Findings from a 6-month efficacy trial in Maharashtra involving iron-biofortified pearl millet
Impact on iron status and physical performance

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Background - the problem

- Globally, 1 in 3 persons are estimated to be anemic (WHO 2014)

- ~50% of anemia is caused by iron deficiency (Kassebaum et al. 2014)

“Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions.”

- World Health Organization, 2014
Anemia & iron deficiency in adolescents

Why is iron deficiency a problem in this group?

- Rapid pubertal growth: lean body mass in males, menarche in females
- Iron requirement doubles from 7-10 y to 11-14 y (WHO 2011)
- Poor diet: > 70% of Indian adolescents get < 50% of RDA for iron (NNMB 2001)

Indian national survey of anemia (ICMR 2010):
- Girls, 12-14 y: 68.7%
- Girls, 15-19 y: 55.8%
- Boys, 15-19 y: 30.2%
Background - potential solutions

Proposed solutions for alleviating the global burden of iron deficiency:

- Supplementation
- Commercial food fortification
- Home fortification - “Sprinkles”
- Dietary diversification/modification
- Biofortification of staple food crops

- Targeted
- Cost-effective
- Sustainable
- Safe
Background- overall biofortification strategy

Do the crops work to improve human health?

Efficacy trials

Provide a scientific basis for scaling up crop delivery
In certain regions, PM intake accounts for > 50% of total cereal consumption (Rao et al. 2006).

At 150-250 g/d, it is a major source of energy in school feeding programs in rural Maharashtra.

Consumed as flatbread (bhakri).
Prescreening:
35% anemia
> 50% iron deficiency
Study objectives

To test the efficacy of iron-biofortified PM in secondary school children.

Specifically, to determine the effect of consuming iron-biofortified PM on:

- Measures of iron status
- Resolution of iron deficiency
- Physical performance
Study design

• Randomized, controlled, masked feeding trial
• Two randomization groups:
  1. Biofortified (ICTP8203): 87 μg Fe/g PM
  2. Control (DG9444): 26 μg Fe/g PM
• Consumed PM over 6 months (140 feeding days) during lunch and dinner meals served at school
Study design

After 4 months:

- Control PM was exhausted, replaced with JKBH778: 51 μg Fe/g PM
- Shev, a savory snack made from PM flour, was introduced

**Combined effect:**
Control group received more Fe in last 2 months vs. first 4 months
288 children screened for Hb and SF

41 excluded due to:
• Age ≠ 12-16 y (2)
• Hb < 8.5 g/dL (2)
• Not interested (37)

247 eligible for baseline blood and randomization to feeding group

34 lost to follow-up:
• Incomplete data (26)
• Left school (8)

Baseline, Oct. 2011

Biofortified (n = 122)  Control (n = 125)

140 feeding days

Biofortified (n = 108)  Control (n = 105)

Endline, Mar. 2012
Milling and storage of pearl millet

- Grain stored in air-conditioned warehouse and milled using separate machines
- Flour stored in stainless steel containers and delivered to hostels every 2-3 days
Preparation of bhakri

- Bhakri prepared in central kitchen of school
- Each woman assigned to make only 1 type of bhakri (biofortified OR control)
- 3 daily weighings: flour women took, dough, prepared bhakri
- Preparation supervised by asst. field coordinator and one RA for each type of bhakri

Cook preparing bhakri in kitchen
Daily recording of intake

- Bhakri consumed ad libitum
- Group of 16-20 children assigned to 1 RA for monitoring meal
- Consumption for each child was recorded at every meal to the 0.25 bhakri
Hematological measurements

Blood samples were obtained at 0, 4, 6 months

- Hemoglobin (Hb)
- Serum ferritin (SF)
- Serum transferrin receptor (sTfR)
- Body iron (TBI) (Cook et al. 2003)

\[
TBI (\text{mg/kg}) = -[\log_{10}(\text{sTfR} / \text{SF})] - 2.8229 / 0.1207
\]

- C-reactive protein (CRP)
- α1-acid glycoprotein (AGP)
- SF adjusted for inflammation (Thurnham et al. 2010)
Baseline characteristics by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Biofortified (n = 122)</th>
<th>Control (n = 124)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR) or %</td>
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</tr>
<tr>
<td>Sex (Female)</td>
<td>38.5%</td>
<td>39.5%</td>
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<tr>
<td>Age (years)</td>
<td>14.0 (12.1, 14.1)</td>
<td>14.0 (13.0, 15.0)</td>
</tr>
<tr>
<td>HAZ &lt; -2</td>
<td>40.0%</td>
<td>38.1%</td>
</tr>
<tr>
<td>BMIZ &lt; -2</td>
<td>40.0%</td>
<td>41.0%</td>
</tr>
<tr>
<td>CRP &gt; 5 mg/L</td>
<td>3.4%</td>
<td>0.0%</td>
</tr>
<tr>
<td>AGP &gt; 1 g/L</td>
<td>6.8%</td>
<td>3.4%</td>
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## Baseline characteristics by treatment group

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<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td>12.5 (11.9, 13.2)</td>
<td>12.5 (11.8, 13.1)</td>
</tr>
<tr>
<td></td>
<td>&lt; 12</td>
<td>28.2%</td>
</tr>
<tr>
<td><strong>Ferritin (ng/mL)</strong></td>
<td>16.3 (10.8, 24.7)</td>
<td>16.4 (10.6, 24.4)</td>
</tr>
<tr>
<td></td>
<td>&lt; 15</td>
<td>45.3%</td>
</tr>
<tr>
<td><strong>Transferrin receptor (mg/L)</strong></td>
<td>1.5 (1.3, 1.8)</td>
<td>1.5 (1.3, 1.7)</td>
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<tr>
<td></td>
<td>&gt; 8.3</td>
<td>11.1%</td>
</tr>
<tr>
<td><strong>Body iron &lt; 0 mg/kg</strong></td>
<td>21.4%</td>
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Iron intake from bhakri + shev, g/d

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<td>Median (IQR)</td>
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<tr>
<td>Total</td>
<td>22.0 (18.4, 25.2)</td>
<td>9.1 (7.7, 10.3)</td>
</tr>
<tr>
<td>Baseline to 4 months</td>
<td>19.6 (16.0, 24.3)</td>
<td>5.2 (4.4, 6.1)</td>
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<tr>
<td>4 to 6 months</td>
<td>24.7 (22.2, 27.3)</td>
<td>15.4 (13.2, 18.0)</td>
</tr>
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Fractional iron absorption in iron-deficient Beninese women (Cercamondi et al. 2013):

- Biofortified (ICTP8203): 7.5%
- Control (DG9444): 7.5%

Calculate how much iron is absorbed and % of requirement met.
Iron requirements were met by biofortified pearl millet

*based on requirement for absorbed iron of \(~1500 \mu g/d\) for 13-15y adolescents (ICMR 2010)
Effect on serum ferritin (SF)

<table>
<thead>
<tr>
<th>Median, ng/mL</th>
<th>baseline</th>
<th>4 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><strong>Biofortified</strong></td>
<td><strong>Control</strong></td>
<td></td>
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</table>

The graph shows the median serum ferritin levels over time for two groups: Biofortified and Control. The levels appear to increase over the 6-month period for both groups, with the Biofortified group showing a higher increase compared to the Control group at 6 months.
Effect on change in serum ferritin (SF)

Biofortified vs Control

0-4 months

4-6 months

Median, ng/mL

0
1
2
3
4
5
6
7
8
9
**Effect on total body iron (TBI)**

TBI (mg/kg) = \[-[\log_{10} (sTfR / SF)] - 2.8229\] / 0.1207

Median, mg/kg

p < 0.05

* Biofortified

* Control
Effect on change in total body iron (TBI)

\[
TBI \text{ (mg/kg)} = -\left[ \log_{10} \left( \frac{sTfR}{SF} \right) \right] - 2.8229 / 0.1207
\]

Median, mg/kg

<table>
<thead>
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<th>0-4 months</th>
<th>4-6 months</th>
</tr>
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<tbody>
<tr>
<td>Biofortified</td>
<td>Control</td>
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0-4 months

4-6 months
Effect on prevalence of anemia and iron deficiency

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<thead>
<tr>
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<th>Biofortified</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Hb &lt; 12 g/dL</td>
<td>RR = 1.78</td>
<td>RR = 1.64</td>
</tr>
<tr>
<td></td>
<td>(0.75, 4.21)</td>
<td>(1.07, 2.49)</td>
</tr>
<tr>
<td>SF &lt; 12 ng/mL</td>
<td>RR = 1.28</td>
<td>RR = 1.92</td>
</tr>
<tr>
<td></td>
<td>(0.79, 2.07)</td>
<td>(1.17, 3.14)</td>
</tr>
<tr>
<td>TBI &lt; 0 mg/kg</td>
<td>RR = 1.28</td>
<td>RR = 1.28</td>
</tr>
<tr>
<td></td>
<td>(0.79, 2.07)</td>
<td>(0.79, 2.07)</td>
</tr>
<tr>
<td>Any ID*</td>
<td>RR = 1.92</td>
<td>RR = 1.92</td>
</tr>
<tr>
<td></td>
<td>(1.17, 3.14)</td>
<td>(1.17, 3.14)</td>
</tr>
</tbody>
</table>

*SF < 12 ng/mL or sTfR > 8.3 mg/L or TBI < 0 mg/kg
Effect on prevalence of anemia and iron deficiency

Children consuming biofortified pearl millet were 92% more likely to resolve any iron deficiency

*SF < 12 ng/mL or sTfR > 8.3 mg/L or TBI < 0 mg/kg
How should efficacy of biofortified crops be assessed?

- Efficacy is typically assessed in terms of impact on biomarkers.
- Benefits of consuming biofortified crops may extend beyond improvements in ferritin or body iron.
- Can biofortified crops improve quality of life?
- Demonstration of an effect of biofortified crops on functional outcomes will allow for a comprehensive assessment of cost-benefit.
Can iron-biofortified PM benefit physical performance?

- Laboratory studies have shown that ID compromises physical performance (Haas & Brownlie 2001)
- ID has been linked to fatigue, impaired aerobic capacity, and low work productivity
- Iron supplementation at therapeutic doses has been shown to improve measures of physical performance
- It is unknown whether low dose iron via consumption of biofortified crops can have similar benefits

Boys fetching water at study site
Objective: To determine whether consumption of iron-biofortified pearl millet for 6 months can improve physical performance in Indian school children

Sample selection: subsample (n=135) of subjects in feeding trial, selected for low iron status

Physical performance measures were performed at 0 and 6 months, before and after feeding trial
Physical performance- measures

1. Aerobic capacity (VO$_{2}\text{max}$)
   - Assesses maximal oxygen uptake at peak exertion on a physical test
   - Measures heart rate, O$_2$ and CO$_2$ at progressive workloads on a cycle ergometer
   - Primary determinant is Hb
Physical performance - measures

2. Work efficiency

- Amount of physiological energy required to perform a given amount of physical work
- Uses ratio of energy expended (from $O_2$ and $CO_2$) to work performed (watts output on ergometer)
- More sensitive to tissue oxidative capacity (ferritin)

Study subject on cycle ergometer
Effect of biofortified pearl millet on physical performance

Change in VO2max (ml/min/kg)
- Change in percent Work Efficiency

Intervention: $p = 0.04$
Intervention: $p = 0.10$
Sex: $p = 0.05$

Biofortified
Control
Sex differences in change in \( \text{VO}_2 \text{max} \)

![Graph showing the change in VO2max for females and males. The y-axis represents the change in VO2max (ml/min/kg) and the x-axis represents gender (Female and Male). The graph indicates a p-value of 0.10.](image)
Summary of findings

In this randomized efficacy trial involving consumption of iron-biofortified vs. control pearl millet by 247 Indian school children ages 12-16 y:

• Baseline anemia (28% Hb < 12 g/dL) and iron deficiency (43% SF < 15 ng/mL) were present

• Iron-biofortified pearl millet:
  • improved iron status by 4 months
  • resolved iron deficiency by 6 months, with greater resolution among those who were more deficient at baseline
  • improved physical work efficiency

These findings suggest that iron-biofortification of pearl millet is an efficacious approach, and should be evaluated for effectiveness.
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Local research staff
Participants

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