Original Communication

Physiological Requirements for Zinc

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Received: January 18, 2011; Accepted: March 21, 2011

Abstract: The estimates of zinc physiological requirements by the International Zinc Nutrition Consultative Group (IZiNCG) in 2004 were conspicuously low in comparison with those estimated by the Institute of Medicine (IOM) in 2001. The objective of this review is to explore the reasons for this gap and to reflect on lessons learned. All estimates of inevitable losses of endogenous zinc, especially intestinal excretion of endogenous zinc, were reviewed. An error in zinc menstrual losses, as well as a minor error in the linear regression of endogenous fecal zinc (EFZ) vs. total daily zinc absorption (TAZ) by IOM, were corrected. The review revealed an error by IZiNCG in selecting two data points for the linear regression of EFZ on TAZ. A second major reason for the "gap" is attributable to weighting of the data in the regression analysis by number of subjects per study by IZiNCG. Adjusting for these factors, together with use of the same reference data for body weights, resulted in satisfactory agreement between the two estimates of physiological requirements. The lessons to be learned from this review are discussed together with suggestions for future action by IOM as well as a constructive role for IZiNCG.

Keywords: Zinc, physiological requirements, factorial estimation

Introduction

This paper offers a critical review and resolution of major discrepancies in two recent estimates of physiological requirements for zinc [1, 2]. An accurate estimate, or reasonable agreement among multiple estimates, of physiological requirements is of critical importance for our understanding of human zinc nutrition and homeostasis. Beyond the core importance of physiological requirements for estimating and understanding human dietary zinc requirements, a strong argument can be made for their value in contributing to our understanding of the zinc status of populations when combined with reliable dietary data. Furthermore, they provide critical reference points for bioavailability studies of zinc in biofortified crops [3].

In 2001, the Institute of Medicine (IOM) published new estimates of physiological zinc requirements [1]. Three years later, the International Zinc Nutrition Consultative Group (IZiNCG) elected to publish alternative estimates [2]. The conspicuous differences between the IZiNCG and IOM estimates have cast a pall over our understanding of zinc homeostasis, and especially zinc requirements, through the latter half of the past decade. The estimated physiological requirement for young children, a critical age group for preventing zinc deficiency, is approximately 30 % lower as estimated by IZiNCG, compared to the figure estimated by IOM [2]. A recent example of the confusion resulting from the disparities between these two estimates has been provided by a recent study in Bangladesh, in which it was calculated that 0 % of young children had zinc-deficient diets based on IZiNCG reference data, while 50 % of these diets were zinc-deficient based on IOM reference data [4]. These are examples of the confusion prevailing at this time. Un-

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til these estimates are reconciled, this confusion will continue to handicap progress in our understanding of how to best prevent and treat human zinc deficiency, now regarded as a public health challenge of global proportions. The objective of the evaluation reported here is to explore why these differences occurred and to seek to reconcile these disparate estimates of human physiological zinc requirements.

Methods

The methods used to determine the total absorbed zinc (TAZ) and intestinal loss of endogenous zinc (EFZ) data were reviewed [5–16], as well as any calculations of EFZ when not reported in the publications. Where substantial errors were detected, data were revised accordingly. Minor errors and discrepancies were not addressed.

The linear regression methods used to analyze the relationship of EFZ to TAZ were evaluated in light of data characteristics and accepted statistical practice [17,18]. In particular, the use of sample size-weighted regression by the IZiNCG was evaluated.

The next step was to re-evaluate estimates of excretion of endogenous zinc from non-intestinal routes and to use these estimates together with the linear regression data for EFZ vs. TAZ in the same modified factorial approach to estimate the physiological zinc requirements adopted by IOM [1], and subsequently by IZiNCG [2]. The differences in estimates of integumental losses were initially ignored as these depended on the deliberate use of different reference data for body weight [2]. However, as a final step, these reference data were standardized to determine the effect of this step on the gap between the two estimates of physiological requirements.

Results

Estimates of intestinal losses of endogenous zinc

Apparent errors in data calculations and discrepancies were discovered in the data used to define the relationship of EFZ to TAZ by both the FNB/IOMand IZiNCG (Figure 1). Most of these were minor in nature and some involved judgments regarding use of data from multiple metabolic study periods. The minor errors and discrepancies, which on balance affected physiological requirement estimates by ≤0.10 mg Zn/day, have not been addressed here. The single exception to this related to the discovery that regression of the EFZ vs. TAZ data used by the IOM produced slightly different physiological requirement estimates than those published (3.3 mg/day and 3.8 mg/day) and the use in this report of the revised estimates of 3.2 mg Zn/day for women and 3.9 mg Zn/day for men.

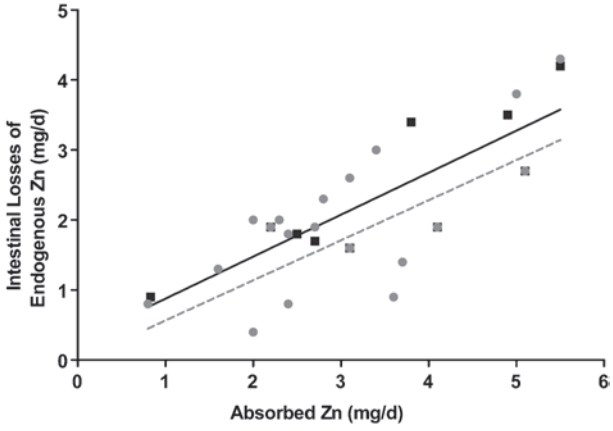

Figure 1: Data and regression lines of intestinal losses of endogenous zinc on absorbed zinc used by the FNB/IOM (black square symbols and solid line) and the IZiNCG (gray circle symbols and dashed line). This graph is similar to Figure 1.3 in the IZiNCG document [2], but shows the actual data used by the IOM which differ slightly from those reported by the IZiNCG.

Data selection judgments were not reevaluated, with the exception of one consequential data selection error. In this case two data points selected for use by the IZiNCG were derived using a misconceived calculation that produced erroneously low EFZ values. The calculation at issue presented EFZ as the difference between true zinc absorption (determined by whole-body counting) and apparent absorption of an orally administered isotope (0.9 mg Zn/day and 0.4 mg Zn/day for the two dietary groups [15]). This calculation provides a measurement of absorbed isotope secreted into the intestine, not total endogenous zinc intestinal losses. The publication [15] also reported the correct calculation of EFZ as the difference between true absorption and apparent elemental absorption measured by conventional metabolic balance (1.5 mg Zn/day and 1.8 mg Zn/day). When the erroneous data were replaced with these values (Figure 2), the IZiNCG estimates of physiological requirements increased from 1.86 to 2.32 mg Zn/day for women and from 2.69 to 3.02 mg Zn/day for men (Figures 3a and 3b). These changes were so large because there were 14 subjects

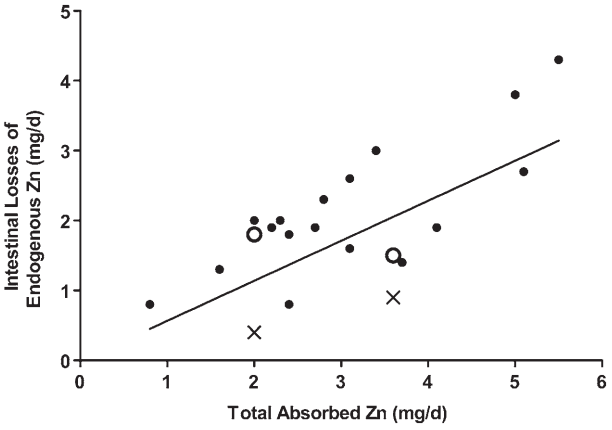

Figure 2: Revision of the intestinal endogenous zinc loss versus absorbed zinc data used by the IZiNCG to replace two erroneous data (x symbols) with more accurate data (open circles) from the same publication. The original IZiNCG regression line is also shown.

in this study and the IZiNCG use of sample size-weighted regression gave these points more weight than most of the other data.

Linear regression methods used to characterize relation of EFZ to TAZ

The IZiNCG used regression analysis wherein the data were weighted by sample size on the expectation that larger samples would produce greater precision and, therefore, ought to be accorded more weight in the analysis [19]. Since 16 of the data were reported with sufficient information to permit the calculation of variance, it was possible to evaluate the appropriateness of the weighting scheme. The correlation between variance and sample size was significant ($r = 0.52$, $P = 0.041$), but the correlation was positive, not negative as would be expected (Figure 4). When "outliers" were removed, the remaining data were uncorrelated ($r = -0.075$, $P = 0.81$). Therefore, weighting by sample size was not supported by the data. When ordinary least squares (unweighted) regression was used instead (Figure 5), the IZiNCG physiological requirement estimates increase to 2.67 mg Zn/day for women and 3.44 mg Zn/day for men (Figures 3a and b). An analysis of the residuals from the ordinary least squares (OLS) regression of the IZiNCG data indicated that error variance was not constant, varying directly with TAZ or EFZ magnitude ($P = 0.0033$). A similar analysis of the regression residuals from the IOM data found the same relationship, though it was

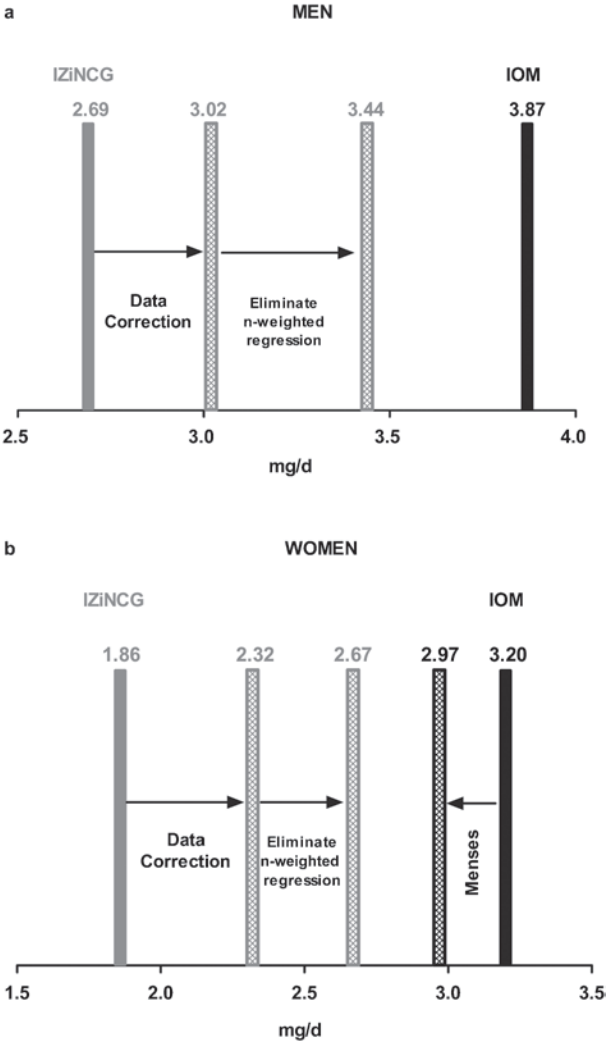

Figure 3: Comparison of estimates of zinc physiological requirements. Bars at the extreme left depict the original IZiNCG estimates of requirements for adult men (3a) and adult women (3b). Corrected original IOM estimates by IOM are depicted by extreme right hand bars. This figure shows the extent to which the gap between these estimates is closed by correction of the IZiNCG data (Figure 2) and the elimination of weighting by number of subjects per data point in regression of endogeous fecal zinc versus total absorbed zinc per day. In addition, Figure 3b shows the extent to which the gap for females is further narrowed by correction of an error by FNB/IOM in the estimate of zinc losses in menses.

not significant ($P = 0.056$). With both the IOM and IZiNCG data, new regression analyses with the data weighted by the reciprocal of variance estimated from the residuals produced physiological requirement estimates that varied by less than 0.1 mg Zn/day from those derived with the OLS regression.

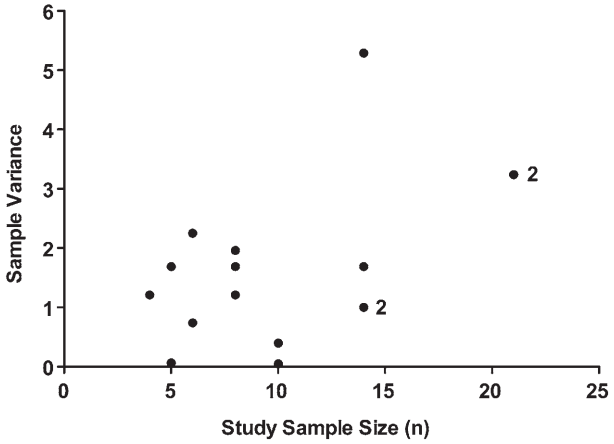

Figure 4: Relation of measured sample variance to sample size for 16 of the data used by the IZiNCG for which sufficient information to calculate the variances was reported. The "2" next to a symbol indicates that the symbol represents two data having the same values. These are cases where a pooled standard deviation was reported for two measurements. Counter to expectation, there is a significant positive correlation between variance and sample size (r = 0.52, *P = 0.*041). When the outlying points are removed, the remaining data are uncorrelated (r = -0.075, *P = 0.*81).

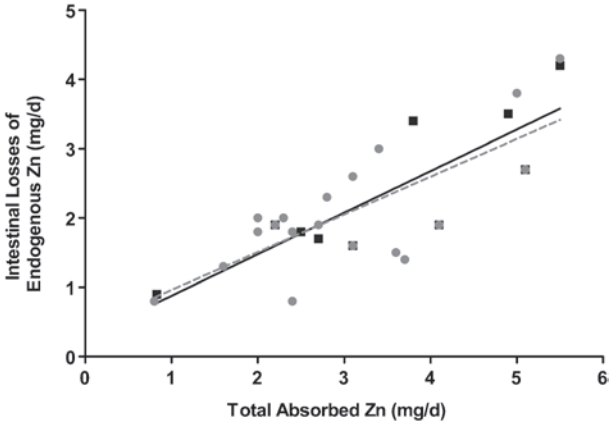

Figure 5: Regressions of IOM (black square symbols and solid line) and IZiNCG (gray circle symbols and dashed line) data after correction of IZiNCG data and elimination of weighted regression.

Estimates of non-intestinal losses of endogenous zinc

As correctly noted by IZiNCG, the value for menstrual losses used by IOM resulted from an error in using the data from their quoted reference [20]. The correction of female menstrual losses from 0.1 to 0.01 mg Zn/day reduced the FNB/IOM estimate of zinc physiological requirement for adult women to 2.97 mg Zn/day (Figure 3b).

The final step of standardizing weight reference data (when added to the modifications of IZiNCG data) closed the gap to 4 % of the original gap for men and 2.5 % of the original gap for women (Figures 6a and 6b).

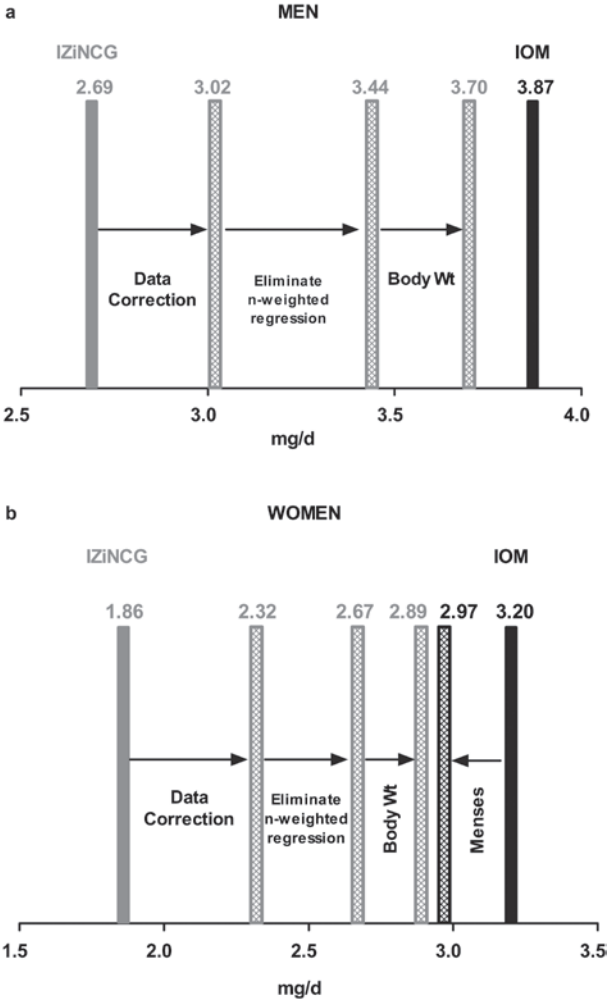

Figure 6: The further reduction in the difference between FNB/IOM and IZiNCG estimates of physiological zinc requirements for adult men (6a) and women (6b) if, in addition to the corrections depicted in Figure 3, the same body weight standards used by FNB/IOM are applied (not as a correction factor) to IZiNCG estimates. The percentages of the gap unaccounted for are 4 % and 2.5 % for men and women, respectively, of the IOM estimates.

Discussion

The results of this review clarify the reasons for the conspicuous gap between the IOM and IZiNCG estimates of physiological zinc requirements. Two major factors, attributable to the IZiNCG, are the selection

of erroneous data and the weighting of data by sample size in the regression analysis. Together, these two factors accounted for 58 % and 62 % of the gaps for men and women, respectively. Correction of an error in estimated menstrual losses by IOM has also helped to close the gap for women.

After these corrections, the remaining difference is attributable to the use of different reference data for adult weights, which in turn impacted estimates of integumental losses, leading to the difference in the data sets. Discussion of the relative merits of the reference data for weight is beyond the scope of this paper; however, it is useful to understand the effect these differences have on estimated physiological zinc requirements.

The data relating EFZ to TAZ have a major role in estimating physiological requirements and, from there, to estimating dietary zinc requirements. The current use of linear regression analysis of EFZ versus TAZ as a core strategy first evolved in establishing the (dietary reference intakes) DRIs for zinc and was subsequently accepted and used by IZiNCG. IOM elected to utilize only adult male data (lack of individual data limiting this to means) because the slope of regression analysis for female data alone was insignificant, with wide confidence intervals. This remains so despite an increase in number of studies [21], likely attributable to the relatively limited range of mean TAZs for the female studies. These calculations were then applied to adult women with appropriate modifications for different estimated losses from non-intestinal routes. IZiNCG elected to use all available data for both genders combined to evaluate the relationship between EFZ and TAZ. Of note, the regression results for these combined data are not significantly different from male alone [21].

Regarding the IZiNCG use of sample size-weighted regression, an examination of measurement variability of a large subset of the IZiNCG data demonstrated that the assumption of a positive relation between measurement precision and sample size was not well founded. This may be attributable to the heterogeneous experimental designs and analytical methods used in the studies from which the data originated. Furthermore, this application of weighted regression strays from the principal use of weighted regression as a remedy for non-constant error variance wherein data are typically weighted by the reciprocal of an estimated error variance function [17, 18]. An appropriate relationship between error variance and sample size would need to exist for the regression technique used by the IZiNCG to be valid; and the measured sample variances indicate that the necessary relationship does not hold with these data. Whatever differences in opinion on the different statistical handling of these data, all can agree that weighting by number was one of two major reasons for differences between the IZINCG and IOM estimates. Another incidental effect of weighting by sample size was that the resulting regression line substantially favored data from females as 62 % of the individual data included were from female subjects.

Several additional items of discussion are relevant to this review. There is a major need for well-designed, stable isotope-based studies to acquire additional data to assist in factorial estimates of zinc requirements, especially experimental data for women, children, and perhaps the elderly. However, an important reminder from this experience is that great care is essential to make appropriate and accurate use of the data already available. This experience also serves as a reminder of the importance of adequate internal and external review not only of a preliminary draft, but of the final document.

This is also an appropriate moment to reflect on which organizations should assume the responsibility of publishing their own versions of estimated physiological and dietary requirements for any nutrient. In this case it is reasonable to question whether it is appropriate to claim to provide "international" estimates when, with the exception of one study of two groups of women [6], virtually all of the data utilized in deriving these estimates were obtained from studies conducted in the United States. IOM DRIs are widely used for international purposes, for example in the recent development of recommendations for zinc fortification of flour [22]. Apart from questions about the validity of current DRI Upper Levels for zinc and lack of a speedy mechanism for correcting an obvious, though minor, error in losses of zinc in menses, the zinc DRIs continue to serve well. It is, however, unfortunate, that the DRI process was insular in its accepted sources of data and it is hoped that this policy has or will change. The Panel on Micronutrients (including zinc) for the DRIs wisely avoided tackling the phytate issue because the necessary data were unavailable at that time, at least in a format that was usable. Sufficient data and an apparently valid model of the impact of dietary phytate on zinc absorption are now available [23, 24] and were supported recently by an independent analysis [25]. This would now be an excellent, indeed urgent, time for the IOM to review the DRIs for zinc and extend these to include the inhibitory effect of dietary phytate. It would also be beneficial to see the IOM less reluctant to recognize the importance of the DRIs beyond North America and be

willing to assume some broader, overt international responsibility as is likely to occur with the guidelines currently being developed by EURRECA (European Micronutrient Recommendations Aligned) [26]. IZiNCG could make a useful contribution by supporting these major established or new initiatives while giving special attention to the impact of pathophysiological and environmental factors, which are likely to be a special burden on zinc requirements in poor, tropical environments.

In conclusion, the outcome of this study designed to determine the extent to which the widely divergent estimates of physiological requirements for zinc by IZiNCG and IOM can be reconciled, has been very reassuring. While factorial methodology may not appear exciting or novel, it remains the only established means of estimating zinc requirements. With the handicap of these differences in estimates behind us, it is time to move on to new horizons including incorporation of phytate into estimates, obtaining the experimental data needed for more direct estimates of zinc requirements for young children with a wide range of phytate intakes, and re-addressing the issue of upper limits for zinc as major goals. An important prerequisite is priority attention to the acquisition of more adequate experimental data, especially that for excretion of endogenous zinc.

Acknowledgements

This work was supported by HarvestPlus (8030) and in part by the International Atomic Energy Agency (IAEA 13254/R3) and the National Institutes of Health (K24 DK083772).

Abbreviations

DRI	Dietary Reference Intakes
EFZ	Endogenous Fecal Zinc
EURRECA	EURopean micronutrient RECommendations Aligned
IOM	Institute of Medicine
IZiNCG	International Zinc Consultative Group
TAZ	Total Absorption of Zinc

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