Human Zinc Requirements

Report of the HarvestPlus Consultation on Physiological and Dietary Zinc Requirements

July 24th 2012
NIH Conference Room 5A01
6100 Executive Boulevard
Rockville, Maryland 20892, USA

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Consultation Funded by:

HarvestPlus
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Introduction

Biofortification is an agricultural strategy that aims to increase the content of selected micronutrients in staple food crops such as maize, pearl millet, rice, wheat and others. Current zinc biofortification efforts are focused on developing high zinc varieties of rice and wheat for Bangladesh, India and Pakistan. The expectation is that when these varieties are consumed by zinc deficient populations, higher zinc intakes will lead to a lower risk of zinc deficiency.

For biofortification to be successful, HarvestPlus has designed its activities along an impact pathway that goes from discovery of "at-risk" target populations and relevant staple crops through to the delivery of micronutrient-enhanced food crops with high acceptance rates by farmers and a measurable improvement in the micronutrient status of target populations.

During its discovery phase, HarvestPlus nutritionists and breeders worked on establishing preliminary minimum zinc nutritional breeding targets for rice and wheat. The key issue was the magnitude of the increase in a crop's zinc content needed to improve the micronutrient status of consumers, particularly women and children. The breeding target and the initial assumptions used to compute them are presented in Table 1. Taking rice as an example, it was estimated that adult women consume an average of 400 grams of raw parboiled milled rice per day. Knowing that the International Zinc Nutrition Consultative Group (IZiNCG) estimated average requirement (EAR) for non-pregnant and non-lactating women is 7 mg/day, HarvestPlus set the target for biofortification to contribute additional zinc through rice that would amount to the equivalent of 40% of the EAR, i.e. an additional 2.9 mg/day. Given rice intake, baseline non biofortified rice zinc content, and 90% retention of zinc (10% of zinc is assumed to be lost after milling and cooking), it was estimated that an additional 8 μg/g of zinc in raw rice was needed to reach that target, thus providing the final breeding target of 24 μg/g.

Table 1. Assumptions used to set target levels for increased zinc content of biofortified staple crop foods

Staple food	**Rice (polished)**	**Wheat (whole)**
Women		
Intake (g/day)	400	400
EAR non-pregnant, non-lactating women (mg/day)[1]	7	7
Target %EAR to achieve through biofortification	~40	~40
Retention[2] (%)	90	90
Baseline content in non-biofortified crop (μg/g)	16	25
Additional content required (μg/g)	8	8
Final target content to achieve in biofortified crop (μg/g)	24	33
Children		
Intake in 4-6 years children (g/day)	200	200
EAR children 4-6 years (mg/day)[1]	4	4
Target %EAR to achieve through biofortification	~40	~40
Retention[2] (%)	90	90
Baseline content in non-biofortified crop (μg/g)	16	25
Additional content required (μg/g)	8	8
Final target content to achieve in biofortified crop (μg/g)	24	33

[1]The IZiNCG estimated average requirements were used. Bioavailability was assumed to be 25%.
[2]Retention refers to the percent amount of zinc that is retained in the crop after processing and cooking

Recognizing that biofortification alone as a strategy to combat zinc malnutrition is likely to be insufficient in covering the entire gap between intakes and requirements, HarvestPlus is seeking to determine levels of coverage of zinc requirements that are likely to produce an effect on zinc status in target groups. As previously shown, HarvestPlus's calculations are based on the IZiNCG EARs but there are also other published international standards that show quite a discrepancy depending on the source used. Tables 2 and 3 below show the zinc physiological requirements (absorbed and retained zinc in the body) and EARs (dietary zinc) currently used by different countries and institutions.

Table 2. Estimated physiological requirements for zinc (absorbed and retained zinc) in adult men and women by source

Source	Physiological requirement (mg/d)	
	Men	Women
World Health Organization (WHO) (1)	1.40	1.00
King (2)	2.50	2.50
United Kingdom (3)	2.20	1.70
Canada (4)	2.10	1.80
IZiNCG (5)	2.69	1.86
Institute of Medicine (IOM) (6)	3.84	3.30

Table 3. Zinc Estimated Average Requirements (dietary zinc) for adults and children by source

	EAR (mg/day)		
Source	Men	Women	Children 4-6 years
Institute of Medicine (IOM)	9.4	6.8	4.0
European Commission (EC)	7.5	5.5	--
Nordic	6.0	5.0	--
Australia/New Zealand	12.0	6.5	3.0
United Kingdom	7.3	5.5	5.0
WHO (moderate bioavailability)	(7.0)	(4.9)	(3.2)
IZiNCG mixed diet	10.0	6.0	3.0
IZiNCG unrefined diet	15.0	7.0	4.0

Source: http://www.serbianfood.info/eurreca/index.php

Recently, researchers engaged in reviewing current physiological zinc requirements offered a possible resolution for the major discrepancies between the IZINCG and the Institute of Medicine (IOM) estimates (7) . Given that these estimates are critical reference points for planning zinc nutrition interventions, HarvestPlus convened a consultation of zinc experts to discuss zinc nutrition and requirements in the context of their effect on rice and wheat breeding programs.

The specific objectives of the consultation were:

1- To recommend zinc requirements for adult women and children 4-6 years of age that will enable HarvestPlus to revise its original breeding target levels for zinc target crops, wheat and rice;

2- To recommend a zinc fractional absorption level (%) for zinc biofortified wheat and polished rice, and

3- To recommend an additional amount of zinc (expressed as percent zinc EAR) from biofortified wheat and rice that has a high probability of producing a measurable impact on zinc status among deficient women and children

The consultation brought together international zinc experts and participants from a variety of backgrounds (pediatric gastroenterology, global nutrition programs, endocrinology, program management, etc.) and institutions (Annex 1). Using information from five background papers (Annex 2) and several oral presentations made during the consultation, experts discussed information on:

- The European Commission (EC)-funded EURECCA project and its work involving collating data on zinc requirements in countries worldwide and systematically reviewing biomarkers of zinc status

- The process used by the IOM to establish Dietary Reference Intakes (DRI) and what needs to be done to trigger the revision of the zinc physiological requirements

- The development of the IZiNCG human zinc requirements and new developments and insights on the reasons for the discrepancies with the IOM estimates

- The development of the IOM human zinc requirements and efforts to reconcile them with the IZiNCG estimates

- New statistical approaches to model the relationship between zinc losses and absorbed zinc

Based on the information provided in the background papers, the presentations, and discussion by the group, experts formulated conclusions on zinc requirements and their application for the assessment of dietary zinc adequacy in general and made specific recommendations for HarvestPlus.

Discussion Points

1. What estimated physiological requirements for adult women and children 4-6 years of age will enable HarvestPlus to revise its original breeding target levels for zinc crops, wheat and rice?

– When setting the preliminary breeding target levels, HarvestPlus based its calculations on the published IZiNCG requirements. These estimates are substantially different from those published by IOM, and the group discussed the main sources of discrepancy between them.

– The group agreed that the main sources of discrepancy can be classified into three general categories and are discussed in the detail below:

  - Differences in the selection and application of available data
  - Differences in statistical modeling of the relationship between zinc intakes and losses
  - Differences in reference body weights for adult men and women

– There are differences in how the IOM and IZiNCG select studies that provide empirical data on endogenous fecal zinc (EFZ) and zinc absorption. The IZiNCG has expanded the selection of studies to those conducted in developing countries for a total of 19 dietary periods whereas IOM had only 10 dietary periods mainly in the US. The IOM included studies with zinc supplements whereas IZiNCG applied restrictions on studies using zinc supplements.

– Data selection judgment was reevaluated during the consultancy and questions were raised about study design or methodologies that are less acceptable now than they were before. Given the advances in stable isotope methodology, one of the issues discussed was the administration of single isotope doses as opposed to double (oral and i.v.) doses, and related considerations about the amount of the dose. The group agreed that there were no fundamental flaws with older single dose studies as long as there is evidence that they were carefully conducted and that very close attention was paid to losses. Very few newer studies with double doses were available and therefore a comparison of calculations based on single dose vs. double dose studies only is not possible at this stage.

– The IZiNCG selection applied restrictions on studies using supplements and included only food based studies. It seems that supplements are not an issue and studies using supplements therefore could eventually be included in the calculation of revised estimates.

- The calculation of both published IOM and IZiNCG estimates currently includes erroneous data. For IOM, there were some errors in data on menstrual losses, which resulted in an overestimation of requirements for women, whereas IZiNCG included data on EFZ estimates that are known to be biased and produced an underestimation of requirements.

- Statistical modeling of the relationship between total absorbed zinc (TAZ) and EFZ differs between IOM and IZiNCG. IZiNCG used regression analysis with data weighted by sample size with the expectation that studies with larger samples produce greater precision. However, a recent reanalysis of the correlation between variance and sample size does not support this assumption and sample weighting therefore cannot be recommended in this case.

- IZiNCG and IOM used different reference body weights for adults. IOM used the US values whereas IZiNCG employed the World Health Organization (WHO) international values.

2. Considering the typical diets of rural Bangladeshi and Indian populations, what zinc fractional absorption level (%) would be more likely to reflect actual absorption from zinc biofortified wheat and polished rice?

- When setting the preliminary target levels, HarvestPlus assumed 25% zinc bioavailability for high zinc wheat (whole wheat flour) and polished rice. However, given that HarvestPlus now has dietary intake data at the individual level from 24-hour dietary recalls and dietary records in selected populations, and that equations are now available to estimate zinc absorption from the diet, a blanket assumption of bioavailability for all individuals can no longer be justified.

- Data from Bangladesh (8) showed that the average phytate: zinc molar ratio for the whole diet was below 15 in both children (11.2) and women (12.0). Preliminary data from Punjab, India, suggests similar ratios.

- The group suggests that HarvestPlus estimate zinc absorption as a function of dietary zinc and phytate in the different populations where the program is being implemented and evaluate zinc adequacy using the physiological requirements directly.

- To estimate zinc absorption, it is recommended that HarvestPlus uses zinc bioavailability algorithms (Miller). This will require that HarvestPlus completes phytate content values in all of its food composition tables.

3. What additional amount of zinc (expressed as percentage coverage of zinc EAR) from biofortified wheat and rice will most likely produce a measurable impact on zinc status among deficient women and children?

- The group proposes that HarvestPlus changes its focus from percentage coverage of IZiNCG EAR with biofortified crops to measuring the shifts in distribution of zinc intake at the current and future crop target levels with varying crop adoption and consumption scenarios as a measure of expected impact.

- The question of by how much the distribution needs to shift or which levels of increased zinc intakes are needed to translate into tangible changes in nutrition and health could not be answered with the current state of knowledge. This is a research priority that remains to be determined.

Conclusions

1. In spite of its limitations, the factorial method continues to offer many advantages and should continue to be used to estimate human zinc requirements.

2. To calculate revised requirements, data points used by the IZiNCG should be used with the exception of two points that are well known to produce erroneously low EFZ values (9). Exclusion of those two data point is not expected to have a great effect on statistical power.

3. Current estimates are based on the back-calculation of absorbed zinc from the regression of zinc losses on absorbed zinc. In the current statistical models, TAZ is not the dependent variable leading to a biased and high variance estimator. It was agreed that it makes more sense statistically to model TAZ as the dependent variable with zinc balance as the independent variable. The revised model would be:

$$(\textit{Absorbed zinc}) = \delta_0 + \delta_1 * (\textit{zinc balance}) + \nu$$

Where zinc balance would be TAZ-EFZ- fixed daily losses from urine, surface and semen or menstrual flow (where total fixed daily losses=1.15 mg for men and 0.80 mg for women)

Zinc requirements would be estimated at balance=0

Estimated zinc requirement = δ_0.

4. Using TAZ as the dependent variable provides the advantage of being able to easily compute confidence intervals around the zinc requirement estimate.

5. Random effects models applied to study-level data are the best option at the moment for modeling the relationship between TAZ and zinc balance. With the random effects model, each study has its own set of coefficients which is an advantage over the previous fixed effects models.

6. Weighting regressions by number of subjects in the sample should be dropped because a recent reanalysis of correlation of the variance and sample size showed that it was not supported by data.

7. The weights to be used in the new regression models should be model based. The group proposes to use a variance of error term proportional to EFZ.

8. The consensus was to use WHO reference body weights for the calculation of revised human zinc requirements. These weights are 65kg for men and 55kg for women.

9. Extrapolation to zinc requirements for children will be based on kg of bodyweight using the WHO references data. Other than that, the procedure remains the same as before.

10. Ideally, the new revised regression models of the relationship between TAZ and zinc balance should use individual level data instead of study level data as this would provide much improved estimates of variance and offer new analyses opportunities. The group acknowledges that gathering individual data may be impossible given the age of some of the studies but it should be attempted nevertheless.

11. Preliminary results from the new regression models indicate that the revised zinc physiological requirement for women may fall between 2.5 and 3.0 mg/day.

Next Steps

1. The new revised zinc physiological requirements are expected to be available for testing by mid December 2012 after the following steps have been completed:

    a. The team from University of Colorado will attempt to get individual level if it is available. Because of the limited sample sizes, many studies have actually published the individual data. Leland Miller has examined it and concluded that most of the remaining missing unpublished individual data could be retrieved by contacting a limited number of researchers. This will be completed by the beginning of December 2012 so the data can be used to revise zinc physiological requirements;

    b. While both teams at UC Davis and University of Colorado obtain the same preliminary results with the new regression models for women's physiological requirements, differences persist in the estimation of men's requirements. This issue will need to be resolved through closer examination of the models and data used for men by mid December 2012;

    c. UC Davis will complete the revision of human zinc requirements based on the conclusions listed above once the procedure is cleared with the team from University of Colorado.

2. When the revised estimates of zinc physiological requirements are available, they should be used to compute the prevalence of zinc inadequacy in populations with known levels of zinc adequacy to test their validity.

3. It is essential that WHO and the Food and Agricultural Organization (FAO) are involved in this effort to revise human zinc requirements. The consultation proposes to explore the possibility of producing an interim statement by WHO based on this report, which is estimated to take about 9 months. If a statement is issued by WHO, this will automatically trigger the revision of WHO zinc requirements in the near future.

4. The European Food Safety Authority (EFSA) NDA Panel has a current mandate from the EC to revise and set new zinc requirements. Although the panel cannot wait for the experts to reach a consensus on revised estimates, they should nevertheless be quickly exposed to early conclusions from the consultancy so that it can feed into their efforts.

5. In the interim, HarvestPlus should assume that the physiologic zinc requirements used to date for setting zinc concentration target levels in rice and wheat will need to be revised upwards. New target levels should be computed assuming physiological requirements for adult women between 2.5 and 3.0 mg/day.

Reference List

1. FAO/WHO. 2002. "Zinc." In *Human Vitamin and Mineral Requirements: Report of a Joint FAO/WHO Expert Consultation* edited by FAO/WHO, 257–270.Rome: FAO.

2. King, J., and J. Turnlund. 1989. "Human Zinc Requirements." In *Zinc in Human Biology,* edited by C. Mills, 335–350. London, UK: Springer.

3. UK Department of Health. 1991. *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. London, UK: Her Majesty's Stationery Office.

4. Ministry of Health and Welfare. 1990. *Recommended Nutrient Intakes for Canadians*. Ottawa, Canada: Canadian Government Publishing Center Supply Services.

5. International Zinc Nutrition Consultative Group (IZiNCG). 2004. *Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control*. Hotz C and Brown KH, eds. *Food and Nutrition Bulletin* 25: S91–S204.

6. IOM. 2001. "Zinc." In *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc,*edited by IOM, 442–501.Washington, DC: National Academy Press.

7. Hambidge, M. K., L. V. Miller, and N. F. Krebs. 2011. "Physiological Requirements for Zinc." *International Journal for Vitamin and Nutrition Research* 81: 72–78.

8. Arsenault, J. E., E. A. Yakes, M. B. Hossain, M. M. Islam, T. Ahmed, C. Hotz, B. Lewis, A. S. Rahman, K. M. Jamil, and K. H. Brown. 2010. "The Current High Prevalence of Dietary Zinc Inadequacy among Children and Women in Rural Bangladesh Could Be Substantially Ameliorated by Zinc Biofortification of Rice." *Journal of Nutrition* 140: 1683–1690.

9. Hunt, J. R., S. K. Gallagher, L. K. Johnson, and G. I. Lykken. 1995. "High- Versus Low-meat Diets: Effects on Zinc Absorption, Iron Status, and Calcium, Copper, Iron, Magnesium, Manganese, Nitrogen, Phosphorus, and Zinc Balance in Postmenopausal Women." *American Journal of Clinical Nutrition* 62: 621–632.

Annex 1 – List of Participants

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Annex 2 – Background Documents

Hambidge, M. K., L. V. Miller, and N. F. Krebs. 2011. "Physiological Requirements for Zinc." *International Journal for Vitamin and Nutrition Research*. 81: 72–78.

Hotz, C. 2009. "The Potential to Improve Zinc Status through Biofortification of Staple Food Crops with Zinc." *Food and Nutrition Bulletin* 30: S172–S178.

IZiNCG. 2004. "Overview of Zinc Nutrition." In *Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control*, edited by C. Hotz and K. H. Brown, S99–S129. *Food and Nutrition Bulletin*.

IZiNCG. 2004. "Research Needs." In *Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control*, edited by C. Hotz and K. H. Brown, S187–S203. *Food and Nutrition Bulletin*.

Nestel, P., H. E. Bouis, J. V. Meenakshi, and W. Pfeiffer W. 2006. "Biofortification of Staple Crops." *Journal of Nutrition* 136: 1064–1067.

Annex 3 – Consultation Agenda

HarvestPlus Zinc Requirements Consultation Agenda
July 24th, 2012

Time: 8:30 AM – 5:30 PM
Location: NIH Building
Conference Room 5A01 (located on the 5th floor),
6100 Executive Blvd.
Rockville, MD 20892

8:30 AM – Breakfast

9:00 AM - Welcome remarks and statement of objectives (Erick Boy)

9:15 AM – Background (the 'EURRECA experience') (Susan Fairweather-Tait)

9:30 AM - Presentation: Overview of DRI Process by Linda Meyers (Chaired by Susan Fairweather-Tait and Rafael Flores-Ayala)

9:45 AM - Presentation: Development of IZiNCG estimates of human zinc requirements, and application for assessment of dietary zinc adequacy by Kenneth Brown (Chaired by Susan Fairweather-Tait and Rafael Flores-Ayala)

10:15 AM – Q & A Session

10:30 AM - Presentation: Looking Ahead Together by Michael Hambidge (Chaired by Susan Fairweather-Tait and Rafael Flores-Ayala)

11:00 AM – Q & A Session

11:15AM - Coffee Break

11:30 AM -Open Discussion on Key Approaches (Chaired by Rafael Flores-Ayala)

12:30PM – Lunch

1:15 PM -Open Discussion Continued (Chaired by Rafael Flores-Ayala)

2:10 PM -Coffee Break

2:30 PM –Summary of open discussion (Rafael Flores-Ayala)

2:40 PM - HarvestPlus Breeding targets and nutritional objectives (Erick Boy)

3:00 PM–Discussion on zinc targets for HarvestPlus

3:30 PM - Coffee Break

3:45 PM–Concluding Remarks (Erick Boy)