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INTERRELATIONSHIPS OF KEY VARIABLES OF HUMAN ZINC HOMEOSTASIS: Relevance to Dietary Zinc Requirements

Michael Hambidge and Nancy F Krebs
Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, Colorado 80262; e-mail: michael.hambidge@uchsc.edu, nancy.krebs@uchsc.edu

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■ **Abstract** Currently, estimates of human zinc requirements depend primarily on a factorial approach. The availability of tracer techniques employing zinc stable isotopes has facilitated the acquisition of data on major variables of zinc homeostasis in addition to those that can be measured with careful metabolic balance techniques. These data have promising potential to facilitate and improve the factorial approach. The thesis proposed in this paper is that realistic estimations of dietary zinc requirements by a factorial approach require attention to the dynamic interrelationships between major variables of zinc homeostasis. This applies especially to the positive relationship between endogenous fecal zinc and total absorbed zinc, which is the essential starting point in estimating physiologic and, from there, dietary requirements.

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INTRODUCTION

The considerable incentive for giving priority to refining our concepts and knowledge of human dietary zinc requirements is attributable to several factors. First is our increasing awareness that not only does human zinc deficiency occur in a variety of circumstances, it also appears to be a public health problem of global proportions (17, 19). Second, in contrast to earlier concepts, the optimal physiologic range for zinc intake and absorption may be limited, with even moderate excess causing previously unexpected and undesirable disruption of normal physiology (54). An obvious corollary to this concern is that it is more prudent to determine requirements as reliably as possible than to simply err on the side of excess in order to minimize the risk of deficiency. Third, zinc deficiency has been well documented in populations in North America (11, 12, 15, 53) whose zinc intake, although not matching earlier recommended daily allowances from the National Academy of Sciences (51), appears to be adequate when compared with standards published more recently (10, 69). Thus, there is an unresolved paradox. On the one hand is the well-documented occurrence of human zinc deficiency in apparently healthy subjects. On the other is evidence, or what appears to be reasonable interpretation of data, from studies of zinc balance (28) or homeostasis/metabolism (59) that zinc requirements are extraordinarily small. If true, this would appear to preclude any practical risk of zinc deficiency except in very special circumstances, for example poor bioavailability.

A first step in addressing this paradox is to achieve a more precise understanding of human zinc requirements in healthy adults whose dietary zinc is of good bioavailability. This chapter focuses on an evaluation of recent data and reevaluation of less recent data on aspects of human zinc homeostasis that are pertinent to estimating human dietary zinc requirements. These data include information utilized by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Food and Nutrition Board of the Institute of Medicine, as well as its Panel on Vitamin A, Vitamin K, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc (10). This recent report included estimated average requirements (EARs) for zinc. From these figures, calculations were also derived for corresponding figures for recommended dietary allowances for individuals and for mean requirements for populations. The purpose of this review is not to derive a specific set of figures but to take a fresh look at the principles underlying the factorial approach to the derivation of EARs for zinc. Any extent to which our examples of estimates of physiologic requirements differ from those published recently by the Food and Nutrition Board reflects differences in the databases utilized. This also serves as a timely reminder of the current need for more extensive experimental data.

OVERVIEW OF ZINC HOMEOSTASIS AND THE ROLE OF ZINC STABLE ISOTOPE TECHNIQUES

This paper is written at a time of real progress in our understanding of zinc metabolism at a subcellular and molecular level, including the identification of several zinc transporters (8) and progress in elucidation of the role(s) of metallothionein in zinc metabolism (45). These advances are leading to better understanding of the complexities of zinc metabolism and homeostasis in each organ and system, with the promise of further progress in the immediate future. At the same time, tracer kinetic studies combined with model-based compartmental analyses are advancing our broad understanding of whole-body zinc metabolism and especially of those pools of zinc that exchange rapidly with zinc in plasma (48, 64). To continue to advance our understanding of human zinc homeostasis and, thence, of human zinc dietary requirements, there is a special need for a clearer understanding of the regulation of zinc metabolism in the gastrointestinal tract. This can be achieved with a combination of molecular/cellular and human tracer/metabolic techniques.

One of the vital variables of zinc homeostasis, and the principal focus of this paper, is endogenous fecal zinc and its interrelationships with total absorbed zinc and with rapidly exchanging zinc pools (EZP). These measurements cannot be achieved simply by measuring total zinc, as is done in the balance technique; they depend on tracer methodology. In this paper, stable isotope techniques are emphasized not only because of our own experience with them, but also because, although still in an early stage of their potential application, these techniques have yielded the information pertinent to the thrust of this paper. The use of stable isotopes for exploring human zinc metabolism is not without potential pitfalls and technical challenges (16). However, it also has advantages, perhaps the most important of which is that three of the five naturally occurring stable isotopes of zinc are in low enough natural abundance to be employed as tracers in studies of human zinc metabolism. In other words, it is feasible to administer three different zinc tracers simultaneously. This means, for example, that different zinc tracers can be administered intravenously and orally on the same day. Within the past decade, as we have gained more experience in their application, zinc stable isotope techniques have been refined considerably. However, the majority of the studies we refer to are first-generation studies, which have depended on the simultaneous acquisition of precise balance data to determine one of the core variables of zinc homeostasis, namely endogenous fecal zinc. It is because of the ability tracer techniques confer to derive simultaneously determinations of zinc absorption and intestinal excretion of endogenous zinc that stable isotope data figure so prominently in this paper. Yet, we need to be sensitive to the limited amount of data, in terms of both number of studies reported and number of subjects per report. We also should be cognizant of the fact that none of the studies have been directed specifically to exploring the interrelationships of key variables of zinc homeostasis as a means of advancing knowledge of dietary zinc requirements. Moreover, in general, individual data are no longer available from several of these studies, which limits their potential to contribute to the goals of this paper.

ALTERNATIVE STRATEGIES FOR ESTIMATING AVERAGE DIETARY ZINC REQUIREMENTS

The importance of efforts to improve knowledge of dietary zinc requirements through refinement of our knowledge of pertinent core features of zinc homeostasis is highlighted by reflecting on the dearth of alternative viable strategies. In particular there is a lack of adequate epidemiological data, including laboratory, functional, and clinical biomarkers of zinc nutritional status (17, 18, 67). Current knowledge of human zinc deficiency is dependent primarily on the results of careful intervention studies, using physiologic rather than pharmacologic quantities of zinc (3, 4, 17, 19). In theory, such studies could yield useful information on dietary zinc requirements, but the extent to which this is possible has been limited by the paucity of dietary data collected. No epidemiologic data can provide information on dietary requirements unless it includes information on dietary zinc intake, including baseline dietary data in intervention studies. Currently, epidemiologic data of one type or another can provide only limited supportive data for estimates of dietary zinc requirements derived from experimental metabolic data, and this is unlikely to change in the near future. Although zinc is by no means unique among the micronutrients in this regard, these limitations of epidemiologic data assume progressively greater importance in parallel with the growing recognition of the extraordinarily exciting biology and of the public health importance of this nutritionally essential element.

FACTORIAL APPROACH

Inevitably, therefore, committees charged with developing estimates of dietary zinc requirements rely primarily on experimental data pertaining to zinc homeostasis (68, 69). Although whole-body turnover rates have been proposed as one means of assessing requirements (30), the information they yield is really limited to a long-term measure of zinc absorption, which, alone, does not define zinc requirements. To achieve reliable estimates of zinc requirements experimentally requires accurate measurements/calculations of the parameters that are required in the factorial equation.

Assuming there is no physiologic need to have positive zinc balance (as in a growing child), physiologic requirements are limited to the absorption of zinc necessary to replace total obligatory endogenous zinc excretion. Dietary zinc requirements can then be calculated by dividing this value for physiologic requirement by the fraction of dietary zinc absorbed. This is the essence of the factorial approach (31). Other strategies for progressing from physiologic requirement, i.e. the amount of required absorbed zinc, to an EAR are also available, especially with the wealth of data that can be derived from zinc stable isotope studies.

INTESTINAL EXCRETION OF ENDOGENOUS ZINC

Substantial quantities of endogenous zinc are secreted into the lumen of the small intestine postprandially (40, 46). This secretion is believed to be primarily via the pancreatic exocrine secretions and possibly the intestinal mucosa (50, 52). A percentage of this endogenous zinc is reabsorbed, which is essential to maintain homeostasis; the remainder is excreted in the feces, where it can be measured with tracer techniques (34).

The feces are the major route of excretion of endogenous zinc, typically approximately twofold that of all other routes combined, but with much variation. This variation results from the regulation, by as-yet-unidentified mechanisms, of the quantity of endogenous zinc excreted by the intestine, in sensitive and rapid response to changes in zinc absorption (26) and status (29). These changes are sustained. Habitually low zinc intakes are associated with impressive evidence of intestinal conservation of endogenous zinc (42). Endogenous fecal zinc varies by as much as an order of magnitude in response to these mechanisms to maintain zinc homeostasis.

The outstanding importance of the regulation of endogenous fecal zinc in the maintenance of zinc homeostasis is apparent when it is considered that this is the only variable of zinc homeostasis known to be regulated by changes in zinc homeostasis. Rates of excretion of endogenous zinc via other organ systems involved with the excretion of endogenous zinc appear to change only with severe dietary zinc restriction (1, 20, 28, 49). Fractional absorption of zinc varies inversely with dietary intake of bioavailable zinc, which has the effect of modulating changes in total absorbed zinc. However, this effect is insufficient to totally stabilize total absorbed zinc at an optimal level, and evidence is not conclusive for a role of zinc status in regulating zinc absorption (9).

Endogenous fecal zinc is the largest and most elusive variable in the determination of physiologic zinc requirements. Endogenous fecal zinc is at the core of the interrelationships between variables of zinc homeostasis that we need to unravel.

NON-INTESTINAL EXCRETION OF ENDOGENOUS ZINC

Although non-intestinal routes of excretion of endogenous zinc are not the primary focus of this paper, they are essential for estimation of physiologic requirements and, therefore, of dietary requirements by the factorial approach.

Kidneys

Current thinking is that the quantity of zinc excreted via the kidney in healthy humans is not affected by dietary zinc intake and absorption except in extreme situations, e.g. zinc deprivation. Therefore, assuming that zinc intake is not very low, the kidneys do not have a discernible regulatory role in the maintenance of zinc homeostasis in normal circumstances. There are, however, unexplained inter-laboratory/investigator differences in the quantities of zinc excreted in the urine, and a more thorough evaluation of this route of excretion over the range of dietary zinc intake and absorption typical for North America and elsewhere is justified before any final conclusion is reached. Meanwhile, the average 24-h urine zinc excretion for 18 studies of young adult males is 0.63 ± 0.15 mg of Zn day^{-1} (1, 2, 7, 14, 21, 25–28, 41, 44, 49, 55, 56, 59, 60, 62, 63). The corresponding figure for 11 studies of young adult females outside the reproductive cycle is 0.44 ± 0.12 mg of Zn day^{-1} (6, 13, 14, 24, 25, 42, 48, 57, 58, 61, 66).

Integument

Data are, understandably, much more limited for zinc excretion via the integument. For current purposes, we rely on two sets of data from the US Department of Agriculture, Agricultural Research Service Grand Forks Human Nutrition Research Center in North Dakota (28, 49), for young adult males whose average excretion was 0.54 mg of Zn day^{-1}. Extrapolation to adult women on a body surface basis gives a corresponding figure of 0.46 mg of Zn day^{-1}. Though cumbersome and fraught with the potential for contamination or incomplete collections, additional data are desirable.

Semen and Menses

Excretion of zinc in semen has been reported to average 0.1 mg day^{-1} (28) but can presumably vary widely depending on the frequency of ejaculation. Higher losses

TABLE 1 Nonintestinal excretion of endogenous Zn

Excretory Route	Men	Women
Urine	0.63	0.44
Integument	0.54	0.46[a]
Semen/menses	0.1	—
Total	1.3	0.9

[a]Extrapolated from male data (28) on basis of surface area. Results are in milligrams of zinc per day.

via this route have been reported (1). Losses in menses, when averaged over the entire month, are negligible, 0.005 mg day^{-1} (20). Excretion of endogenous zinc via nonintestinal routes when zinc intake is restricted is considered below.

Altogether, the nonintestinal losses of endogenous zinc are substantial and may approximate the intestinal endogenous losses when zinc intake and absorption are relatively low. Current knowledge, however, indicates that they do not have the same vital regulatory role and, indeed, may be regarded as a constant over a substantial range of zinc intake and absorption. This probably encompasses the value for absorption that is necessary to match physiologic requirements. Subject to further information, approximate values are given in Table 1.

METABOLIC BALANCE

Data for each of the nonintestinal routes of excretion of endogenous zinc are acquired as part of a comprehensive approach to determining zinc balance. The factorial method for estimating zinc requirements is obviously dependent on information derived from complete, as opposed to the more typical crude, balance studies, in which the only endogenous losses measured are those in feces and urine. Because endogenous fecal zinc is not measured with the metabolic balance technique, it is not possible to determine the physiologic requirement for zinc by this means. Despite this limitation, it is theoretically possible to estimate an average dietary requirement by plotting either crude balance or net (apparent) absorption vs dietary zinc for subjects consuming their habitual diets or who have had adequate time to adapt to a change in diet prior to the balance study. In the case of crude balance, it is necessary to adjust for endogenous losses other than feces and urine; it is also necessary to adjust balance in the case of net absorption for zinc excretion in urine. The limited application of this approach to the estimation of dietary zinc requirements, despite a wealth of literature data, appears to stem from awareness of the propensity for errors. These do not need to be of great magnitude, compared with that of the overall measurements of intake and excretion, to provide substantially different results and, thus, different interpretations.

Mean results of zinc balance studies for men and women are illustrated in Figures 1*a* and *b*, respectively. These results are for crude balance, i.e. dietary zinc − (fecal zinc + urine zinc). The calculated adjustments required for other endogenous zinc losses for men and women are indicated. For men, there was no discernible relationship between balance and diet zinc over a range of intake from less than 2 up to 20 mg day^{-1}, a circumstance that first received attention several years ago (31). For women, there is, as would be expected for both genders, a significant positive linear regression for balance vs dietary zinc, but with a wide confidence interval.

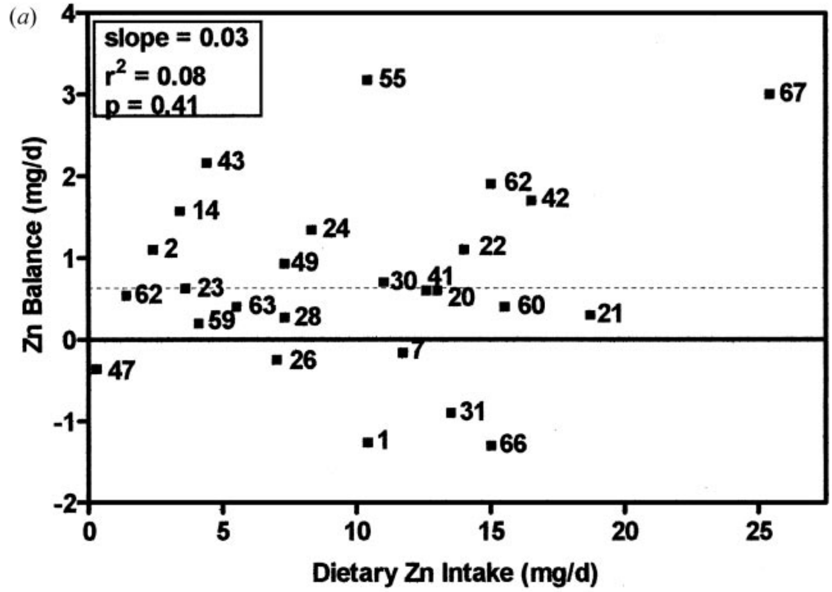
Figure 1*a* suggests that zinc balance is maintained over a wide range, including a remarkably low intake (28), of zinc. This is in contrast to a theoretical pattern of continuing variation in body zinc (5) over a low-to-high normal range of intake, the evidence for which is discussed later.

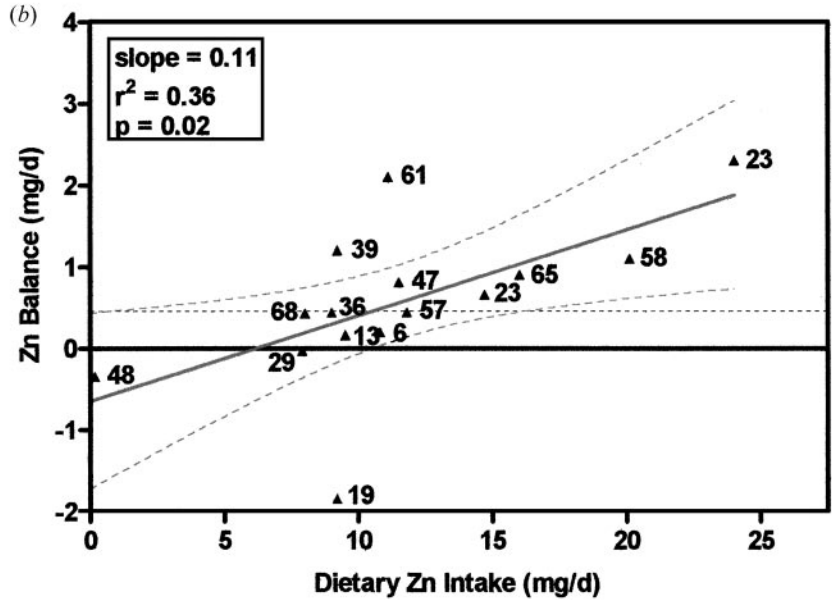
ADAPTATION

Adaptation has received considerable attention in discussion and interpretation of metabolic studies designed to explore aspects of whole-body zinc homeostasis with or without a goal of estimating dietary zinc requirements. This attention has been driven, in part, by the goal of understanding to what extent humans can adapt to dietary restriction. The ability to adapt has been evaluated primarily on the basis of zinc metabolic balance. Whether this yardstick is adequate, even if all sources of loss are considered, is questionable. One example of why we regard the restoration of balance following introduction of a zinc-restricted diet as inadequate evidence of satisfactory adaptation is the positive association between habitual dietary zinc intake (47) or absorbed zinc (42) and the total quantity of zinc in those combined pools of zinc that exchange readily (within 3 days) with zinc in plasma (EZP). "Adaptation" to a restricted intake is, therefore, not without a cost, which may be limited to diminution of short-term zinc stores as hepatic metallothionein (43), but which could also involve impairment of zinc-dependent physiology. Thus, even if, when dietary zinc is restricted, balance is eventually fully restored, this does not necessarily imply that this level of intake is optimal. At the very least, the individual may be ill-equipped to deal with those stress situations that may require more zinc.

Another reason adaptation has attracted attention is that most experimental metabolic studies of humans on low zinc intakes have depended on a period of experimental zinc depletion in closely supervised metabolic units. Dietary restriction

Figures 1*a* and 1*b* Zn balance vs dietary Zn. Means for young adult men (*a*) and women (*b*). (Solid lines) Crude zero balance (excretion in feces and urine only); (dotted lines) adjusted zero balances accounting for estimated losses via integument/semen/menses. Data from extensive, but not exhaustive, literature search (high phytate diets excluded). Numbers denote references.


(a)
slope = 0.03
r^2 = 0.08
p = 0.41
Zn Balance (mg/d)
Dietary Zn Intake (mg/d)


(b)
slope = 0.11
r^2 = 0.36
p = 0.02
Zn Balance (mg/d)
Dietary Zn Intake (mg/d)

has been of variable duration, and there has been, and continues to be, considerable discussion and uncertainty on how long it takes to fully "adapt" to the zinc-restricted diet.

An aspect of adaptation that is especially pertinent to the theme of this paper relates to the strategy that has been used to derive a value for endogenous fecal zinc for the factorial approach to estimating physiologic requirements. Specifically, the excretion of endogenous zinc on a nearly zinc-free diet has been extrapolated back to zero time to derive an estimate of obligatory intestinal excretion of endogenous zinc in the nonadapted state (1). This figure, however, presumably depends on initial zinc status prior to introduction of the severely zinc-restricted diet. The World Health Organization (WHO) (69) proposed two numbers for endogenous fecal zinc to use in the calculation of physiologic requirements: one for the fully adapted individual ("basal") and one for the nonadapted ("normative"). Although it is not entirely clear how the precise numbers were derived, they were based on the same concept of adapted and nonadapted on a zinc-free or low-zinc diet. The differences in the critical value for endogenous fecal zinc derived from this approach are in sharp contrast to those derived from the approach developed in this paper, as is illustrated below (see Figure 6).

INTERRELATIONSHIP BETWEEN ENDOGENOUS FECAL ZINC AND TOTAL ABSORBED ZINC

This section considers the evidence for a strong positive interrelationship between the quantity of exogenous dietary zinc absorbed and the quantity of endogenous zinc excreted via the intestine. Physiologically, this may not be a direct cause-effect relationship. Rather, it is assumed that total absorbed zinc affects zinc "status," which, in turn, rapidly affects endogenous fecal zinc. The positive correlations we have observed between total absorbed zinc and EZP (the size of the combined pools of zinc that exchange with zinc in plasma within 3 days) (36, 39, 42) and between EZP and endogenous fecal zinc (36, 39, 42) are consistent with this viewpoint.

The relationship between endogenous fecal zinc and total absorbed zinc, although indirect and of uncertain variability, offers new insight into strategies for improving the factorial approach to the estimation of human zinc dietary requirements and is the principal focus of this paper. Before considering the paradigm that we propose and the experimental evidence in support of this paradigm, it is useful to contrast alternative paradigms for the relationship between endogenous fecal zinc and total absorbed zinc and their implications.

The line of equality in Figure 2, for example, depicts the theoretical relationship between total endogenous losses and total absorbed zinc if zinc requirements are zero (i.e. if no endogenous losses occur) at zero intake absorption and if there is no accumulation of body zinc as total absorbed zinc increases progressively from zero. In this model, endogenous zinc excretion is matched by total absorbed zinc at

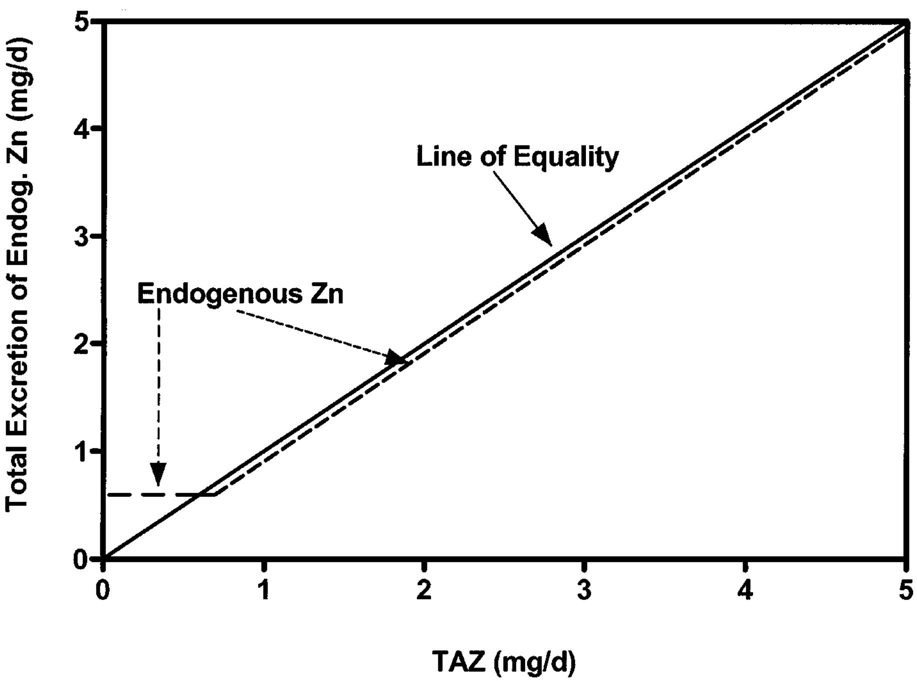


Figure 2 Theoretical models for relationship between total excretion of endogenous Zn and total absorbed Zn that do not fit experimental data. See text for description. TAZ, total absorbed zinc.

all levels of absorption. It is generally accepted, however, that there is an obligatory loss of endogenous zinc at zero absorption. Although the experimental evidence for this is still limited, it is also physiologically likely. The most useful information we have on this topic is provided by two carefully conducted and detailed balance studies on almost zinc-free diets that were undertaken by Baer & King (1) and Hess et al (20) in the 1970s and 1980s. One of these studies involved women fed a diet providing 0.15 mg of Zn day^{-1}. The other involved young men fed a diet providing 0.28 mg of Zn day^{-1}. Although tracer studies were not included, the windows for possible total absorbed zinc and endogenous fecal zinc values were extremely narrow, and it needed only minor extrapolation back to the *y*-axis (zero total absorbed zinc) for endogenous fecal zinc or for urine zinc calculations at zero intake/absorption. Depending, to a small extent, on the precise FAZ and the slope of endogenous fecal zinc vs total absorbed zinc used to extrapolate to the *y*-axis, the figure for endogenous fecal zinc at zero total absorbed zinc is approximately 0.25 mg of Zn day^{-1}. The mean urine loss was 0.14 mg of Zn day^{-1}. There are conflicting data on whether integumental losses decrease at very restricted zinc intakes (28, 49). Assuming they remain about the same as for urine (28), for obligatory losses at zero intake these would add another 0.14 mg of Zn day^{-1}, giving a total of 0.53 mg of Zn day^{-1}. Losses in semen have been reported to account for 9% of total endogenous zinc excretion at an intake of 1.4 mg of Zn day^{-1} (22), increasing the calculated total obligatory excretion of zinc at zero total absorbed zinc to 0.6 mg of Zn day^{-1}. Figures for females are a little lower. If we accept these figures, we require a different model than the line of equality, one hypothetical example of which is depicted in Figure 2.

An assumption that appears to have been made, if not explicitly stated, in estimating physiologic requirements is that endogenous losses calculated at zero intake/absorption do not change until total absorbed zinc has increased sufficiently to meet physiologic requirements. This model is also depicted in Figure 2, which also assumes that, once total absorbed zinc is sufficient to meet physiologic requirements, any further increases are paralleled by identical increases in endogenous excretion in order to maintain balance. The implications of this assumption with respect to the resulting extremely low estimate of requirements has been "softened" considerably by two factors. One has been the use of generous figures, in view of the data just discussed, for endogenous zinc excretion at zero intake. The second has been to calculate a figure for endogenous fecal zinc at zero intake for nonadapted individuals (1), as discussed earlier. This has provided the basis for the "normative" figures of the WHO (69). Before leaving Figure 2, it should be noted that there is no experimental data to support this model, however appealing theoretically.

EXPERIMENTAL DATA ON THE INTERRELATIONSHIP BETWEEN ENDOGENOUS FECAL ZINC AND TOTAL ABSORBED ZINC

The general pattern of the interrelationship between endogenous fecal zinc and total absorbed zinc has been elucidated in animal models, including research by Weigand & Kirchgessner (65). This work clearly demonstrated that both total absorbed zinc and endogenous fecal zinc increased as dietary zinc increased. Replotting the data indicates that the slope of the regression of endogenous fecal zinc and total absorbed zinc was similar to that discussed below for human studies. Moreover, endogenous fecal zinc progressively increased with increasing total absorbed zinc at total absorbed zinc levels lower than those required for maximal growth in young rats, i.e. lower than physiologic requirements. The significance of this observation is discussed below.

In our experience with zinc stable isotope studies of healthy subjects (not consuming a high phytate diet), a strongly positive correlation between endogenous fecal zinc and total absorbed zinc has been consistent both in infants (33, 35–38) and in adults (42). This is illustrated in Figure 3, which depicts data from young nulliparous Chinese women consuming their habitual diet (42). One of the

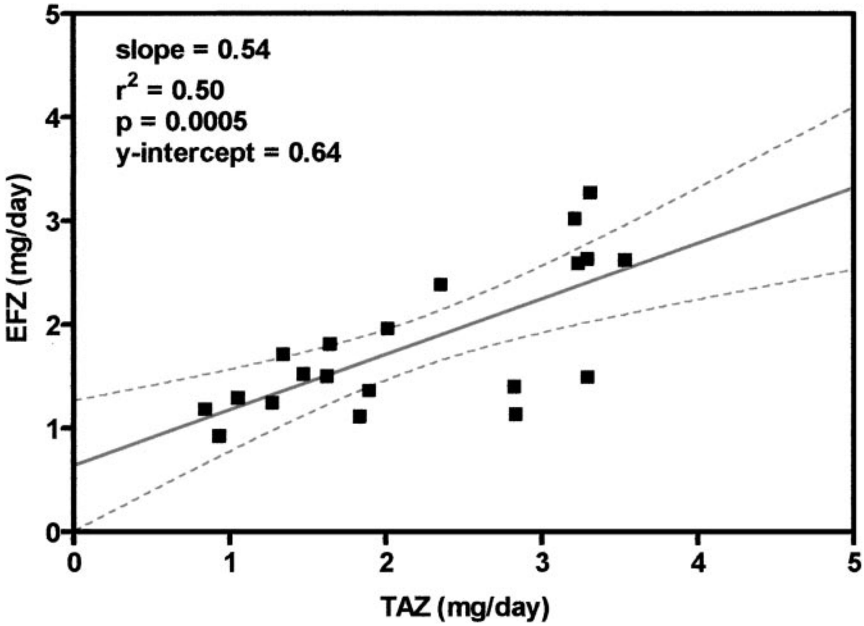


Figure 3 Endogenous fecal Zn (EFZ) vs total absorbed Zn (TAZ) for young Chinese women (42). Linear regression with 95% confidence intervals shown.

valuable features of this plot is that it includes numerous unusually low values for total absorbed zinc, the result of the habitually low-zinc diet of the rural Chinese women included in this study. The importance of these low total absorbed zinc values and several features of this plot are discussed further below.

Although in the 1980s, a positive correlation between endogenous fecal zinc and total absorbed zinc was proposed for humans, this was not backed by experimental data and, moreover, was only thought likely at levels of total absorbed zinc above those required to maintain balance (31). However, a number of zinc stable isotope studies in the 1980s and 1990s (Table 2) have provided a new perspective. These have included the following: measurements of FAZ; information on dietary zinc, allowing calculation of total absorbed zinc; and either measurement of (26) or information that allowed calculation of endogenous fecal zinc in the same week as for total absorbed zinc (22–25, 32, 41, 48, 59–63). In no instance was the relationship between endogenous fecal zinc and total absorbed zinc a specified and major objective of the study. In most instances individual data are not available. However, plotting of the means for these studies has proved to be an interesting and informative exercise. The duration ranged from 1 week to 6 months (Table 2), with one study being undertaken while subjects were on their habitual diet. Although the briefer studies left some question about the completeness of adaptation to the

TABLE 2 Summary of zinc stable isotopes studies included in Figure 4

Reference	Year	Gender	Diet Zn (mg/day)	Duration	Type of Diet[a]	No.
26	1984	M	7.1	1 week	Food	1
62	1984	M	15.0	7 weeks	LF/Zn++	4
63	1985	M	16.5	3 weeks	Food	6
63	1985	M	5.5	7 weeks	Food	6
60	1986	M	15.5	2 months	SP/Zn++	6
59	1991	M	5.7	1 week	SP/Zn++	5
59	1991	M	0.9	5 weeks	SP	5
25	1992	M	14.0	2 weeks	Food	14
41	1993	M	12.6	4 weeks	Food	8
41	1993	M	4.1	6 months	SP	8
61	1991	F	11.8	9 weeks	SP	4
61	1991	F	9.0	9 weeks	SP	4
25	1992	F	7.8	9 weeks	Food	14
24	1998	F	11.1	8 weeks	Food	21
24	1998	F	9.2	8 weeks	Food	21
32	1996	M/F	10.7	3 weeks	Food	8
48	2000	M/F	9.2	Habitual	Food	4
60	1986	Elderly M	15.5	12 weeks	SP	6
23	1995	Elderly F	13.0	7 weeks	Food	14
23	1995	Elderly F	6.7	7 weeks	Food	14

[a]LF, liquid formula; SP, semipurified; Zn++, Zn supplement added.

experimental diet, for the shorter intervals in Table 2, the diet in these studies did not differ much in zinc content from the subjects' habitual diet. Diet zinc ranged from 0.9 to 16.5 mg day^{-1}, i.e. very low to high "normal" and the bioavailability of zinc from all diets is judged to be average to high.

The plot of mean endogenous fecal zincs vs mean total absorbed zincs for these studies is given in Figure 4. The similarities between this plot and that for individual Chinese women is noted. The data for young males, which comprise the single largest subgroup of studies, are identical to those utilized by the Food and Nutrition Board, Institute of Medicine (10). The linear regression for this group had a slope of 0.60, $r^2 = 0.73$, y-intercept $= 0.27$, and $P = 0.002$. The salient features of these plots of endogenous fecal zinc vs total absorbed zinc are discussed below.

Together, these data provide substantial support for a positive linear relationship between endogenous fecal zinc and total absorbed zinc. This raises a number of questions and also invites a new and quantitatively important concept in the

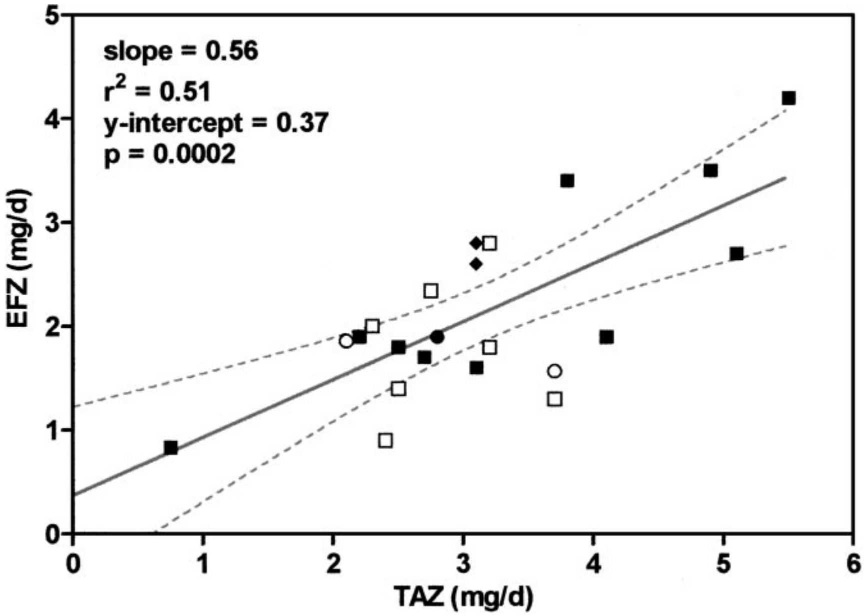


Figure 4 Linear regression of mean endogenous fecal Zn (EFZ) vs mean total absorbed Zn (TAZ) for adults. Regression includes means for young males (solid squares), young females (open squares), elderly males (solid circles), elderly females (open circles), and young mixed gender (solid diamonds).

calculation of physiologic requirements for zinc. These issues are addressed in the following sections.

SALIENT FEATURES OF THE REGRESSION EQUATIONS FOR ENDOGENOUS FECAL ZINC VS TOTAL ABSORBED ZINC

Several features of the experimental plots of endogenous fecal zinc vs total absorbed zinc, including those illustrated in Figures 3 and 4, are relevant to the theme of this paper.

Linearity

These plots are linear, with no suggestion that any other regression would provide a better fit and no indication of an inflexion point over the range of total absorbed zinc included in the experimental data.

The Same Linear Relationship Between Endogenous Fecal Zinc and Total Absorbed Zinc is Maintained at Low Levels of Total Absorbed Zinc

The study with an animal model (65) demonstrated that endogenous fecal zinc increased with increases in zinc intake and in total absorbed zinc at levels of intake that were lower than those required to maintain maximal growth. In other words, endogenous fecal zinc increased progressively with increases in total absorbed zinc at levels of total absorbed zinc that were less than those required for matching physiologic requirements. These data are not supportive of the model depicted in Figure 2 but are supportive of the strategy proposed in this paper for estimating physiologic requirements.

The human data are a little more difficult to interpret because there is no general agreement for figures for physiologic requirements. Our calculations of these are discussed below, but we will assume an extremely conservative theoretical figure of 2.0 mg of Zn day^{-1} for young adult women. Figures 3 and 4 between them include a substantial number of points below this number that appear to fit well with the overall linear regressions. These observations are consistent with those in the study of the animal model discussed above (65). Together they provide support for the hypothesis that endogenous fecal zinc increases with increasing total absorbed zinc at levels of total absorbed zinc that are lower than those needed to match physiologic requirements. Considered from another viewpoint, these observations indicate that not only is there an obligatory excretion of zinc by the intestine on zinc-free diets, there is also an obligatory increase in endogenous fecal zinc in tandem with total absorbed zinc, even at levels of total absorbed zinc that are less than those needed to match physiologic requirements. Once again, they are not consistent with the model in Figure 2.

Y-Axis Intercept

Without putting weight on an observation obtained by extrapolating linear regressions of endogenous fecal zinc vs total absorbed zinc beyond measured data points, it is not without interest that the *y*-intercepts derived from these extrapolations were in good agreement with the calculations of endogenous fecal zinc at zero absorption already discussed. These intercepts are, therefore, supportive of the legitimacy of these calculations and are also consistent with the hypothesis that the linear regression between endogenous fecal zinc and total absorbed zinc holds even to the very lowest levels of absorption.

Slope of Linear Regression of Endogenous Fecal Zinc on Total Absorbed Zinc

A feature of the linear regression of endogenous fecal zinc on total absorbed zinc in our infant studies and in Figures 3 and 4 has been the consistency of the slope between 0.5 and 0.6. Considering that the relationship between these two key variables is presumably indirect rather than direct, this consistency is perhaps unexpected and requires further careful research. Further research is also a priority because of the implications of this slope, which contrasts notably with the model depicted in Figure 2 for values of total absorbed zinc both below and above those that correspond to physiologic requirements and, therefore, which contrasts with current published concepts of zinc homeostasis. These implications are discussed in the next section.

IMPLICATIONS OF EXPERIMENTAL DATA ON THE RELATIONSHIP BETWEEN ENDOGENOUS FECAL ZINC AND TOTAL ABSORBED ZINC

Current data on urine zinc excretion (which merit further research) reveal no evidence to suggest that the kidneys have any role in maintaining zinc homeostasis except at extremely high and low intakes. The same applies to the integument and testes, and indeed, it is difficult to envisage that these organs, zinc excretion via which is subject to so much individual and environmental variation, have a serious regulatory role rather than an incidental impact on zinc homeostasis. Given these circumstances, a slope for the regression of endogenous fecal zinc on total absorbed zinc of <1 implies a positive zinc balance when the total absorbed zinc exceeds the physiologic requirement. It also implies a negative balance when total absorbed zinc is insufficient to match physiologic requirements. In other words, these data suggest that there is a very narrow range at which balance is truly achieved, rather than a plateau over a wide range of intake and absorption.

This concept of an optimal point is illustrated in Figure 5, which is based on the endogenous fecal zinc and total absorbed zinc data depicted in Figure 4, together with a calculated constant (Table 1) for nonintestinal endogenous losses for women.

The line of equality between endogenous losses and total absorbed zinc in Figure 5 is identical to the line depicted in Figure 2. Except at low levels of zinc intake, which are associated with diminution of nonintestinal excretion of endogenous zinc (indicated by interrupted line in Figure 5), these nonintestinal losses are added as a constant, giving a line parallel to the linear regression for endogenous fecal zinc on total absorbed zinc, and 0.9 mg of Zn day^{-1} (Table 1) above this regression. This plot indicates that the physiologic requirement is one specific number that is the value for total absorbed zinc at which the line of equality intersects the line depicting total endogenous zinc losses. This particular set of data indicates a calculated physiologic requirement for young women of 2.8 mg of Zn day^{-1}. This figure is obviously considerably higher than that which would be derived from the model depicted in Figure 2, unless the value for endogenous zinc losses at zero absorption were to be set arbitrarily high.

The mixed-gender endogenous fecal zinc vs total absorbed zinc regression was used in Figure 5 because of the lack of adequate female data apart from the data

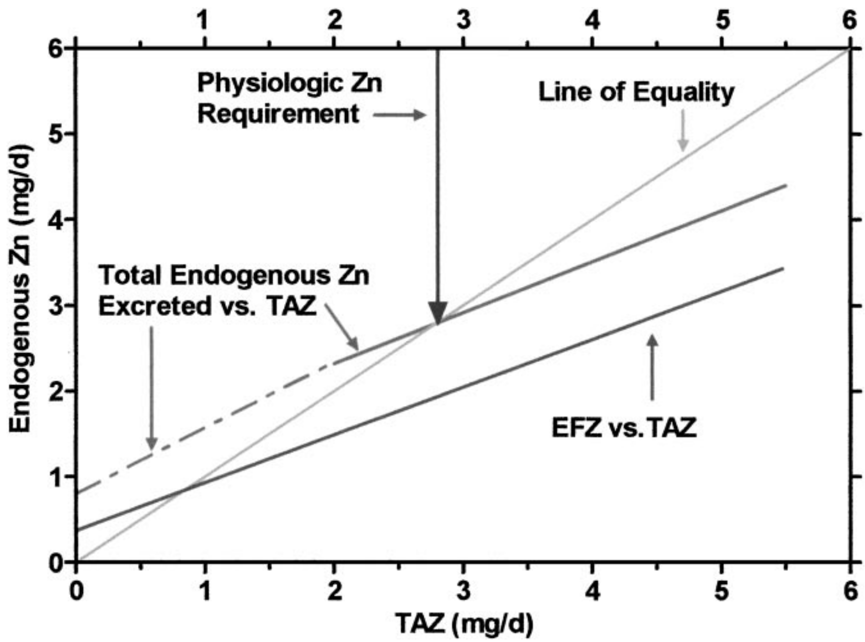


Figure 5 Estimation of physiologic requirement for Zn for young women: Calculated nonintestinal excretion of endogenous Zn for women added to linear regression for endogenous fecal zinc (EFZ) vs total absorbed zinc (TAZ) (Figure 3) to give calculated total endogenous Zn excretion. Intercept of that line with the line of equality is the estimated physiologic requirement (2.8 mg of Zn day^{-1}).

from the China study (42). Substituting the latter regression gives an estimated physiologic requirement of 3.4 mg of Zn day^{-1}. These differences are likely to reflect the limitations of available data rather than being related to gender. For the experimental data available at this time, total absorbed zinc explains only about 50% of variance in endogenous fecal zinc. It will require high-quality prospective studies to determine how much higher this figure might be if experimental limitations are minimized.

ALTERNATIVE SCENARIOS THAT WOULD RESULT IN MUCH LOWER ESTIMATES OF PHYSIOLOGIC REQUIREMENTS

Two alternative scenarios merit consideration. The first is related to the reduced urine zinc excretion (1, 20, 28), the reduced zinc per ejaculation (22), and the probable reduction in integumental zinc (28) at very low levels of zinc intake (i.e. <3–4 mg day^{-1} with further reduction at <0.3 mg day^{-1}). Given this lower excretion of zinc via nonintestinal routes, does the line of equality intersect the line for total endogenous zinc losses at a level of total absorbed zinc much lower than that suggested in Figure 5, even if the positive linear regression of endogenous fecal zinc on total absorbed zinc does extend to the *y*-axis? After all, there is evidence that crude balance is not far from being achieved with a zinc intake as low as 0.28 mg day^{-1} (1). Is it not, therefore, reasonable to anticipate that just a little more zinc intake would be sufficient to achieve balance? Currently, there are insufficient experimental data to totally exclude this possibility. However, given the data that are available, and making some estimates in converting data related to dietary intake into data related to absorption, it appears that this does not occur. Approximate calculated total endogenous zinc losses at low intakes of zinc are depicted by the interrupted line in Figure 5.

The second scenario that could give lower figures for calculated physiologic requirements is to utilize the model depicted in Figure 2. This, in fact, has been the "standard" approach to factorial calculations, even if not necessarily explicitly stated in these terms. A standard figure has been used for endogenous fecal zinc and, therefore, for total endogenous excretion of zinc (30, 31, 69). As illustrated in Figure 6, if the dynamic interrelationship between endogenous fecal zinc and total absorbed zinc is not considered, the calculated obligatory loss of endogenous zinc via the intestine is much lower. A conceptual difference in the approach described in this paper is that there is no attempt to determine a number for obligatory endogenous fecal zinc, which is then used as part of a sum to estimate physiologic requirements. Rather, this figure for endogenous fecal zinc is only apparent retrospectively from the plot (Figures 5 and 6). Instead of being a static number, endogenous fecal zinc will be found to vary substantially with physiologic requirements and, therefore, with several factors, including gender, that affect physiologic requirement.

ESTIMATED AVERAGE REQUIREMENTS

With the information already in hand from the same zinc stable isotope/metabolic studies, it is straightforward to progress from the calculated physiologic requirement to derive the estimated average requirement (EAR). The simplest and most direct route is to plot total absorbed zinc vs dietary zinc intake using data from the same study. From this plot, the dietary zinc that corresponds to the total absorbed zinc that matches the physiologic requirement is the EAR. Alternatively, fractional absorption can be plotted vs total absorbed zinc, and the "critical" figure for fractional absorption that corresponds to the total absorbed zinc matching physiologic requirement can be determined. The physiologic requirement divided by this figure for fractional absorption gives the EAR.

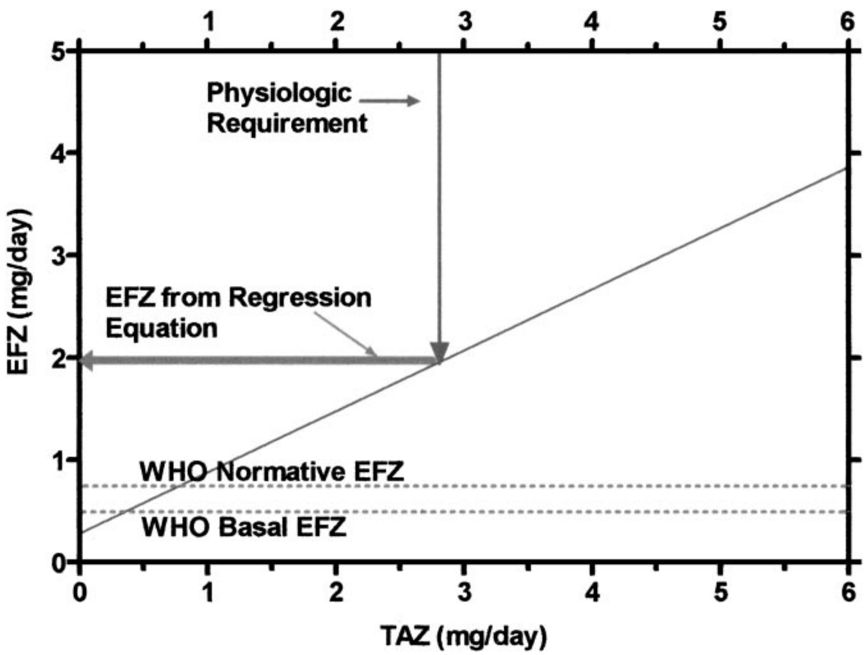


Figure 6 Comparison of endogenous fecal zinc (EFZ) at level of physiologic requirement determined by method described in paper with basal and normative EFZ figures for the World Health Organization (WHO) (69).

CONCLUDING REMARKS

Well-designed zinc stable isotope tracer/metabolic studies can provide a wealth of information on zinc homeostasis, which is invaluable in refining estimates of physiologic and dietary requirements. To derive optimal value from such studies, data analyses should include a special focus on the interrelationships between major variables of zinc homeostasis. These include, especially, the interrelationship between endogenous fecal zinc and total absorbed zinc. Maximal use should be made of individual data, rather than limiting data analyses to comparisons of means, and more research is needed that includes individuals on a range of zinc intake and absorption, including the challenging task of achieving this at low levels of diet zinc. Prospective studies are essential to confirm the validity of utilizing the interrelationship between endogenous fecal zinc and total absorbed zinc in factorial calculations of dietary zinc requirements and to explore if and how we might improve further on the approach proposed in this paper. Meanwhile, we conclude that although the numbers are not yet definite, they represent a distinct advance conceptually in tackling the enigma of intestinal excretion of endogenous zinc in factorial calculations of dietary zinc requirements.

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