Efficacy and Other Nutrition Evidence for Iron Crops

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After plant breeders have successfully developed varieties of selected staple foods with increased iron content, the edible portion of the food crops must be tested for a number of qualities before the crops can be considered for introduction into the food supply. Efficacy, or the demonstration of a significant impact on the nutritional status of human subjects who consume the staple food under controlled experimental conditions, must be demonstrated. To date, three iron-biofortified staple foods, beans (in Mexico and Rwanda), rice (in the Philippines), and pearl millet (in India) have been tested for efficacy in populations that consume the foods as a major component of their normal diet. A systematic evaluation of all of the iron biofortification efficacy studies follows.

Methodology for Establishing Efficacy

All four efficacy studies previously completed have employed a randomized, controlled, experimental study design. The iron-biofortified or similar control food was prepared to local tastes. Human research subjects consumed the foods for between 100 and 270 days, depending on the study, and meal consumption was monitored that allowed observation of dietary iron intakes from the total diet as well as precise quantification of the iron consumed from the biofortified staple food of interest. All studies assessed iron status (blood hemoglobin, serum ferritin, soluble transferrin receptor, and total body iron) at baseline and again at the end of the feeding trial, and analysis focused on the feeding group difference in the change in iron status between baseline and endline.

Proof-of-Concept and Nutrition Evidence

The first efficacy study demonstrated “proof-of-concept” when consumption of iron-biofortified rice for 9 months resulted in an increase in serum ferritin and total body iron in non-anemic Filipina religious sisters. Biofortified pearl millet was evaluated in secondary school children from western Maharashtra, India. A significant improvement in serum ferritin and total body iron was observed in iron-deficient adolescent boys and girls after consuming pearl millet flat bread twice daily for four months. The prevalence of iron deficiency was reduced significantly in the high iron group, and for those children who were iron deficient at baseline, significantly more (64%) resolved their deficiency by six months. This study demonstrated that iron-biofortified pearl millet is effective in improving iron status in children.

Biofortified beans were tested for efficacy in two different populations. Mexican primary school children were observed to have improved transferrin receptor levels after consuming biofortified black beans for 105 days; however, the acute phase protein, serum ferritin, did not improve, primarily because of high levels of infection in this population. In Rwanda, iron-depleted university women showed a significant increase in hemoglobin and total body iron after consuming biofortified beans for 4.5 months.

Variance in Iron Concentration

Iron concentration in the consumed portion of the staple food was lowest in rice [9.8 micrograms/kilogram (µg/kg)] compared to the other three crops (86–97 µg/kg). The combination of relatively higher iron concentration and the large quantities of staple food consumption of Rwandan beans and Indian pearl millet contributed 68% and 123%, respectively, to meeting the physiological requirements (EAR) for iron in these populations.

Potential Factors Influencing Variation

Iron biofortification of select staple food crops has been shown to be efficacious when feeding trials followed specified guidelines to ensure: a) adequate iron concentration difference exists between high-iron and control foods used in the study, b) subjects were iron deficient at baseline, c) sufficient consumption of the staple food was documented, d) adequate time elapsed to see a response, and e) appropriate biomarkers of iron status were used. Other factors can influence demonstration of efficacy. They include low iron bioavailability in the staple food due to inhibitors of absorption in the diet, other non-staple food sources of iron in the diet, and the health and nutritional status of the study subjects.

Improving Future Efficacy Studies

The strength of the findings for each study and across all four studies will be evaluated relative to the iron status of the study population at baseline, their inflammation status, dietary components that might affect absorption, dose of absorbable iron consumed, subject compliance, and length of feeding time. These results will be discussed in terms of how to improve future efficacy studies for iron biofortified crops.

Continued Research and Challenges

Further analysis will focus on the effect of improving iron status on physical and cognitive performance in order to assess costs versus benefits of iron biofortification. Also, HarvestPlus and its partners will be analyzing the ability of iron biofortified beans and pearl millet to reduce the prevalence of iron deficiency in populations. Challenges remain to breed for crops with higher levels of biologically available iron that approach the levels seen in the Rwanda and India studies.

Comparison of Results from Four Iron Biofortification Efficacy Studies[1]

Staple Food Crop (Location)	**Rice (Philippines)**		**Beans (Rwanda)**		**Beans (Mexico)**		**Pearl Millet (India)**	
Subjects	**Adult Females**		**Adult Females**		**Children (M+F)**		**Youth (M+F)**	
Experimental Group	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**
Number of Subjects	69	69	116	118	269	166	99	98
Hemoglobin (g/dL)	0.11	0.09	0.3*	-0.10	0.00	0.60	-0.14	-0.15
Ferritin (µg/L)	1.1*	-4.27	4.04	2.65	3.20	5.20	5.7*	1.2
Transferrin Receptor (mg/L)	0.35	-0.15	-0.26	0.09	-0.10*	0.10	0.19	0.21
Body Iron (mg/kg)	0.63*	-0.25	1.36*	0.43			0.83*	0.02
Sample Description	Non-anemic (Hb>12g/dL) at baseline		Low ferritin (<20 µg/L) at baseline		Low morbidity/ low inflammation schools		Low ferritin (<15µg/L) at baseline	

[1] Values reflect change in iron status indicator from baseline to endline.
*Significant difference between high- and low-iron groups, Wilcoxon 2-group comparison test, $p<0.05$

Iron Intakes from Biofortified Staple Food

Staple Food Crop (Location)	**Rice (Philippines)**		**Beans (Rwanda)**		**Beans (Mexico)**		**Pearl Millet (India)**	
Experimental Group	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**
Iron Content								
Concentration (µg/kg-dry)	9.8	1.9	86	51	95	55	87	30
Intake from Staple (mg/d)	1.8	0.4	13.5	8.0	4.7	2.6	17.6	5.7
Percent of Total Dietary Iron	18	5	64	46	26	19	90	81
Iron Intake Relative to Requirements								
Percent Iron Absorption[1]	7.3	7.3	7.3	9.2	5.0	5.0	7.4	7.5
Absorbable Iron (µg/d)	134	30	986	737	233	132	1300	428
EAR for Iron (µg/d)[2]	1460		1460		800		1060	
Percent EAR from Staple	9	2	68	51	29	17	123	40

[1] Iron absorption estimates: Philippines rice from by Beard et al. (J Nutr 137:1741;2007); Rwanda beans from Petri et al. (*J Nutr 143:1219;* 2013); Mexico beans estimated from algorithm of Hallberg and Hulthen (*AJCN* 71:147;2000); pearl millet from Cercamondi et al. (*J Nutr* 143:1376; 2013)
[2] EAR = Estimated Average Requirement (from Institute of Medicine, Food and Nutrition Board, *Dietary Reference Intakes-DRI, US National Research Council, 2001*).