and about 41.3% of city's populace
121	resides in urban slums <sup>15</sup> .
122	
123	Study design
124	We will conduct a randomised controlled trial in which children (12 to 18 months old)
125	will be fed complementary foods prepared using iron- and zinc-biofortified pearl
126	millet (FeZn-PM) or the comparator conventional pearl millet for nine months.
127	Depending on the number of children in the appropriate age group, slums will be
128	selected for the intervention. The participants will be children between the ages of 12
129	and 18 months at enrolment and will be selected based on the following selection
130	process:

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2 3 4	131	1. Census: Before the study begins, a census will be conducted in the identified
5 6	132	slums to gather information on the age of the child, sex, and location.
7 8	133	
9 10	134	2. Screening Phase 1: Non-invasive data will be collected on: dietary allergies, use
11 12 13	135	of iron or zinc dietary supplements, availability of a caregiver, and if the caregivers
14 15	136	are planning to stay in the slum for the duration of the study. If the children were not
16 17	137	dewormed recently, they will be dewormed using liquid albendazole at the study
18 19	138	clinic.
20 21	139	
22 23 24	140	3. Screening Phase 2: Blood will be collected during this phase of screening to
25 26	141	measure complete blood counts including haemoglobin. Anthropometry and dietary
27 28	142	intake will also be measured at this phase of screening.
29 30	143	
31 32 33	144	4. Randomization: The Cornell Statistical Consulting Unit will generate the random
34 35	145	allocation sequence using a statistical software package; the randomization
36 37	146	sequence key will not be available to any study personnel until follow-up is over.
38 39	147	Randomization will be allocated at the individual level. Individuals will be randomized
40 41 42	148	in blocks of 60. Pearl millet food products will coded by study arm (Arm 1: iron- and
42 43 44	149	zinc-biofortified pearl millet; Arm 2: conventional pearl millet). Children will be
45 46	150	randomized to either study arm and will be monitored to ascertain they consume the
47 48	151	assigned food product throughout the study duration. The midline measurement
49 50	152	(including biological sample collection and cognitive assessment) will be a random
51 52 53	153	serial sample taken at any month (months 2-7) between the baseline and endline
54 55	154	measurements <sup>16</sup> . Longitudinal midline random serial sampling is often used in
56 57	155	population pharmacokinetic research and has been shown to be a useful strategy for
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iron fortification efficacy studies, by describing the pattern of iron repletion<sup>16</sup>. 156

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1 2

## 158 Intervention and comparator

159 The intervention is iron-and-zinc-biofortified pearl millet (ICTP-8203) developed by 160 the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and 161 the comparator is a conventional pearl millet that is commercially available on the 162 market. The comparator was chosen because it is similar to the intervention in all 163 aspects, except for iron and zinc content, which allows direct analysis of the impact 164 of the iron-and-zinc-biofortified variety on our outcome measurements. We expect a 165 child to consume an average of 60 g of pearl millet per day, depending on the age of the child<sup>12</sup>.

166

- 167
- 168 Inclusion and exclusion criteria

169 Inclusion criteria

- 170 Participants included in this study will be 12-18-month-old male and female children
- 171 with haemoglobin concentration greater than 9 g/dL, living in urban slums of

172 Mumbai.

173

174 Exclusion criteria

175 Children will be excluded if: (1) they are younger than 12 months old or older than 18

- 176 months at enrolment; (2) their haemoglobin concentration is less than 9 g/dL or
- 177 haemoglobinopathy is present (as indicated via abnormal peak via hemoglobin
- variant analysis): (3) they show signs of severe malnutrition (a weight-for-length, 178
- 179 length-for-age, and/or weight-for-age z-score less than -3)<sup>17</sup>; (4) their caregiver
- 180 reports known diagnosis of diseases such as HIV/AIDS, malaria, tuberculosis, and/or

1		
2 3 4	181	dengue fever; (5) their caregiver is unavailable to bring the child to the feeding
5 6	182	centre during follow-up; (6) the child has any known dietary allergies; and (7) their
7 8	183	caregivers are not planning to stay in the study area over the period of follow-up; (8)
9 10 11	184	prior (within the past one year) or current consumption of iron or zinc supplements.
12 13	185	Children who are severely anemic will be referred to physicians at the Center for the
14 15	186	Study of Social Change (CSSC).
16 17	187	
18 19	188	Informed consent process
20 21 22	189	Research Assistants will obtain informed consent from caregivers in the following
23 24	190	stages:
25 26	191	1) Screening Phase 1: Based on the census data, informed consent for the
27 28	192	collection of data regarding child's date of birth, deworming, morbidities, and
29 30 21	193	dietary history will be collected. Based on the child's eligibility at this stage,
31 32 33	194	the child's caregiver will then be requested to return for the collection of
34 35	195	biological specimens.
36 37	196	2) Screening Phase 2: Informed consent will be collected from the caregivers for
38 39	197	collection and storage of biological samples, as well as anthropometry
40 41 42	198	screening. Blood will be collected to assess haemoglobin concentration.
43 44	199	Anthropometric measurements and dietary intake will be collected from
45 46	200	caregivers and their children to determine maternal and participant nutritional
47 48	201	status and nutrient intake, respectively.
49 50	202	3) Intervention: If a child is deemed eligible, the caregiver will be requested to
51 52 53	203	provide informed consent for the child's enrolment into the trial including
54 55	204	consent to storage of biological samples. All trial details will be explained
56 57 58 59	205	orally and in the written local language (Hindi) by study staff, and each
60		

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2 3	206	informed consent process will be recorded by video as per the requirement of
4 5 6	207	the Government of India. Our anticipated date of enrolment of the first
7 8	208	participant is June 1, 2017.
9 10	209	
11 12	210	Feeding Centre
13 14 15	211	Feeding centres will be located near to where children and their caregivers'
16 17	212	dwellings are clustered, to ensure that travel time from their home to the feeding
18 19	213	centre is within walking distance.
20 21	214	
22 23 24	215	Sample size estimation
25 26	216	Our estimates of sample size are based on assumptions about mean values and
27 28	217	associated variation in haemoglobin and serum ferritin. We expect 50-75% of these
29 30	218	children to have iron and zinc deficiency based on published literature <sup>18</sup> and
32 33	219	preliminary results (unpublished). We assume that absorbed iron will be transferred
34 35	220	to body stores in the liver, and that the change in liver stores is reflected in changes
36 37	221	in serum ferritin at a rate of 8 $\mu$ g/L of serum ferritin per mg liver iron in non-anaemic,
38 39	222	iron-depleted children. Iron-deficient, anaemic (haemoglobin < 11 g/dL <sup>19</sup> ) children
40 41 42	223	will have their absorbable iron directed to haemoglobin synthesis in the early months
43 44	224	of the feeding trial. We estimate that the children consuming biofortified FeZn-PM
45 46	225	will demonstrate a gain of 1 g/dL in haemoglobin concentrations in 2.4 months,
47 48	226	whereas children consuming control pearl millet will need more than 9 months to
49 50 51	227	demonstrate the same increase. To detect a significant increase in ferritin from the
52 53	228	baseline value of 1.79 (6 mg/L) with standard deviation of 1.2, in the experimental
54 55	229	group, compared to the control group, at a power of 80% and 5% significance level,
56 57 58	230	96 participants will be required. We propose a larger sample size of n=250, to

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ensure adequate power for other outcomes of interest in this study, including
immune and cognitive function, as well as biomarkers for zinc status which are les
defined than biomarkers of iron status.
Follow-up
Assessments
At baseline, we will assess anthropometry; collect blood for analysis of hemoglobir
iron, zinc, CRP, and AGP biomarkers in the blood (all blood analyses other than
complete blood counts will be performed in batch at the end of the trial to minimize
error); nutrient intake measures using multiple-pass 24-h dietary recall <sup>20</sup> for childre
and food frequency questionnaire for the mothers; socio-economic and demograpl
information; and morbidity history. Follow-up will continue for 9 months and will
include monthly anthropometric measurements, morbidity assessments, and 24-ho
dietary recall. Additionally, we will conduct cognitive testing and collect blood and
stool samples to measure immune function at three time points (baseline, midline
and endline). The midline time point will be a random serial sample that occurs
between the baseline and endline measures. Children may visit the CSSC clinic at
any time during the trial for healthcare treatment.
Administration of intervention
Both arms will receive complementary foods prepared with pearl millet three times
per day, 6 days per week, for 9 months. Each day, the child's caregiver will bring the
child to their feeding centre. Two meals will be consumed at the feeding centre; the
third meal may be consumed at home. The food intake of the two meals at the
feeding centre will be measured directly before and after consumption. To measure

immune and cognitive function, as well as biomarkers for zin defined than biomarkers of iron status. Follow-up Assessments At baseline, we will assess anthropometry; collect blood for a iron, zinc, CRP, and AGP biomarkers in the blood (all blood) complete blood counts will be performed in batch at the end error); nutrient intake measures using multiple-pass 24-h die and food frequency questionnaire for the mothers; socio-eco information; and morbidity history. Follow-up will continue for include monthly anthropometric measurements, morbidity as dietary recall. Additionally, we will conduct cognitive testing a stool samples to measure immune function at three time poir and endline). The midline time point will be a random serial s between the baseline and endline measures. Children may v any time during the trial for healthcare treatment. Administration of intervention Both arms will receive complementary foods prepared with p per day, 6 days per week, for 9 months. Each day, the child's child to their feeding centre. Two meals will be consumed at

third meal may be consumed at home. The food intake of the

feeding centre will be measured directly before and after con

256	the intake of the third meal, the weight of unconsumed food from the third meal will
257	be measured the next morning at the feeding centre. To assure adherence,
258	healthcare workers will follow up with the participants' caregivers and record reasons
259	for non-adherence. Throughout the study, we will conduct periodic analysis of
260	random samples of both grain varieties and prepared food products to ensure food
261	safety and quality of the intervention.
262	
263	Primary outcome measurements
264	Biomarkers of iron and zinc status
265	We will determine if biofortified pearl millet improves iron and zinc status compared
266	to children who consume non-biofortified pearl millet. Specifically, iron and zinc
267	status will be assessed by measuring concentrations of haemoglobin (Hb), serum
268	ferritin, serum transferrin receptor (sTfR) and plasma zinc at enrolment (baseline),
269	midline, and endline. Additionally, we will measure concentrations of inflammatory
270	biomarkers C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP), as iron
271	and zinc biomarkers can be influenced by inflammation.
272	
273	Growth
274	We will assess change in a child's growth as another outcome variable to determine
275	if iron-biofortified pearl millet reduces the risk of underweight (weight-for-age z score
276	< -2), wasting (weight-for-height z-score < -2) and stunting (length-for-age z score < -
277	2) during the 9-month intervention period, compared to those who receive non-
278	biofortified pearl millet. We will measure weight (kg) and length (cm) at baseline and
279	monthly throughout follow-up. The weight and length data will be converted to
280	weight-for-age, weight-for-length z-score, and length-for-age z-score. The growth

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2 3 4	281	rates (as kg/month, and cm/month) will also be compared from the absolute
5 6	282	measurements after controlling for age.
7 8	283	
9 10 11	284	Additional outcomes
11 12 13	285	Immune function
14 15	286	We will directly assess immune function by measuring superoxide burst and
16 17	287	proteolytic capacities of macrophages and monocytes. Activity index will be
18 19	288	assessed by comparing mean substrate and calibration florescence <sup>21</sup> . Additionally,
20 21 22	289	we will collect morbidity data, including changes in types and frequencies of
22 23 24	290	morbidities such as diarrhoeal illness, pneumonia, and any chronic disease
25 26	291	throughout follow-up during each clinic visit. Caregivers will also be encouraged to
27 28	292	come to the clinic at any time for any health-related reasons, which will also be
29 30	293	recorded. This will provide an accurate representation of both innate and adaptive
31 32 33	294	immunity in participants.
34 35	295	
36 37	296	Cognitive function
38 39	297	To determine if consumption of biofortified pearl millet improves child cognitive
40 41 42	298	function, compared to consumption of non-biofortified pearl millet, we will assess
43 44	299	aspects of cognition that (1) previously have been shown to be sensitive to the
45 46	300	effects of early iron and/or zinc deficiency, or (2) draw heavily upon brain structures
47 48	301	or processes thought to be vulnerable to early iron and/or zinc deficiency, based on
49 50	302	studies of animal models and humans. Thus, we will assess multiple specific aspects
51 52 53	303	of memory, attention and processing speed using automated eye trackers <sup>22</sup> . In
54 55	304	addition, we will also assess higher-level, integrative cognitive abilities, including
56 57	305	problem-solving and exploratory behavior and global aspects of attention and
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inattention during free play with toys<sup>23</sup>. Finally, we will conduct a Developmental
Assessment Scales for Indian Infants (DASII)<sup>24</sup> test at the baseline, intermediate and
endline assessments to obtain a broad measure of attainment of developmental
milestones that can be used to compare the characteristics of our cohort with those
reported in the literature.

311

# 312 Data collection and storage

313 Prior to data collection, the field staff will be trained on ethical and data collection 314 procedures. The data collection tools will be translated from English into the local 315 languages of Hindi and back-translated into English to retain the original meaning. 316 The Hindi regional language versions of the guestionnaire will be used for data 317 collection. A pre-testing of the data collection tools will be evaluated before the main intervention; the protocol of data collection forms to be used is shown in Figure 1. 318 319 The data collection will begin with baseline measurements in January 2017. All data 320 will be collected on iPads or laptops for the proposed project. We will use a mobile 321 electronic data capture (mEDC) framework on the iOS platform, Connedct, that was 322 created by our Database Developer and is being used in our ongoing efficacy trial in Mumbai<sup>25</sup>. A secure server will be utilized for uploading, storing, and accessing the 323 324 data. This will enable real-time feedback and error checking, as well as eliminate 325 errors associated with data entry, facilitating faster data analysis and rapid 326 dissemination of results. All information will be kept confidential. All names will be 327 removed from the data for analysis. The identifying codes and linked names will be 328 securely stored in password-protected computers. All data will be securely stored on 329 a third-party server, which will have limited access by study team members. All 330 biological specimens will be collected by trained medical professionals and

331	evaluated by certified laboratories within Ind	lia. Biological specimens will be stored
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- appropriately throughout the duration of the study and after the study for future
- analysis. All study investigators will have access to the final trial dataset via
- 334 contractual agreements.

Кеу	Н	HarvestPlus Database Form Collection Protocol											
			DHACE 2										
req, daily	FNA												
Outside EDC; manual	Before Screening	1-2 months before baseline		FOLLOW-UP									
Not req	1	2	2	4	5	6	7	9	0	10	11	12	
Forms:	Census	Pre- Screening, Screening	Baseline [pre- feeding]	1	Midline 2	Midline 3	Midline 4	Midline 5	Midline 6	Midline 7	8	9 Endline [post- feeding]	
Phase 1 - Screening													
Census (OED)											-	$\square$	
Consent for Prescreening												$\square$	
Prescreening/RA Form												$\square$	
Consent for Screening													
Screening_Lab/Clinic													
Screening_Anthropometry													
Screening_RectalSwab													
Screening_WHZ_Zscore													
Consent for Trial													
Phase 2 - Trial													
FollowUp_Baseline_SES													
FollowUp_Lab													
FollowUp_RectalSwab													
FollowUp_Cognition													
FollowUp_Morbidity													
FollowUp_Anthropometry													
FollowUp_Feeding1													
FollowUp_TB_Contact_Inves	tigation												
FollowUp_IYCF													
FollowUp_FFQ													
FollowUp_Recall				_									
AER (Adverse Event Report)													
DropOut {Termination}													
Other Issue (Not in draft form database)			:::::::					11111	1			1.1.1.1.1	

Notes:

The midline measurements will take place in months 2-7.

336 Figure 1. Form Collection Protocol.

# 338 Data analysis

339 We will use an intention-to-treat approach to determine the effect of biofortified pearl

- 340 millet on the outcomes described above. Advanced analysis will use mixed models
- 341 to account for pearl millet-based complementary foods consumed and use each

# M<sup>5</sup>Open: first published as 10.1136/bmjopen-2017-017631 on 14 November 2017. Downloaded from http://bmjopen.bmj.com/ on June 11, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Pa Pa

block of 60 as a random effect. We will also plot dose-response curves using restricted cubic splines that will help us detect any threshold effects, as well as non-linear associations. Nonparametric tests, such as the Hodges-Lehmann test, will be used where relevant, as in the case of non-normally distributed variables including serum ferritin. Study monitoring board A Data Safety Monitoring Board (DSMB) will be established for this study and will include experts representing Cornell University and SNDT University who are not directly involved in the trial. The DSMB members will oversee the study and will periodically monitor the progress and outcomes of the intervention. Reporting of adverse events All significant adverse events will be reported by the study physican via an Adverse Event/Serious Adverse Event Report (AE/SAE) form. When an adverse event occurs, study physicians, research assistants and/or other study personnel will

358 undertake all necessary precautions to ensure the safety and well-being of the

359 participant. Participants will not receive any compensation or payment for taking part

360 in the study. Every participant will be insured for the duration of the trial in the

361 unexpected and unlikely event of any adverse event relating to the study.

362 The following study protocol endpoints will be considered to define safety and

363 efficacy outcomes, and establish un-blinding and stopping guidelines in this trial, as

364 per the discretion of the DSMB: 1. Diagnosis of the development of severe acute

365 malnutrition such as Kwashiorkor; 2. Occurrence of all-cause death. Demonstration

366 of efficacy, namely a significant beneficial effect on mortality and other adverse

**BMJ Open** 

367 outcomes will be used as a guideline to determine if the study should be un-blinded,368 stopped, or terminated.

371 ETHICS AND DISSEMINATION

Before conducting interviews or allowing participation in the study, written informed consent will be obtained from each participant's guardians/primary caregivers, and research assistants will record each granting of informed consent using audio and video techniques. We will also assess children's current nutritional status to ensure that our study provision is at least comparable to the children's daily dietary intake, which is generally lower than ideal for growth and health, according to preliminary data. Severely anaemic (<7 g/dL) and malnourished children will be provided with appropriate medical care and referred to CSSC. The caregivers will be assured of the confidentiality and anonymity of reports and publications generated from this study. Participation will be voluntary, and participants will be assured that refusal to participate in the study will not impact their access to care. The ethical clearance for this study has been obtained from the Institutional Review Board of Cornell University; Intersystem Biomedical Ethics Committee (ISBEC) of SNDT University, Mumbai, India; and the Institutional Review Board of St. John's Research Institute, Bangalore, India. This study has received clearance from Health Ministry's Screening Committee (HMSC) of the ICMR. The results of this study will be disseminated at several research conferences and as published articles in peer-reviewed journals. The present study protocol was prepared in accordance to the SPIRIT statement<sup>26</sup>. This trial has been registered at Clinicaltrials.gov (registration number NCT02233764) and the Clinical Trials Registry of India (CTRI) (reference

3 4	392	number REF/2014/10/007731, CTRI number CTRI/2015/11/006376).
5 6	393	
7 8	394	
9 10	395	REFERENCES
11	396	1. Black MM, Quigg AM, Hurley KM, et al. Iron deficiency and iron-deficiency anemia
13	397	in the first two years of life: strategies to prevent loss of developmental
14	398	potential. Nutrition reviews 2011;69 Suppl 1:S64-70. doi: 10.1111/j.1753-
15 16	399	4887.2011.00435.x
10	400	2. Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and
18	401	overweight in low-income and middle-income countries. The Lancet
19	402	2013;382(9890):427-51. doi: 10.1016/s0140-6736(13)60937-x
20	403	3. Vucic V, Berti C, Vollhardt C, et al. Effect of iron intervention on growth during
21	404	gestation, infancy, childhood, and adolescence: a systematic review with
22	405	meta-analysis. Nutrition reviews 2013;71(6):386-401. doi:
23	406	10.1111/nure.12037
24	407	4. Cole CR, Lifshitz F. Zinc nutrition and growth retardation. <i>Pediatr Endocrinol Rev</i>
25	408	2008:5(4):889-96.
26	409	5. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. The
27	410	Journal of nutrition 2001:131(2S-2):616S-33S: discussion 33S-35S.
28	411	6. Black MM. The evidence linking zinc deficiency with children's cognitive and motor
29 30	412	functioning. The Journal of nutrition 2003;133(5 Suppl 1):1473S-6S.
31	413	7. Lawless J. Latham M. Kinoti S. et al. Iron Supplementation Improves Appetite and
32	414	Growth in Anemic Kenvan Primary School Children Journal of Nutrition
33	415	1994·124·645-54
34	416	8 Kanani S RH P Symposium Supplementation with Iron and Folic Acid Enhances
35	417	Growth in Adolescent Indian Girls. Journal of Nutrition 2000:130(452S-455S)
36	418	9 Walter T Olivares M Pizarro F et al Iron anemia and infection Nutrition
37	419	reviews 1997:55(4):111-24
38	420	10 Georgieff MK Long-term brain and behavioral consequences of early iron
39	421	deficiency. Nutrition reviews 2011:69 Suppl 1:543-8 doi: 10.1111/i 1753-
40 11	422	4887 2011 00432 x
41	423	11 HE B Plant Breeding- A New Tool for Eighting Microputrient Malputrition Am
43	423	Soc Nutr Sci 2002:511-3
44	425	12 Accentability of iron biofortified pearl millet among young children in urban slums
45	425	of Mumbai India Experimental Biology: 2016: San Diego, EASEB journal :
46	420	official publication of the Edderation of American Societies for Experimental
47	427	
48	420	Diology. 12 Kodkapy PS Ballad DM Mahantahatti NS at al Diafartification of poarl millet
49	429	is. Noukarry bs, beliau Rivi, inarianshelli NS, et al. bioloninication of pean miller
50	430	these minerals shows physiologic requirements in young shildren. The Journal
51	431	of putrition 2012:142(0):1490.02 doi: 10.2045/in.112.176677
52 53	432	0/ 1/0(1/0/1/2013, 145(9), 1469-95, 001, 10.5945/j11, 115, 176077
54	433	14. Research EGolicow. Nument Requirements and Recommended Dietary
55	434	Allowances for Indians. In: Research EGotiColli, ed. Hyderabad, India:
56	435	National institute of Nutrition, indian Council of Medical Research, 2009.
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2	436	15 Chandramouli C. HOUSING STOCK, AMENITIES & ASSETS IN SLUMS -
4	437	CENSUS 2011 (Presentation) In: Affairs MoH ed India: Census of India
5	438	2011
6	439	16. Andersson M. Theis W. Zimmermann MB. et al. Random serial sampling to
7	440	evaluate efficacy of iron fortification: a randomized controlled trial of
8	441	margarine fortification with ferric pyrophosphate or sodium iron edetate <i>Th</i>
9	442	American journal of clinical nutrition 2010;92(5):1094-104 doi:
10	443	10.3945/aicn 2010.29523
12	444	17 de Onis M Onvango A Borghi F et al Worldwide implementation of the WH
13	445	Child Growth Standards. <i>Public health nutrition</i> 2012:15(9):1603-10. doi:
14	446	10 1017/S136898001200105X
15	447	18 Kapoor D Agarwal KN Sharma S et al. Iron status of children aged 9-36
16	448	months in an urban slum Integrated Child Development Services project in
17	449	Delhi Indian Pediatr 2002:39(2):136-44
18	450	19 WHO/NMH/NHD/11 1 Haemoglobin concentrations for the diagnosis of anae
19	451	and assessment of severity 2011
20	452	20 Rizek RL Pao FM Dietary intake methodology L USDA surveys and support
21	453	research The Journal of nutrition 1990 120 Suppl 11:1525-9
23	454	21 Podinovskaja M. VanderVen BC. Yates RM, et al. Dynamic quantitative assay
24	455	of phagosomal function Curr Protoc Immunol 2013:102:Unit 14 34 doi:
25	456	10 1002/0471142735 im1434s102
26	457	22 Aslin R B M Automated Corneal-Reflection Eve Tracking in Infancy-
27	458	Methodological Developments and Applications to Cognition <i>Infancy</i>
28	459	2004.6.155-63 doi: 10.1207/s15327078in0602 1
29	460	23 Ruff HA Lawson KR Parrinello R et al Long-term stability of individual
30	461	differences in sustained attention in the early years <i>Child Dev</i> 1990:61(1):
32	462	75
33	463	24 Phatak P. Developmental Assessment Scales for Indian Infants (DASII)- Revi
34	464	Baroda Norms Manual Pune: Anand Agencies 1998
35	465	25 Ruth C. Yu FA. Huev SL. et al. ConnEDCt: A Development Framework for
36	466	Mobile Electronic Data Capture in Disconnected Communities Computer
37	467	Science and Education in Computer Science 2016 12:219-33
38	468	26 Chan A-W Tetzlaff I Altman D et al SPIRIT 2013 Statement- Defining
39	469	Standard Protocol Items for Clinical Trials Ann Intern Med 2013:158:200-7
40 /1	470	
42	170	
43	471	AUTHORS' CONTRIBUTIONS: Saurabh Mehta Julia L. Finkelstein and Jere D.
44	17 1	
45	472	Haas designed the study, conceived the research questions and prepared the stu
46	172	
47	473	protocol. Saurabh Mehta is the principal investigator of the study and Julia I
48	170	
49 50	474	Finkelstein Jere D. Haas, Shobha A. Udipi, Padmini Ghugre, Richard L. Canfield
51	., .	
52	475	Anura V Kurpad and Ramesh D Potdar and are co-investigators. Caleb Ruth
53		
54	476	developed the database used in this trial and provided feedback on the protocol
55	170	
56	477	Samantha L. Huev and Sudha Venkatramanan contributed to the editing of the
5/		
38 50		
59 60		

2	478	protocol and will supervise the data collection under the guidance of the
2	479	investigators. All authors read and approved the final protocol.
2	480	
2	481	ABBREVIATIONS: AGP: Alpha 1-acid glycoprotein; CRP: C-reactive protein; Fe:
2	482	Iron; FeZn-PM: iron- and zinc-enhanced variety of pearl millet; ICRISAT:
2	483	International Crops Research Institute for the Semi-Arid Tropics; ICTP8203-Fe: high
2	484	iron and zinc-biofortified pearl millet variety; ICMR: Indian Council of Medical
2	485	Research; ISBEC: Intersystem Biomedical Ethics Committee; IRB: Institutional
2	486	Review Board; KHS: Kasturba Health Society; PM: Pearl Millet; RDA:
2	487	Recommended Daily Allowance; SJRI: St. John's Research Institute; SNDT:
2	488	Shreemati Nathibai Damodar Thackersey; sTfR: Serum transferrin receptor; Zn: Zinc
2	489	
2	490	FUNDING: This work was supported by HarvestPlus, grant number 2014H8302.
2	491	Address:
2	492	HarvestPlus
4	493	2033 K St NW
2	494	Washington, DC 20006
2	495	
2	496	CONFLICTS OF INTEREST: SM is an unpaid board member for a diagnostic start
4	497	up focused on developing point-of-care assays for nutritional status informed by his
2	498	research as a faculty member at Cornell University. All other authors report no
2	499	conflict of interest.
ľ	500	

Key		HarvestPlus Database Form Collection Protocol											
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req, daily	FNA	SE I					PHAS						
Outside EDC; manual not req	Before Screening	1-2 months before baseline	FOLLOW-UP										
Visit	# 1	2	3	4	5	6	7	8	9	10	11	12	
Forms:	Census	Pre- Screening, Screening	Baseline [pre- feeding]	1	Midline 2	Midline 3	Midline 4	Midline 5	Midline 6	Midline 7	8	9 Endline [post- feeding]	
Phase 1 - Screening													
Census (OED)													
Consent for Prescreening													
Prescreening/RA Form													
Consent for Screening													
Screening_Lab/Clinic													
Screening_Anthropometry	la l												
Screening_RectalSwab													
Screening_WHZ_Zscore													
Consent for Trial													
Phase 2 - Trial													
FollowUp_Baseline_SES													
FollowUp_Lab													
FollowUp_RectalSwab													
FollowUp_Cognition													
FollowUp_Morbidity													
FollowUp_Anthropometry													
FollowUp_Feeding1													
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FollowUp_FFQ													
FollowUp_Recall													
AER {Adverse Event Repo	ort)												
DropOut {Termination}													
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# Notes:

The midline measurements will take place in months 2-7.

Figure 1. Form Collection Protocol.



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

# SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	ltem No	Item NoDescriptionrmation112aTrial identifier and registry name. If not yet registered, name of intended registry2bAll items from the World Health Organization Trial Registration Data Set3Date and version identifier	Addressed on page number
Administrative inf	ormatior		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_3,16
	2b	All items from the World Health Organization Trial Registration Data Set	_yes
Protocol version	3	Date and version identifier	_N/A
Funding	4	Sources and types of financial, material, and other support	_19
Roles and	5a	Names, affiliations, and roles of protocol contributors	_1, 18
responsibilities	5b	Name and contact information for the trial sponsor	_19
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_15
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1 2					
3	Introduction				
- 5 6 7	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	_5, 6	
B a		6b	Explanation for choice of comparators	_7, 8	_
10	Objectives	7	Specific objectives or hypotheses	_6	
2  3  4	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	_6	
6	Methods: Participar	nts, inte	erventions, and outcomes		
7 8 9	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	_6	
0 1 2	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9	
3 4 5	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	_9,10	-
0 7 8 9		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	_11	_
0 1 2		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_12	_
3 4		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_10,11	_
5 6 7 8 9	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	_11-13	
0 1 2 3	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	_x (see attached figure)	
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2 3 4	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10,11	
5 6 7	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_10,11	
, 8 9	Methods: Assignme	ent of ir	nterventions (for controlled trials)		
10 11	Allocation:				
12 13 14 15 16	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_7	
17 18 19 20 21	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_7,8	
22 23 24	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_7	
25 26 27	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_4	
28 29 30		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_N/A	
31 32	Methods: Data colle	ection,	management, and analysis		
33 34 35 36 37 38	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_12-14	
39 40 41 42		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_12, 16	
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45 46 47 48	l əb ənpidqsזפסוldi	g əonəb <sub>ı</sub>	רטנפכנפd by copyright, including for uses related to text and data mining, A it raining, and similar technologies. Enserghement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.	Open: first published as	LMf

Page 2	25 of	26
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2 3 4 5 6	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_14	
0 7 8 9	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_15	
10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_15	
12 13 14		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_15	
15 16	Methods: Monitorin	ıg			
17 18 19 20 21 22	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_15	
23 24 25		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_16	
26 27 28	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_15,16	
29 30 31	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_15	
32 33	Ethics and dissemi	nation			
34 35 36 37	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_16,17	
38 39 40 41 42	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_16,17	
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2 3 4	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_9,10	
ວ 6 7		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_9,10 _	
o 9 10 11	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_14,15	
12 13 14	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_20	
15 16 17	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_15	
18 19 20	Ancillary and post- trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_16	
21 22 23 24	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_16,17	
25 26		31b	Authorship eligibility guidelines and any intended use of professional writers	_N/A	
27 28 29		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_17	
30 31	Appendices				
32 33 34	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_attached	
35 36 37	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15	
38 39 40 41 42 43	<ul> <li>*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation &amp; Elaboration for important clarification on the items.</li> <li>Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons</li> <li><u>"Attribution-NonCommercial-NoDerivs 3.0 Unported</u>" license.</li> </ul>				
44 45 46 47 48	الا Open: first published as 10.1136/bmjopen-2017 <sub>1</sub> 0176315.90164667015,Downloaded from http://pmjoge.bmjoge.bmjogunor. Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies. 2				

# **BMJ Open**

# Effect of Iron- and Zinc-Biofortified Pearl Millet Consumption on Growth and Immune Competence in Children Aged 12-18 Months in India—Study Protocol for a Randomised Controlled Trial

Journal:	BMJ Open
Manuscript ID	bmjopen-2017-017631.R1
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Complete List of Authors:	Mehta, Saurabh; Cornell University ; Institute for Nutritional Sciences, Global Health, and Technology Finkelstein, Julia; Cornell University , Division of Nutritional Sciences; St John's Research Institute Venkatramanan, Sudha; Cornell University , Division of Nutritional Sciences Huey, Samantha; Cornell University , Division of Nutritional Sciences Udipi, Shobha; Kasturba Health Society Ghugre, Padmini; SNDT Women's University, Food Science and Nutrition Ruth, Caleb; Data Performance LLC Canfield, Richard; Cornell University , Division of Nutritional Sciences Kurpad, Anura; St John's Research Institute, Nutrition Potdar, Ramesh; Centre for the Study of Social Change Haas, Jere; Cornell University , Division of Nutritional Sciences
<b>Primary Subject Heading</b> :	Global health
Secondary Subject Heading:	Nutrition and metabolism, Paediatrics, Public health
Keywords:	Biofortification, zinc, iron, pearl millet, children, growth

SCHOLARONE<sup>™</sup> Manuscripts

# **BMJ Open**

1	Effect of Iron- and Zinc-Biofortified Pearl Millet Consumption on Growth and		
1	Immune Competence in Children Aged 12 18 Months in India—Study Protocol		
2	for a Pandomicod Controlled Trial		
3	for a Randomised Controlled That		
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5	Saurabh Menta'' <sup>2</sup> , Julia L. Finkelstein'' <sup>2</sup> , Sudha Venkatramanan', Samantha L.		
6	Huey', Shobha A. Udipi⁴, Padmini Ghugre⁵, Caleb Ruth °, Richard L. Canfield',		
7 8	Anura V. Kurpad <sup>3</sup> , Ramesh D. Potdar <sup>7</sup> , Jere D. Haas <sup>1</sup>		
9	<sup>1</sup> Division of Nutritional Sciences, Cornell University, Ithaca, New York, US		
10	<sup>2</sup> Institute for Nutritional Sciences, Global Health, and Technology, Cornell University,		
11	Ithaca, New York, US		
12	<sup>3</sup> St. John's Research Institute, Bangalore, India (SJRI)		
13	<sup>4</sup> Kasturba Health Society Medical Research Centre (KHS-MRC), Mumbai, India		
14	<sup>5</sup> Shreemati Nathibai Damodar Thackersey, Women's University (SNDT), Mumbai,		
15	India		
16	<sup>6</sup> Data Performance LLC, Ithaca, New York, US		
17	<sup>7</sup> Center for the Study of Social Change, Mumbai, India (CSSC)		
18			
19	Corresponding Author (Principal Investigator):		
20	Saurabh Mehta, MBBS, ScD		
21	314 Savage Hall, Ithaca, New York 14853, US		
22	Phone: +1-607-255-2640; Fax: +1-607-255-1033		
23	E-mail: smehta@cornell.edu		
24			
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26 Keywords: Biofortification, iron, zinc, pearl millet, children, growth

27 ABSTRACT

Introduction: Biofortified crops represent a sustainable agricultural solution for the
widespread micronutrient malnutrition in India and other resource-limited settings.
This study aims to investigate the effect of the consumption of iron and zincbiofortified pearl millet by children on biomarkers of iron and zinc status and growth

33 outcomes. Additionally, we will assess immune function in the participants.

Methods and Analysis: We will conduct a randomised controlled feeding trial in identified slums of Mumbai, India among 250 children between 12-18 months of age for 9 months. Children will be randomised to receive the biofortified pearl millet (FeZn-PM, ICTP8203-Fe) or non-biofortified pearl millet. Anthropometric and morbidity data will be gathered every month for 9 months. Biological samples will be collected at baseline, midline, and endline, to assess iron and zinc status, including haemoglobin, serum ferritin, serum transferrin receptor, serum zinc, C-reactive protein, and alpha-1 acid glycoprotein. Biological samples will be archived for future analyses. The midline measurement will be a random serial sample between baseline and endline. Immune function will be assessed at each time point by the measurement of T cell counts in a subset, respectively.

Ethics and Dissemination: This study has obtained clearance from the Health
Ministry Screening Committee (HMSC) of the Indian Council of Medical Research
(ICMR). Ethical clearance has been obtained from Cornell University's Institutional
Review Board, the Inter System Biomedica Ethics Committee (ISBEC), and St.
John's Research Institute Institutional Ethics Review Board. The results of this study

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52 will be disseminated at several research conferences and as published articles in

- 53 peer-reviewed journals.
- 54
  - 55 Registration Details: Clinicaltrials.gov registration number: NCT02233764
  - 56 (registered on September 4, 2014). Clinical Trials Registry of India (CTRI), reference
- e. J/0773'). 57 number REF/2014/10/007731, CTRI number CTRI/2015/11/006376.
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23 3M.Open: first published as 10.1136/bmjopen-2017-017631 on 14 November 2017. Downloaded from http://bmjopen.bmj.com/ on June 11, 2025 at Agence Bibliographique de I Enseignement Superieur (ABES) . ge Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies. P

2 3	59	Strengths and limitations of this study
4 5 6	60	This is the first longitudinal randomised controlled trial to determine the
7 8	61	efficacy of consuming complementary foods prepared using iron- and zinc-
9 10	62	biofortified pearl millet on both nutritional status and functional outcomes,
11 12	63	among children 12-18 months of age.
13 14 15	64	The longitudinal random midline serial sampling strategy both increases
16 17	65	sensitivity and the power of the proposed study, while reducing cost and
18 19	66	invasiveness (by decreasing the number of biological samples from each
20 21 22	67	participant).
22 23 24	68	Data will be collected on iPads or laptops, using a mobile electronic data
25 26	69	capture system framework on the iOS platform. This will decrease the
27 28	70	potential for error in data entry compared to standard written hard copies of
29 30 31	71	forms, and allow direct uploading of data using a secure server.
32 33	72	One limitation of this study is that blinding may not be possible due to
34 35	73	potential sensory differences between the crops used in the two arms of the
36 37	74	study.
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/9	INTRODUCTION
80	The burden of iron and zinc deficiency
81	Deficiencies in iron and zinc are two of the most important public health problems
82	globally <sup>12</sup> . The role of iron deficiency in growth and development of children has
83	been demonstrated in several studies <sup>3-5</sup> . Similarly, it appears that a major fraction of
84	stunting can be explained by zinc deficiency <sup>6</sup> . Suboptimal iron and zinc status also
85	impairs immune functioning through a number of mechanisms, including a reduction
86	in the proportion of circulating T-lymphocytes and lymphocyte proliferative
87	responses <sup>7</sup> . Cognitive deficits are also observed with iron and zinc deficiency,
88	including irreversible impairments in neurological and psychomotor development of
89	children <sup>8</sup> .
90	
91	Iron and zinc biofortification
92	Biofortification has the potential to be a more sustainable and cost-effective
92 93	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and
92 93 94	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and supplementation, to address micronutrient malnutrition among vulnerable
92 93 94 95	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and supplementation, to address micronutrient malnutrition among vulnerable populations <sup>9</sup> . In India, staple crops such as pearl millet ( <i>Pennisetum glaucum</i> ) are
92 93 94 95 96	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and supplementation, to address micronutrient malnutrition among vulnerable populations <sup>9</sup> . In India, staple crops such as pearl millet ( <i>Pennisetum glaucum</i> ) are consumed as part of the daily diet, particularly in Maharashtra, Gujarat, Rajasthan,
92 93 94 95 96 97	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and supplementation, to address micronutrient malnutrition among vulnerable populations <sup>9</sup> . In India, staple crops such as pearl millet ( <i>Pennisetum glaucum</i> ) are consumed as part of the daily diet, particularly in Maharashtra, Gujarat, Rajasthan, and Karnataka. The iron and zinc concentration in biofortified PM is reported to be
92 93 94 95 96 97 98	Biofortification has the potential to be a more sustainable and cost-effective approach compared to other strategies such as diet diversification, fortification, and supplementation, to address micronutrient malnutrition among vulnerable populations <sup>9</sup> . In India, staple crops such as pearl millet ( <i>Pennisetum glaucum</i> ) are consumed as part of the daily diet, particularly in Maharashtra, Gujarat, Rajasthan, and Karnataka. The iron and zinc concentration in biofortified PM is reported to be 70-85 and 35-40 parts per million (ppm), respectively <sup>10</sup> . Previous research from
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103 respectively<sup>10</sup>, and would help meet 50 to 70% of the recommended dietary

104	allowances (RDA) for children between 1-3 years of age. These estimates are based
105	on the RDA recently recommended for Indians by the Indian Council of Medical
106	Research <sup>13</sup> .
107	
108	The primary objectives of this randomised controlled trial are to study the effect of
109	the daily consumption of high iron- and zinc-biofortified pearl millet on biomarkers of
110	iron and zinc status, as defined by haemoglobin, serum ferritin, serum transferrin
111	receptor, serum zinc, and C-reactive protein; and growth, as defined by length-for-
112	age, weight-for-age and weight-for-length, among 12-18-month-old children, as
113	compared to children receiving conventional pearl millet. Additionally, we will assess
114	the effect of high iron- and zinc-biofortified pearl millet by 12-18-month-old children
115	on immune outcomes.
116	
117	METHODS AND ANALYSIS
118	Study setting
119	This study will take place in identified slums of urban Mumbai, Maharashtra, India.
120	Mumbai is the commercial capital of India, and about 41.3% of city's populace
121	resides in urban slums <sup>14</sup> .
122	
123	Study design
124	We will conduct a randomised controlled trial in which children (12 to 18 months old)
125	will be fed complementary foods prepared using iron- and zinc-biofortified pearl
126	millet (FeZn-PM) or the comparator conventional pearl millet for nine months.
127	Caregivers will be requested to provide three (3) informed consent forms for 1)
128	census; 2) screening; and 3) enrolment into the trial. Caregivers who provide all

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2 3 4	129	three consents are considered eligible to be screened. Details of the informed
5 6	130	consent process can be found the section "Informed consent process."
7 8	131	
9 10	132	1. Census: Before the study begins, a census will be conducted in the identified
11 12 13	133	slums to gather information on the age of the child, sex, and location.
14 15	134	
16 17	135	2. Screening: Consent will be obtained for screening and inclusion in the trial. Non-
18 19	136	invasive data will be collected on: dietary allergies, use of iron or zinc dietary
20 21	137	supplements, availability of a caregiver, and if the caregivers are planning to stay in
22 23 24	138	the slum for the duration of the study. Anthropometry and dietary intake will also be
25 26	139	measured. If the children were not dewormed recently, they will be provided liquid
27 28	140	albendazole by the study physician at the study clinic (5 mL of syrup equivalent to
29 30	141	200 mg per dose). If still eligible for the study, blood will be collected to measure
31 32 33	142	complete blood counts including haemoglobin for further determination of eligibility.
34 35	143	
36 37	144	3. Randomization: A statistician from the Cornell Statistical Consulting Unit will
38 39	145	generate the random allocation sequence using a statistical software package (SAS
40 41	146	version 9.4); the randomization sequence key will be blinded to all study personnel
42 43 44	147	except the study statistician and the Filemaker database developer until follow-up is
45 46	148	over. Randomization will be allocated at the individual level. Individuals will be
47 48	149	randomized in blocks of 60. Pearl millet food products will coded by study arm (Arm
49 50	150	1: iron- and zinc-biofortified pearl millet; Arm 2: conventional pearl millet). Children
51 52 53	151	will be randomized to either study arm and will be monitored to ascertain they
54 55	152	consume the assigned food product throughout the study duration. The midline
56 57	153	measurement (including biological sample collection) will be a random serial sample
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- Longitudinal midline random serial sampling is often used in population
- pharmacokinetic research and has been shown to be a useful strategy for iron
- fortification efficacy studies, by describing the pattern of iron repletion<sup>15</sup>.

# Intervention and comparator

The intervention is iron-and-zinc-biofortified pearl millet (ICTP-8203) developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the comparator is a conventional pearl millet that is commercially available on the market. The comparator was chosen because it is similar to the intervention in all aspects, except for iron and zinc content, which allows direct analysis of the impact of the iron-and-zinc-biofortified variety on our outcome measurements. The nutrient composition of biofortified and the conventional pearl millet flour are presented in Table 1. We expect a child to consume an average of 60 g of pearl millet per day, depending on the age of the child<sup>11</sup>. 

Table 1: Nutrier varieties	nt composition o	f pearl millet
	Biofortified	Control
	(per 100 g	(per 100 g
	flour)	flour)
Moisture (g)	6.89	7.16
Fat (g)	7.88	8.74
Protein (g)	13.46	13.46
Carbohydrate	30.22	30.98
(g)		
Energy (Kcal)	246	256
Ash (g)	1.99	1.63
Phytate (mg)	876.59	998.44
Iron (mg)	6.64	2.56
Zinc (mg)	4.43	1.24

Inclusion and exclusion criteria Inclusion criteria Participants included in this study will be 12-18-month-old male and female children with haemoglobin concentration greater than or equal to 9 g/dL, living in urban slums of Mumbai. Exclusion criteria Children will be excluded if: (1) they are younger than 12 months, 0 days old or older than 18 months, 30 days at enrolment; (2) their haemoglobin concentration is less than 9 g/dL or haemoglobinopathy is present (as indicated via abnormal peak via hemoglobin variant analysis and confirmed by CSSC physicians); (3) they show signs of severe malnutrition (a weight-for-length -3)<sup>16</sup>; (4) their caregiver reports prior known diagnosis of HIV, tuberculosis, or current diagnoses of HIV, malaria, tuberculosis, and/or dengue fever; (5) their caregiver is unavailable to bring the child to the feeding centre during follow-up; (6) the child has any known dietary allergies; and (7) their caregivers will leave the study site for greater than 4 weeks during the follow-up period; (8) prior (within the past one year) or current consumption of iron or zinc supplements. Children who are severely anemic will be referred to physicians at the Center for the Study of Social Change (CSSC). Informed consent process Research assistants will obtain informed consent from caregivers. Three consent forms will be collected at the Screening Visit: 1) Pre-Screening Consent (non-invasive guestionnaires to determine age eligibility, use of dietary supplements. availability of caregiver, etc. as described in Exclusion Criteria above; 2) Screening Consent (invasive procedures including blood collection and anthropometric

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204	measurements to assess malnutrition); and 3) Intervention Consent (including
205	baseline procedures such as a full doctor's exam and detailed background
206	questionnaires).
207	
208	Feeding Centre
209	Feeding centres will be located near to where children and their caregivers'
210	dwellings are clustered, to ensure that travel time from their home to the feeding
211	centre is within walking distance.
212	
213	Sample size estimation
214	Our estimates of sample size are based on assumptions about mean values and
215	associated variation in haemoglobin and serum ferritin. We expect 50-75% of these
216	children to have iron and zinc deficiency based on published literature <sup>17</sup> and
217	preliminary results (unpublished). We assume that absorbed iron will be transferred
218	to body stores in the liver, and that the change in liver stores is reflected in changes
219	in serum ferritin at a rate of 8 $\mu$ g/L of serum ferritin per mg liver iron in non-anaemic,
220	iron-depleted children. Iron-deficient, anaemic (haemoglobin < 11 g/dL <sup>18</sup> ) children
221	will have their absorbable iron directed to haemoglobin synthesis in the early months
222	of the feeding trial. We estimate that the children consuming biofortified FeZn-PM
223	will demonstrate a gain of 1 g/dL in haemoglobin concentrations in 2.4 months,
224	whereas children consuming control pearl millet will need more than 9 months to
225	demonstrate the same increase. To detect a significant increase in ferritin from the
226	baseline value of 1.79 (6 mg/L) with standard deviation of 1.2, in the experimental
227	group, compared to the control group, at a power of 80% and 5% significance level,
228	96 participants will be required.

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- 5 6	230	Follow-up
7 8	231	Assessments
9 10	232	At baseline, we will assess anthropometry; collect blood for analysis of hemoglobin,
11 12 13	233	iron, zinc, CRP, and AGP biomarkers in the blood (all blood analyses other than
13 14 15	234	complete blood counts and T cell counts will be stored at -80 and then performed in
16 17	235	batch at the end of the trial); nutrient intake measures using multiple-pass 24-h
18 19	236	dietary recall <sup>19</sup> for children and mothers; socio-economic and demographic
20 21	237	information; and morbidity history. Follow-up will continue for 9 months and will
22 23 24	238	include monthly anthropometric measurements, morbidity assessments, Infant and
25 26	239	Young Child Feeding Questionnaire <sup>20</sup> , and 24-hour dietary recall <sup>19</sup> . Both maternal
27 28	240	and infant dietary data will be collected via paper forms and entered into the CS
29 30	241	Dietary System Rel. 1.10, to calculate energy and nutrient intakes. Additionally, we
31 32 33	242	will collect rectal swab or stool samples to determine microbiome composition in
34 35	243	potential future ancillary analyses. The midline time point will be a random serial
36 37	244	sample that occurs between the baseline and endline measures. Children may visit
38 39	245	the CSSC clinic at any time during the trial for healthcare treatment. All blood
40 41 42	246	collection procedures will include the use a local anaesthetic (Prilox- Lidocaine and
42 43 44	247	Prilocaine cream) to decrease pain, and a Vein Finder device to illuminate the veins
45 46	248	to better identify the injection site.
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49 50	250	Administration of intervention
51 52 53	251	Both arms will receive complementary foods prepared with pearl millet three times
54 55	252	per day, 6 days per week, for 9 months. Culturally acceptable pearl millet based
56 57 58 59	253	complementary foods were developed by SNDT University and the acceptability of

these food products was tested on the caregivers and the children from the slums of
Mumbai<sup>10</sup>. We will conduct a run-in/pilot phase of the study with 1-3 selected feeding
centres.

> Each day, the child's caregiver will bring the child to their feeding centre. Two meals will be consumed at the feeding centre; the third meal may be consumed at home. The food intake of the two meals at the feeding centre will be measured directly before and after consumption. To measure the intake of the third meal, the weight of unconsumed food from the third meal will be measured the next morning at the feeding centre. To assure adherence, healthcare workers will follow up with the participants' caregivers and record reasons for non-adherence. Throughout the study, we will conduct periodic analysis of random samples of both grain varieties and prepared food products to ensure food safety and quality of the intervention. Primary outcome measurements Biomarkers of iron and zinc status We will determine if biofortified pearl millet improves iron and zinc status compared to children who consume non-biofortified pearl millet. Specifically, iron and zinc status will be assessed by measuring concentrations of haemoglobin (Hb), serum ferritin, serum transferrin receptor (sTfR) and plasma zinc at enrolment (baseline), midline, and endline. Additionally, we will measure concentrations of inflammatory biomarkers C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP), as iron

- and zinc biomarkers can be influenced by inflammation.

278 Growth

We will assess change in a child's growth as another outcome variable to determine

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if iron-biofortified pearl millet reduces the risk of underweight (weight-for-age z score
< -2), wasting (weight-for-height z-score < -2) and stunting (length-for-age z score < -
2) during the 9-month intervention period, compared to those who receive non-
biofortified pearl millet. We will measure weight (kg) and length (cm) at baseline and
monthly throughout follow-up. The weight and length data will be converted to
weight-for-age, weight-for-length z-score, and length-for-age z-score. The growth
rates (as kg/month, and cm/month) will also be compared from the absolute
measurements after controlling for age.
Additional outcomes
Immune function
We will assess immune function by measuring T cell counts and vaccine responses.
Additionally, we will collect morbidity data, including changes in types and
frequencies of morbidities such as diarrhoeal illness, pneumonia, and any chronic
disease throughout follow-up during each clinic visit. Caregivers will also be
encouraged to come to the clinic at any time for any health-related reasons, which
will also be recorded. This will provide an accurate representation of both innate and
adaptive immunity in participants.
Cognitive function
To determine if consumption of biofortified pearl millet improves child cognitive
function, compared to consumption of non-biofortified pearl millet, we will assess in a
subset of children aspects of cognition that (1) previously have been shown to be
sensitive to the effects of early iron and/or zinc deficiency, or (2) draw heavily upon

brain structures or processes thought to be vulnerable to early iron and/or zinc deficiency, based on studies of animal models and humans. Thus, we will assess multiple specific aspects of memory, attention and processing speed using automated eve trackers<sup>21</sup>. In addition, we will also assess higher-level, integrative cognitive abilities, including problem-solving and exploratory behavior and global aspects of attention and inattention during free play with toys<sup>22</sup>. 

### Data collection and storage

Prior to data collection, the field staff will be trained on ethical and data collection procedures. The protocol of data collection forms to be used is shown in Figure 1. All data except dietary data will be collected on iPads or laptops for the proposed project. We will use a mobile electronic data capture (mEDC) framework on the iOS platform, *Connedct*, specifically designed for this project<sup>23</sup>. A secure server will be utilized for uploading, storing, and accessing the data. This will enable real-time feedback and error checking, as well as eliminate errors associated with data entry, facilitating faster data analysis and rapid dissemination of results. All information will be kept confidential. All names will be removed from the data for analysis. The identifying codes and linked names will be securely stored in password-protected computers. All data will be securely stored on a third-party server, which will have limited access by study team members. All biological specimens will be collected by trained medical professionals and evaluated by certified laboratories within India. Biological specimens will be stored appropriately throughout the duration of the study and after the study for future analysis. 

### Data analysis

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329	We will use an intention-to-treat approach to determine the effect of biofortified pearl
330	millet on the outcomes described above. Advanced analysis will use mixed models
331	to account for pearl millet-based complementary foods consumed and use each
332	block of 60 as a random effect. We will also plot dose-response curves using
333	restricted cubic splines that will help us detect any threshold effects, as well as non-
334	linear associations. Nonparametric tests, such as the Hodges-Lehmann test, will be
335	used where relevant, as in the case of non-normally distributed variables including
336	serum ferritin.
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338	Study monitoring board
220	A Data Safaty Manitoring Poard (DSMP) will be actablished for this study and will

A Data Safety Monitoring Board (DSMB) will be established for this study and will include experts representing Cornell University and SNDT University who are not directly involved in the trial. The DSMB members will oversee the study and will periodically monitor the progress and outcomes of the intervention.

# 44 Reporting of adverse events

All adverse events will be reported by the study physician via an Adverse
Event/Serious Adverse Event Report (AE/SAE) form. When an adverse event
occurs, study physicians, research assistants and/or other study personnel will
undertake all necessary precautions to ensure the safety and well-being of the
participant.

The following study protocol endpoints will be considered to define safety and
efficacy outcomes, and establish un-blinding and stopping guidelines in this trial, as

353 per the discretion of the DSMB: 1. Diagnosis of the development of severe acute

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354	malnutrition such as Kwashiorkor; 2. Occurrence of all-cause death. Demonstration	
355	of efficacy, namely a significant beneficial effect on mortality and other adverse	
356	outcomes will be used as a guideline to determine if the study should be un-blinded,	
357	stopped, or terminated.	
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360	ETHICS AND DISSEMINATION	
361	Before conducting interviews or allowing participation in the study, written informed	
362	consent will be obtained from each participant's guardians/primary caregivers, and	
363	research assistants will record each granting of informed consent using audio and	
364	video technology. Severely anaemic (<7 g/dL) and malnourished children will be	
365	provided with appropriate medical care and referred to CSSC. The caregivers will be	
366	assured of the confidentiality and anonymity of reports and publications generated	
367	from this study. Participation will be voluntary, and participants will be assured that	
368	refusal to participate in the study will not impact their access to care. The ethical	
369	clearance for this study has been obtained from the Institutional Review Board of	
370	Cornell University; Intersystem Biomedical Ethics Committee (ISBEC) of SNDT	
371	University, Mumbai, India; and the Institutional Review Board of St. John's Research	
372	Institute, Bangalore, India. This study has received clearance from Health Ministry's	
373	Screening Committee (HMSC) of the ICMR. The results of this study will be	
374	disseminated at several research conferences and as published articles in peer-	
375	reviewed journals. The present study protocol was prepared in accordance to the	
376	SPIRIT statement <sup>24</sup> . This trial has been registered at Clinicaltrials.gov (registration	
377	number NCT02233764) and the Clinical Trials Registry of India (CTRI) (reference	
378	number REF/2014/10/007731, CTRI number CTRI/2015/11/006376).	

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3	379	
4 5	380	
6	300	
7	381	REFERENCES
8	001	
9 10	382	1. Black MM, Quigg AM, Hurley KM, et al. Iron deficiency and iron-deficiency anemia
11	383	in the first two years of life: strategies to prevent loss of developmental
12	384	potential. Nutrition reviews 2011;69 Suppl 1:S64-70. doi: 10.1111/j.1753-
13	385	4887.2011.00435.x
14	386	2. Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and
15	387	overweight in low-income and middle-income countries. The Lancet
10	388	2013;382(9890):427-51. doi: 10.1016/s0140-6736(13)60937-x
18	389	3. Vucic V, Berti C, Vollhardt C, et al. Effect of iron intervention on growth during
19	390	gestation, infancy, childhood, and adolescence: a systematic review with
20	391	meta-analysis. <i>Nutrition reviews</i> 2013;71(6):386-401. doi:
21	392	10.1111/nure.12037
22	393	4. Lawless J, Latham M, Kinoti S, et al. Iron Supplementation Improves Appetite and
23	394	Growth in Anemic Kenyan Primary School Children. Journal of Nutrition
24 25	395	1994;124:645-54.
26	396	5. Kanani S, RH P. Symposium Supplementation with Iron and Folic Acid Enhances
27	397	Growth In Adolescent Indian Girls. <i>Journal of Nutrition</i> 2000;130(452S-455S)
28	398	6. Cole CR, Lifshitz F. Zinc nutrition and growth retardation. Pediatr Endocrinol Rev
29	399	ZUU0,0(4).009-90. Z. Welter T. Oliveres M. Dizerre F. et al. Iron enemie, and infection. <i>Nutrition</i>
30	400	7. Waiter T, Olivares M, Pizarro F, et al. Iron, anemia, and infection. <i>Nutrition</i>
31	401	P Coargioff MK Long term brain and behavioral consequences of early iron
२ २२	402	deficiency. Nutrition reviews 2011:60 Suppl 1:5/3.8 doi: 10.1111/j.1753
34	403	4887 2011 00/32 v
35	405	9 HE B Plant Breeding- A New Tool for Fighting Microputrient Malnutrition Am Soc
36	406	Nutr Sci 2002:511-3
37	407	10 Huev SI Venkatramanan S Udipi SA et al Acceptability of Iron- and Zinc-
38	408	Biofortified Pearl Millet (ICTP-8203)-based Complementary Foods Among
39 40	409	Children in an Urban Slum of Mumbai. India. Frontiers in Nutrition
40	410	2017:4(August 2017, Article 39):1-10, doi: 10.3389/fnut.2017.00039
42	411	11. Acceptability of iron biofortified pearl millet among young children in urban slums
43	412	of Mumbai, India. Experimental Biology; 2016; San Diego. FASEB journal :
44	413	official publication of the Federation of American Societies for Experimental
45	414	Biology.
46	415	12. Kodkany BS, Bellad RM, Mahantshetti NS, et al. Biofortification of pearl millet
47 48	416	with iron and zinc in a randomized controlled trial increases absorption of
49	417	these minerals above physiologic requirements in young children. The Journal
50	418	of nutrition 2013;143(9):1489-93. doi: 10.3945/jn.113.176677
51	419	<ol><li>I.C.M.R. Nutrient Requirements and Recommended Dietary Allowances for</li></ol>
52	420	Indians. In: Research EGotICoM, ed. Hyderabad, India: National Institute of
53 54	421	Nutrition, Indian Council of Medical Research, 2009.
04 55	422	14. Chandramouli C. HOUSING STOCK, AMENITIES & ASSETS IN SLUMS -
56	423	CENSUS 2011 (Presentation). In: Affairs MoH, ed. India: Census of India,
57	424	2011.
58		
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2		
3	425	15 Andersson M Theis W Zimmermann MB et al Random serial sampling to
4	426	evaluate efficacy of iron fortification: a randomized controlled trial of
5	420	margaring fartification with forrig pyraphaephate or addium iron additate. The
6	427	A marganne formation with terric pyrophosphale of sodium non edetate. The
7	428	American journal of clinical nutrition 2010;92(5):1094-104. doi:
8	429	10.3945/ajcn.2010.29523
0	430	16. de Onis M, Onyango A, Borghi E, et al. Worldwide implementation of the WHO
9 10	431	Child Growth Standards, Public health nutrition 2012;15(9);1603-10, doi:
10	432	10 1017/\$136898001200105X
10	132	17 Kanoor D. Agarwal KN. Sharma S. et al. Iron status of childron agod 0.36
12	433	17. Rapool D, Ayaiwai RN, Sharina S, et al. Iron status of childler ayeu 9-50
13	434	months in an urban sium integrated United Development Services project in
14	435	Delhi. Indian Pediatr 2002;39(2):136-44.
15	436	18. WHO/NMH/NHD/11.1. Haemoglobin concentrations for the diagnosis of anaemia
10	437	and assessment of severity. 2011
17	438	19. Rizek RL, Pao EM. Dietary intake methodology I. USDA surveys and supporting
18	439	research The Journal of nutrition 1990:120 Suppl 11:1525-9
19	100	20. Organization WH Indicators for assossing infant and young child fooding
20	440	20. Organization with indicators for assessing infant and young child recurry
21	441	
22	442	21. Aslin R, B M. Automated Corneal-Reflection Eye Tracking in Infancy-
23	443	Methodological Developments and Applications to Cognition. Infancy
24	444	2004;6:155-63. doi: 10.1207/s15327078in0602_1
25	445	22. Ruff HA, Lawson KR, Parrinello R, et al. Long-term stability of individual
26	446	differences in sustained attention in the early years. <i>Child Dev</i> 1990.61(1):60-
27	447	75
28	117	23 Puth C. Vu EA. Huov SL, at al. ConnEDCt: A Dovelonment Framework for
29	440	25. Ruth C, Tu EA, Huey SL, et al. Connector A Development Framework for
30	449	Mobile Electronic Data Capture in Disconnected Communities. Computer
31	450	Science and Education in Computer Science 2016;12:219-33.
32	451	24. Chan A-W, Tetzlaff J, Altman D, et al. SPIRIT 2013 Statement- Defining
33	452	Standard Protocol Items for Clinical Trials. Ann Intern Med 2013;158:200-7.
34	453	
35		
36	454	AUTHORS' CONTRIBUTIONS: Saurabh Mehta, Julia L. Finkelstein and Jere D.
37	434	AUTTICKS CONTRIBUTIONS. Saurabit Menta, Julia E. Thirkeistein and Jere D.
38		
39	455	Haas designed the study, conceived the research questions and prepared the study
40		
41	456	protocol. Saurabh Mehta is the principal investigator of the study and Julia L.
42		
43	457	Finkelstein, Jere D. Haas, Shobha A. Udipi, Padmini Ghugre, Richard L. Canfield,
44	107	
45	150	Anura V, Kurnad, and Ramoch D. Potdar are co investigators. Calob Puth
46	450	Anura V. Kurpau, anu Ramesh D. Poluar are co-investigators. Caleb Ruth
47		
48	459	developed the database used in this trial and provided feedback on the protocol.
49		
50	460	Samantha L. Huey and Sudha Venkatramanan contributed to the editing of the
51		, ,
52	461	protocol and will supervise the data collection under the guidance of the
53	101	protocol and will supervise the data concetion under the guidance of the
54	1(2)	in continutory. All with our word and encoursed the final protocol
55	462	investigators. All authors read and approved the final protocol.
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# **BMJ Open**

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APPREVIATIONS: ACD: Alpha 1 agid gluganratain: CDD: C repoting protain: For
ABBREVIATIONS: AGP. Alpha T-acid glycoprotein, CRP. C-reactive protein, Fe.
Iron; FeZn-PM: Iron- and zinc-enhanced variety of pearl millet; ICRISAT:
International Crops Research Institute for the Semi-Arid Tropics; ICTP8203-Fe: high
iron and zinc-biofortified pearl millet variety; ICMR: Indian Council of Medical
Research; ISBEC: Intersystem Biomedical Ethics Committee; IRB: Institutional
Review Board; KHS: Kasturba Health Society; PM: Pearl Millet; RDA:
Recommended Daily Allowance; SJRI: St. John's Research Institute; SNDT:
Shreemati Nathibai Damodar Thackersey; sTfR: Serum transferrin receptor; Zn: Zinc
FUNDING: This work was supported by HarvestPlus, grant number 2014H8302.
Address:
HarvestPlus
2033 K St NW
Washington, DC 20006
CONFLICTS OF INTEREST: SM is an unpaid board member for a diagnostic start
up focused on developing point-of-care assays for nutritional status informed by his
research as a faculty member at Cornell University. All other authors report no
conflict of interest.
Figure Legends:
Figure 1. Form collection protocol.

468	Research; ISBEC: Intersystem Biomedical Ethics Committee; IRB: Institutional
469	Review Board; KHS: Kasturba Health Society; PM: Pearl Millet; RDA:
470	Recommended Daily Allowance; SJRI: St. John's Research Institute; SNDT:
471	Shreemati Nathibai Damodar Thackersey; sTfR: Serum transferrin receptor; Zr
472	
473	FUNDING: This work was supported by HarvestPlus, grant number 2014H830
474	Address:
475	HarvestPlus
476	2033 K St NW
477	Washington, DC 20006
478	
479	CONFLICTS OF INTEREST: SM is an unpaid board member for a diagnostic
480	up focused on developing point-of-care assays for nutritional status informed b
481	research as a faculty member at Cornell University. All other authors report no
482	conflict of interest.
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484	Figure Legends:
485	Figure 1. Form collection protocol.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Ксу		HarvestPlus D				atabase Form Collection Protocol							
req													
	req, daily	FIA	SE I					PHAS					
	req, random (once) Outside EDC; manual not reg	Before Screening	1-2 months before baseline				F	OLLO	W-UP				
	Visit #	1	2	3	4	5	6	7	8	9	10	11	12
Forms:		Census	Pre- Screening, Screening	Baseline [pre- feeding]	1	Midline 2	Midline 3	Midline 4	Midline 5	Midline 6	Midline 7	8	9 Endline [post- feeding]
Phase 1 - So	creening												
Census (OEI	D)												
Consent for	Prescreening												
Prescreening	g/RA Form												
Consent for	Screening												
Screening_L	ab/Clinic												
Screening_A	nthropometry												
Screening_R	RectalSwab												
Screening_W	VHZ_Zscore												
Consent for	Trial												
Phase 2 - Tr	ial												
FollowUp_Ba	aseline_SES												
FollowUp_La	ab												
FollowUp_Re	ectalSwab												
FollowUp_Co	ognition												
FollowUp_M	orbidity												
FollowUp_Ar	nthropometry												
FollowUp_Fe	eding1												
FollowUp_TE	3_Contact_Inves	tigation											
FollowUp_IY	CF												
FollowUp_FF	=Q												-
FollowUp_Re	ecall												
AER {Advers	e Event Report)												
DropOut (Ter	rmination}										::::::		
Other Issue {	}												

# Notes:

The midline measurements will take place in months 2-7.

Figure 1. Form collection protocol.

159x146mm (300 x 300 DPI)





Standard Protocol Items: Recommendations for Interventional Trials

# SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

110		page number
ormation		
1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_1
2a	Trial identifier and registry name. If not yet registered, name of intended registry	_3,16
2b	All items from the World Health Organization Trial Registration Data Set	_yes
3	Date and version identifier	_N/A
4	Sources and types of financial, material, and other support	_19
5a	Names, affiliations, and roles of protocol contributors	_1, 18
5b	Name and contact information for the trial sponsor	_19
5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_18
5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_15
	Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.	
	2a 2b 3 4 5a 5b 5c 5d	Image: Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym         Image: Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym         Image: Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym         Image: Descriptive title identifying the study design, collection Data Set         Image: Date and version identifier         Image: Date and contact information for the trial sponsor         Image: Date and version identifier         Image: Date and version identifier

2 3 4	Introduction				
5 6 7	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	_5, 6	
3		6b	Explanation for choice of comparators	_7, 8	
,  0	Objectives	7	Specific objectives or hypotheses	_6	
2 3 4	Trial design	ign 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)			
5 6	Methods: Participa	nts, inte	erventions, and outcomes		
7 8 9	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	_6	
0 1 2 2	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9	
5 4 5 5	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	_9,10	
7 3 9		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	_11	
)   <u>2</u>		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_12	
} 		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_10,11	
5 6 7 8 9	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	_11-13	
) 1 2 3	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	_x (see attached figure)	
4 5			Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.	2	
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Pag	e 23 of 25		BMJ Open	
1 2 3 4	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including	10,11
4 5 6 7 8 9 10 11 12 13 14 15 16	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_10,11
	Methods: Assignm	ent of i	nterventions (for controlled trials)	
	Allocation:			
	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_7
17 18 19 20 21	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_7,8
21 22 23 24 25 26 27 28 29 30 31 23 34 35 36 37 89 40 41 23 44 546 47 48	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_7
	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_4,7
		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_N/A
	Methods: Data coll	ection,	management, and analysis	
	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_12-14
		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_12, 16
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2 3	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality	_14	_
4 5 6			(eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol		
7 8 9	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_15	-
10 11		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_15	_
12 13 14		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_15	_
15 16	Methods: Monitorin	ıg			
17 18 19 20 21 22	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_15	
23 24 25		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_16	
26 27 28	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_15,16	
29 30 31	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_15	
32 33 34	Ethics and dissemi	nation			
35 36 37	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_16,17	
38 39 40 41 42	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_16,17	
43 44 45			ריסנפכנפם מא כסףארופתנ, וחכועמותם זסר עצפג רפואנפם נס נפאנ אחם מאנא הוחותם, או נראוחותם, אחם גווהוואר נפכחחסוספופג.		4
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2 3 4	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_9,10
5 6 7 8		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_9,10 _
9 10 11	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_14,15
12 13 14	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_20
15 16 17	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_15
18 19 20	Ancillary and post- trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_16
21 22 23 24	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_16,17
25 26		31b	Authorship eligibility guidelines and any intended use of professional writers	_N/A
27 28 29		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_17
30 31	Appendices			
32 33 34	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_attached
35 36 37	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15
38 39 40 41	*It is strongly recomm Amendments to the p " <u>Attribution-NonComm</u>	nended protoco mercial	I that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarifica I should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Co -NoDerivs 3.0 Unported" license.	ation on the items. ommons
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# **Correction:** Effect of iron and zinc-biofortified pearl millet consumption on growth and immune competence in children aged 12–18 months in India: study protocol for a randomised controlled trial

Mehta S, Finkelstein JL, Venkatramanan S, *et al.* Effect of iron and zinc-biofortified pearl millet consumption on growth and immune competence in children aged 12–18 months in India: study protocol for a randomised controlled trial. *BMJ Open* 2017;7:e017631. doi: 10.1136/bmjopen-2017-017631.

There are typographical errors in the author affiliations: 1. Affiliation number 3: Division of Nutrition, St John's Research Institute, Bangalore, Maharashtra, India. should be: Division of Nutrition, St John's Research Institute, Bengalaru, Karnataka, India 2. Affiliation number 5: Department of Food Science and Nutrition, Shreemati Nathibai Damodar Thackersey, Women's University (SNDT), Mumbai, India. should be: Department of Food Science and Nutrition, Shreemati Nathibai Damodar Thackersey (SNDT) Women's University, Mumbai, Maharashtra, India. 3. Affiliation number 7: Center for the Study of Social Change, Mumbai, India. should be: Center for the Study of Social Change, Mumbai, Maharashtra, India

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BMJ Open 2018;8:e017631corr1. doi:10.1136/bmjopen-2017-017631corr1



# **Correction:** Effect of iron and zinc-biofortified pearl millet consumption on growth and immune competence in children aged 12–18 months in India: study protocol for a randomised controlled trial

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The previous version of this manuscript contains an error in using both ICTP-8203 and ICTP-8203Fe as only ICTP-8203Fe. Both designations should read as Dhanashakti.

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