Iron Bioavailability From Maize And Beans: A Comparison Of Human And Caco-2 Cell Measurements

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ABSTRACT

Background: An *in vitro* digestion/Caco-2 cell model may predict iron bioavailability to humans, but direct comparisons are lacking. **Aims:** To confirm previous *in vitro* iron bioavailability differences between two maize varieties and between white vs. colored beans, directly comparing human and Caco-2 cell results. **Methods:** Two randomized, 2 x 2 factorial, 29-d experiments compared women's iron absorption (n=26) from two maize varieties (ACR vs. TZB) or great northern vs. pinto beans (n = 13). Each food was served with and without orange juice to provide ascorbic acid. Nonheme iron bioavailability was determined from 2-wk whole body and erythrocyte retention of extrinsically added radioiron tracers, and compared with Caco-2 cell results from identical meals. **Results:** In contrast to results with previous harvests, *in vitro* results predicted no difference in iron availability between the maize varieties. Maize varieties did not affect percent iron absorption, but women tended (p = 0.06) to absorb 4-7 µg more iron/meal from ACR compared to TZB, because of a slightly higher corn iron content (0.8 vs. 0.7 mg/meal, respectively, p <0.0001). Contrary to *in vitro* predictions, iron absorption did not differ between bean varieties. As predicted *in vitro* for all but the pinto beans, ascorbic acid increased iron absorption from all foods by at least 3 times (p<0.0001). **Conclusion:** Caco-2 cell results correctly predicted ascorbic acid enhancement of iron bioavailability to humans from maize and great northern beans, but incorrectly predicted color-associated differences between bean varieties and their interaction with ascorbic acid. Further refinement of the Caco-2 *in vitro* model to correspond with the human results would provide an efficient and economical means of screening foods for possible biofortification.

INTRODUCTION

Objective: To directly compare *in vivo* human and *in vitro* Caco-2 cell measurements of iron bioavailability from maize and beans, by testing Caco-2 predictions of:

- improved iron bioavailability from TZB, a newer maize variety with higher production potential, compared with ACR, a common Nigerian maize (1).
- better bioavailability and better ascorbic acid enhancement using white beans (e.g. great northern), compared with darker beans (e.g., pinto beans).

METHODS

- **Experiments:** Two 2X2 factorial designs
  - ACR or TZB maize (50 g as porridge), with or without ascorbate from orange juice
  - Pinto or great northern beans (100 g as soup with garlic and onion spices), with or without ascorbate
- **Subjects:** healthy non-anemic women
  - Maize study (n=26); serum ferritin 28 (3-248) µg/L
  - Bean study (n=13); serum ferritin 31 (6-93) µg/L
- ***In vivo* human absorption** measured using radioiron tracers extrinsically added to foods, with 2-wk retention determined from erythrocyte incorporation and whole body counting.
- ***In vitro* bioavailability** measured with a Caco-2 cell model based on cellular ferritin formation after exposure to enzyme-digested and dialyzed foods. Absorption ratios with / without ascorbate estimated (2) as:

ln(human absorption ratio) = 0.6401 X ln(Caco-2 absorption ratio)

- **Calculated bioavailability** also estimated with the algorithm by Hallberg et al. (3), adjusting for only phytic acid and ascorbic acid (not polyphenols/tannins)

Composition of Maize Meals

50 g dry maize cooked as porridge with 15 g sucrose

	TZB Maize	ACR Maize	p-value
Iron (mg)	0.71 ± 0.01	0.84 ± 0.02	<0.0001
Calcium (mg)	16 ± 1.6	17 ± 1.1	0.56
Zinc (mg)	1.3 ± 0.1	1.7 ± 0.1	<0.01
Phytate (mg)	418 ± 6.2	446 ± 5.8	<0.001
Tannic Acid Equival. (mg/meal)	6.4 ± 1.3	5.7 ± 1.1	0.52
For meals with added orange juice:			
Ascorbic Acid (mg)	39 ± 3	39 ± 3	---
Ascorbic Acid: Fe (molar ratio)	17	15	---

Mean ± SD (n=3)

Composition of Bean Meals

100 g dried beans cooked as soup with salt, onion, & garlic powders

	Grt Northern	Pinto	p-value
Iron (mg)	1.9 ± 0.2	2.1 ± 0.2	<0.20
Calcium (mg)	78 ± 3.1	58 ± 2.3	<0.0001
Zinc (mg)	1.1 ± 0.1	1.2 ± 0.1	0.25
Phytate (mg)	289 ± 0.3	270 ± 1.7	<0.001
For meals with added orange juice:			
Ascorbic Acid (mg)	112 ± 8	112 ± 8	---
Ascorbic Acid: Fe (molar)	19	17	---

Mean ± SD (n =3)

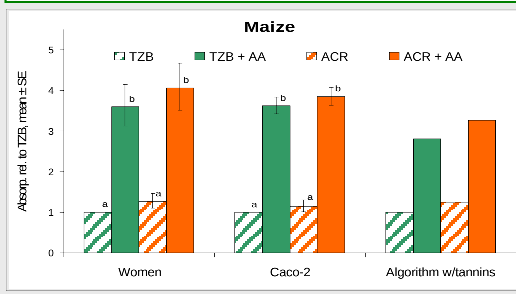
RESULTS

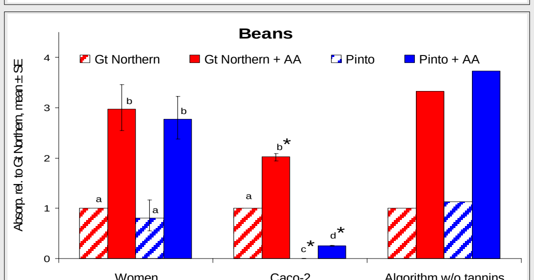
In vivo and *in vitro* assessments of iron bioavailability from maize and bean meals with and without orange juice

MAIZE	TZB	TZB+AA	ACR	ACR+AA	p values		
Women's iron absorption: (n = 26)					Variety	Ascorbate	VxA
% observed	2.0 (1.6, 2.5)a[1]	7.3 (5.8, 9.1)b	2.2 (1.7, 2.7)a	7.0 (5.6, 8.7)b	NS	0.0001	NS
µg/meal observed	14 (11, 18)a	51 (41, 64)b	18 (15, 23)a	58 (46, 73)b	NS	0.0001	NS
%, normalized[2]	2.4 (2.1, 2.9)a	8.8 (7.4, 10.4)b	2.6 (2.2, 3.1)a	8.4 (7.1, 10.1)b	NS	0.0001	NS
Caco-2 cell ferritin (ng/mg pro)	5.7 ± 0.5a	41.7 ± 1.3B	7.6 ± 1.7a	45.8 ± 1.5b	0.04	0.0001	NS
Algorithm w/ tannins, % abs[3]	4.3	10.2	4.6	10.1	--	--	--
BEANS	Gt Northern	Gt Northern+AA	Pinto	Pinto+AA			
Women's iron absorption: (n = 13)							
% observed	2.2 (1.7, 3.0)a[1]	6.6 (4.9, 8.9)b	1.6 (1.2, 2.1)a	5.4 (4.0, 7.3)b	NS	0.0001	NS
µg/meal observed	42 (31, 56)a	124 (92, 166)b	33 (25, 45)a	116 (86, 155)b	NS	0.0001	NS
%, normalized[2]	3.0 (2.3, 4.0)a	9.0 (6.8, 11.9)b	2.1 (1.6, 2.8)a	7.4 (5.6, 9.7)b	NS	0.0001	NS
Caco-2 cell ferritin (ng/mg pro)	53.4 ± 2.5a	159.1 ± 4.9b	0 ± 0c	6.0 ± 0.2d	0.0001	0.0001	0.0001
Algorithm w/o tannins, % abs[3]	6.0	20.0	6.1	20.2	--	--	--

[1] Geometric mean (-1 SEM, +1 SEM) or arithmetic mean ± SEM. [2] Absorption was normalized to a serum ferritin of 23 µg/L.
[3] Algorithm calculations for beans only were modified from those of Hallberg et al. (3), by eliminating adjustments for polyphenol/tannin content which could not be analytically assessed.

Relative Iron Bioavailability





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CONCLUSIONS

- Women's iron bioavailability from maize was low, and the maize varieties did not differ *in vitro* or *in vivo*.
- Adding ascorbic acid to maize meals tripled iron bioavailability (p<0.0001), both *in vivo* and with Caco-2 cells *in vitro*, but the enhancement was less by algorithm.
- Women's iron absorption was low and not different between great northern and pinto beans, and ascorbate tripled iron absorption (p<0.0001) from both.
- The similar bioavailability between bean varieties was predicted by the algorithm based on phytic and ascorbic acid contents, but the algorithm became less predictive when also based on polyphenol content.
- In contrast, the Caco-2 cell model predicted much greater iron bioavailability from great northern than from pinto beans, interacting with ascorbic acid to double predicted bioavailability from great northern, but with considerably less influence from pinto beans.
- Overall, human iron absorption results confirmed the *in vitro* predictions of ascorbic acid enhancement with maize, but did not verify the *in vitro* difference between great northern and pinto beans.

LITERATURE CITED

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