INTRODUCTION
Multiple micronutrient deficiencies often coexist in populations prone to malnutrition. Although this may often just reflect a food supply that provides insufficient amounts of several micronutrients, some evidence has also emerged to suggest that the status of one micronutrient has a direct effect on the apparent status of other micronutrients. HarvestPlus reviewed evidence for such interactions among zinc, iron, and vitamin A (Hess, Thurnham, and Hurrell 2005), and although many interesting associations were elucidated, the causality of such interactions was by no means definitive.

It was of particular interest to HarvestPlus to assess whether the potential benefits of provitamin A biofortified crops on vitamin A status could be extended to include benefits to iron and zinc status as well. Specifically, we were interested in determining whether increased provitamin A intake would increase iron and zinc absorption from the diet.

Alterations in intestinal permeability, an indicator of impaired intestinal function, are widespread in tropical populations. Although some studies suggest interactions between intestinal permeability and micronutrient status or bioavailability, little direct information was available. These associations were of interest to HarvestPlus because intestinal health might be an important conditioning factor for the interpretation of studies measuring the absorption of micronutrients from biofortified foods among potential beneficiary populations in tropical settings.

A HarvestPlus study was conducted in Bangladesh to determine the efficacy of consumption of orange-fleshed sweet potato (OFSP) to improve vitamin A status of vitamin A-deficient Bangladeshi women. A previous study conducted in Bangladeshi men indicated that vitamin A stores were improved by the intervention, but the OFSP was pureed and had vegetable fat added—conditions that might lead to greater vitamin A uptake but did not represent the way sweet potato might normally be prepared in
this population. Therefore, the present study in women was designed to determine efficacy when OFSP was served with or without added fat.

We benefited from this large trial by including several substudies that would explore additional hypotheses relevant to food-based vitamin A interventions. Namely, these hypotheses were whether vitamin A intakes, vitamin A status, or impaired intestinal integrity affected the absorption of zinc or iron. The results of several of these substudies are summarized in this brief report. Additional details can be found in the individual project reports.

**STUDY RATIONALE**

Although plasma concentrations of zinc and vitamin A appear to be unrelated in well-nourished populations, they tend to be correlated in populations with coexisting zinc and vitamin A deficiencies (Shingwekar, Mohanram, and Reddy 1979). The cause and effect of this correlation needs to be elucidated. Plausible biological mechanisms for a positive effect of zinc on vitamin A status or metabolism have been proposed, but zinc supplementation trials in humans have produced inconsistent changes in vitamin A status (Christian and West 1998), possibly because of differences in the zinc or vitamin A status of the study populations.

Some animal studies also suggest a mechanism by which vitamin A may have a positive effect on zinc status. Studies in animal models found that either plasma zinc concentration or zinc absorption were impaired among vitamin A-deficient animals and that zinc absorption was increased when vitamin A was administered (Sklan, Halevy, and Donoghue 1987; Bauman and Berzin 1976). This response may be attributed to an effect of vitamin A supplementation on zinc transport from the intestine into the body (Berzin and Bauman 1987; Markov Iu and Berzin 1990). However, the effect of vitamin A intake and status on zinc absorption in humans remains unknown. The present study hypothesized that increased vitamin A intake from preformed or provitamin A could increase the absorption of zinc from food.

Several studies have shown a relationship between alterations of the intestinal mucosa and micronutrient deficiencies (Goto et al. 1999; Chen et al. 2003; Maggini et al. 2007). The direction of such a relationship has been difficult to elucidate; while increased intestinal permeability could impair nutrient absorption, deficiencies of certain nutrients could affect the integrity of the intestinal mucosa as well (Maggini et al. 2007). Several studies have found that vitamin A supplementation decreased intestinal permeability in infants, but little information is available on the etiology of altered intestinal permeability in adults. It is also uncertain whether consumption of foods with high beta-carotene content would have similar effects as preformed vitamin A on
intestinal mucosal function. The study design also allowed us to explore possible effects of change in vitamin A status on intestinal permeability.

METHODS
The main study was designed as a randomized, community-based intervention trial to compare the effects of three diets containing different amounts and sources of vitamin A served to vitamin A-depleted women. A 60-day period of controlled feeding used the following test diets: orange-fleshed sweet potato (OFSP), orange-fleshed sweet potato plus vegetable oil (OFSP + fat), white-fleshed sweet potato plus a vitamin A capsule (WFSP + Vit A), or white-fleshed sweet potato (WFSP-control). A total of 150 nonpregnant, nonlactating women 18–45 years of age from low-income, peri-urban neighborhoods in Mirpur, Dhaka, Bangladesh were recruited to participate in the main study. Women with marginal vitamin A deficiency (plasma retinol concentrations <1.05 mmol/L), plasma C Reactive Protein (CRP) <5 mg/L, and Hemoglobin ≥90 g/L were eligible for the study.

Of the women who participated in the OFSP, WFSP + Vit A, or WFSP-control groups, 51 participated in the substudies for zinc and iron absorption and intestinal permeability. The women’s zinc and iron absorption from a traditional rice-based diet plus the assigned sweet potato treatment and intestinal function were assessed before and after 60 days of controlled feeding. Zinc and iron absorption from standard rice-based meals was assessed using stable isotope tracer techniques. To disaggregate the effects of vitamin A intake and vitamin A status on zinc absorption, absorption was assessed during two consecutive days from test meals with and without added vitamin A. This was done for all study diet types. Intestinal permeability was evaluated with the double-sugar test, and total body vitamin A pool size was quantitatively measured using the deuterated retinol dilution technique.

The study protocol was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and the Human Subjects Protection Committee of the University of California, Davis (UC Davis).

SUMMARY OF KEY RESULTS
Interactions between vitamin A sources and zinc absorption:
- The mean fractional zinc absorption from meals with vitamin A added was 11 percent lower than the mean fractional zinc absorption from meals with no vitamin A at baseline [27.8 ± 7.6 (SD) vs. 31.1 ± 18.9%, p < 0.004]. A marginal effect of feeding group was observed on the difference in zinc absorption between the two meals at baseline (p = 0.062), but no significant differences in the post-hoc analysis of this effect were found.
When zinc absorption was measured again after the 60 days of controlled feeding, the effect of vitamin A added to the standard test meal was similar to that observed at baseline. The mean fractional zinc absorption from meals with vitamin A added was 9 percent lower than mean absorption from meals with no vitamin A \([29.9 \pm 6.6 \text{ (SD)} \text{ vs. } 32.8 \pm 7.9\%\text{, } p < 0.002]\). Feeding group allocation did not affect this relationship \((p = 0.73)\).

No correlation between zinc absorption from any of the two meals and any indicator of vitamin A status was found either at baseline or after the controlled feeding period.

**Interactions between vitamin A sources and iron absorption:**

- There was no effect of the intervention on iron bioavailability in any of the treatment groups: geometric mean iron absorption from Bangladeshi meals without vitamin A added was 3.0 percent \((\text{CI: } 2.2, 4.2)\) at baseline and 2.8 percent \((\text{CI: } 2.2, 3.6)\) after the intervention. This difference was not statistically significant \((p = 0.445)\).
- Iron absorption correlated with iron status as measured by plasma ferritin \((r = -0.50; p = 0.001)\), total body iron \((r = -0.54; p = 0.0005)\), and transferrin receptors \((r = 0.45; p = 0.005)\).
- Additional results on iron absorption from the meals with and without vitamin A added are pending.

**Effect of intestinal permeability on zinc and iron absorption:**

- The overall prevalence of altered intestinal permeability \((L/M \text{ ratio }> 0.07)\) in the studied population was 27 percent. L/M ratio negatively correlated with CRP \((r = -0.30; p = 0.044)\).
- Fractional zinc absorption in individuals with altered intestinal permeability was higher than in individuals with no alterations of the intestinal mucosa \([36.1 \pm 9.4\% \text{ vs. } 24.9 \pm 10.3\%, p = 0.038]\). Zinc absorption marginally correlated with the lactulose:creatinine ratio \((p = 0.07)\).
  - The increased zinc absorption when intestinal permeability is altered could be due to more zinc diffusing through the paracellular pathway in the ileum, which seems to be the site of higher absorption in animal models.
- Iron absorption from meals did not differ in individuals with normal and increased intestinal permeability \([2.6\% \text{ (CI: } 1.8, 3.8) \text{ vs. } 2.9\% \text{ (CI: } 1.4, 5.8), p = 0.79]\). These results suggest that the regulation of iron absorption is not disturbed by alterations in intestinal permeability.
  - No correlation between iron absorption and any indicator of intestinal function was found.
Effect of daily consumption of orange-fleshed sweet potato or vitamin A supplements on intestinal permeability:

- The prevalence of altered intestinal permeability decreased slightly in all treatment groups after the intervention, being statistically significant from baseline only in the group receiving WFSP + oil capsule ($p = 0.009$).
  - When expressed as a percent of women who recovered from increased intestinal permeability through the intervention, this recovery rate was higher in the groups consuming OFSP and vitamin A supplements.
- When comparing the indicators of intestinal permeability post-intervention, there were no significant differences among treatment groups.
- Study results indicate that daily consumption of OFSP or vitamin A supplements providing the recommended daily intake of vitamin A have no effect on the prevalence of increased intestinal permeability, L:M urinary recovery ratio, or other indicators of intestinal function in vitamin A-depleted women. The small, final sample size and the lack of effect of our intervention on vitamin A status does not allow us to make any definitive conclusions about the role of vitamin A from different sources on the integrity of the intestinal mucosa.

CONCLUSIONS

Long-term (i.e., 60 days) intake of supplementary vitamin A, either as preformed vitamin A or provitamin A in the amounts provided in these studies, does not appear to affect the bioavailability of iron or zinc among marginally vitamin A-deficient Bangladeshi women. However, it was observed that vitamin A added to a single day’s test diet had an inhibitory effect on zinc absorption, compared to the control diet. Altered intestinal permeability does not appear to be a significant confounding factor of iron absorption among these women. However, zinc absorption was increased with this condition. Caution should be used in applying these results as the loss of endogenous zinc may also be increased in this condition by the same mechanisms. The effects of vitamin A on intestinal permeability were not definitive, but the results for recovery from altered intestinal permeability are suggestive of a potential therapeutic effect of preformed or provitamin A. Prospective case-control studies with sufficient statistical power are warranted to elucidate these outcomes.
REFERENCES


**Note:** The data presented in this brief have not yet been reviewed for publication in a scientific journal, and the interpretation of results is subject to change following peer review.

**AUTHORS**
Ana B. Pérez-Expósito¹, Leslie R. Woodhouse², Kazi M. Jamil³, Marjorie J. Haskell¹, Bakhtiar Hossain³, Mohammed A. Wahed³, Gry Barfod⁴, Munirul M. Islam³, Maleka Jamil³, Janet M. Peerson¹, Kenneth H. Brown¹

¹ Department of Nutrition and Program in International and Community Nutrition, University of California, Davis

² USDA/ARS Western Human Nutrition Research Center

³ International Centre for Diarrhoeal Disease Research, Bangladesh

⁴ Department of Geology, University of California, Davis