A dynamic model for predicting growth in zinc-deficient stunted infants given supplemental zinc

Meryl E Wastney,[1] *Christine M McDonald,*[2] *and Janet C King*[2]

[1]Metabolic Modeling Services, Blenheim New Zealand and [2]Children's Hospital Oakland Research Institute, Oakland, CA

ABSTRACT

Background: Zinc deficiency limits infant growth and increases susceptibility to infections, which further compromises growth. Zinc supplementation improves the growth of zinc-deficient stunted infants, but the amount, frequency, and duration of zinc supplementation required to restore growth in an individual child is unknown. A dynamic model of zinc metabolism that predicts changes in weight and length of zinc-deficient, stunted infants with dietary zinc would be useful to define effective zinc supplementation regimens.

Objective: The aims of this study were to develop a dynamic model for zinc metabolism in stunted, zinc-deficient infants and to use that model to predict the growth response when those infants are given zinc supplements.

Design: A model of zinc metabolism was developed using data on zinc kinetics, tissue zinc, and growth requirements for healthy 9-mo-old infants. The kinetic model was converted to a dynamic model by replacing the rate constants for zinc absorption and excretion with functions for these processes that change with zinc intake. Predictions of the dynamic model, parameterized for zinc-deficient, stunted infants, were compared with the results of 5 published zinc intervention trials. The model was then used to predict the results for zinc supplementation regimes that varied in the amount, frequency, and duration of zinc dosing.

Results: Model predictions agreed with published changes in plasma zinc after zinc supplementation. Predictions of weight and length agreed with 2 studies, but overpredicted values from a third study in which other nutrient deficiencies may have been growth limiting; the model predicted that zinc absorption was impaired in that study.

Conclusions: The model suggests that frequent, smaller doses (5–10 mg Zn/d) are more effective for increasing growth in stunted, zinc-deficient 9-mo-old infants than are larger, less-frequent doses. The dose amount affects the duration of dosing necessary to restore and maintain plasma zinc concentration and growth. *Am J Clin Nutr* 2018;107:808–816.

Keywords: growth, infants, model, simulation, zinc

INTRODUCTION

Zinc deficiency, which affects ∼25% of the global population, is one of the most prevalent nutritional problems (1). It is a primary cause of poor growth and development in children (2). Zinc deficiency can arise from insufficient zinc intake, poor zinc absorption, or excessive zinc losses (3). A meta-analysis of 55 studies reported that zinc supplementation significantly increased weight and linear growth, reduced the incidence of diarrhea by 20%, lowered respiratory infections by ∼15%, and decreased mortality in infants >1 y of age by 18% (4). The WHO recommends the use of multiple micronutrient powders containing zinc for home fortification of complementary foods consumed by infants 6–23 mo old in nutritionally vulnerable populations (5, 6).

Zinc supplementation programs recommend criteria for identifying target populations and effective strategies for zinc delivery (7). A WHO/UNICEF/International Atomic Energy Agency/International Zinc Nutrition Consultative Group interagency group recommended that populations at risk of zinc deficiency can be determined by the prevalence of low zinc intakes, low serum zinc concentrations, or a low height-for-age (i.e., stunting) (8). Serum zinc concentrations alone should not be used to diagnose zinc deficiency because serum zinc concentrations do not decline with marginal intakes, between 4 and 6 mg/d, and a decline in serum zinc concentrations may reflect inflammation or infection rather than low zinc intakes (9). Length-for-age is considered the best functional growth outcome for estimating zinc deficiency because it is the primary response to increased zinc intake (10).

Currently, there is no evidence-based method to determine the amount, frequency, and duration of zinc supplementation required to improve the growth of stunted infants who are considered to be zinc deficient. We hypothesized that mathematical models of zinc metabolism would be an effective strategy

Supported by the Bill & Melinda Gates Foundation.

Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to MEW (e-mail: wastneym@metabolic-modeling-services.com).

Abbreviations used: LAZ, length-for-age *z* score; RBC, red blood cell; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.

Received November 20, 2017. Accepted for publication January 17, 2018.

First published online May 2, 2018; doi: https://doi.org/10.1093/ajcn/nqy020.

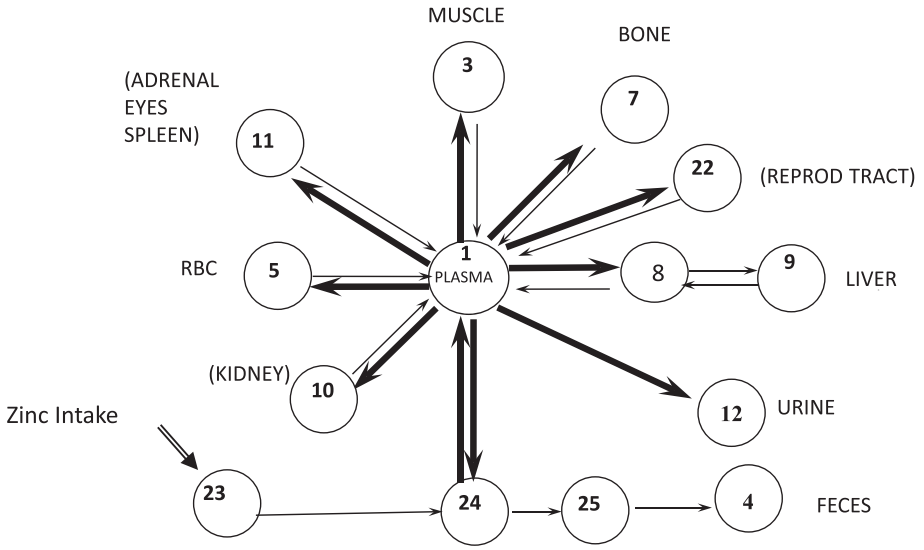

FIGURE 1 A compartmental model for zinc kinetics in infants. Circles represent pools (the numbers in the circles are arbitrary); the arrows represent the movement of zinc between pools. Compartments 23, 24, and 25 are located in the gastrointestinal tract. Labels by the pools were determined from adult studies with zinc radiotracer that allowed external counting over the liver and thigh (muscle and bone) areas. Labels in parentheses were identified from animal studies (26). The bold arrows indicate that these were functions that changed over time in response to zinc intake. The open arrow represents site of entry of zinc in diet and by supplement. RBC, red blood cell; Reprod, reproductive.

for identifying protocols to improve growth in zinc-deficient infants. Mathematical models are used routinely to develop dosing regimens for drugs, but they have also been used to determine intakes to relieve a nutrient deficiency, namely vitamin A (11). Metabolism is a highly regulated, complex system, and perturbing one aspect causes a cascade of homeostatic responses. Those numerous responses make it difficult to predict the effect of changing zinc intakes on the homeostatic responses, but modeling and simulation procedures can be used to predict the outcomes (12). Mathematical models of zinc metabolism have been developed for neonates (13, 14), children (15), and adults (16–18). In addition, some aspects of zinc metabolism (i.e., absorption and endogenous excretion) have been measured in infants fed different amounts of zinc (19). However, a dynamic model of zinc metabolism that would provide a basis for protocols to replenish zinc pools in zinc-deficient infants does not exist. The aim of this study was to develop a dynamic model for zinc metabolism in infants and to use that model to predict the growth response when stunted, zinc-deficient infants are given zinc supplements.

METHODS

Modeling was performed by using the WinSAAM software (NIH) (20, 21). Initially, a kinetic, or steady state, model was created for healthy 9-mo-old infants (described below). This model was expanded into a dynamic model by adding functions that describe the changes in zinc absorption and excretion with changes in zinc intake, and that represent growth. Parameter values of the model were then set to represent stunted, zinc-deficient infants by using the WHO definition of stunting as length-for-age z score (LAZ) <-2 SDs of the WHO Child Growth Standards median (22) and of zinc deficiency as plasma zinc <65 μg/dL (23). Then, various amounts of zinc supplementation were simulated by using the "QO feature" of WinSAAM (21). This feature sets the value of a compartment to specified values at specified times and is useful for simulating drug dosing regimens (24). In this study, the QO feature enabled variations of the amount, timing, and duration of zinc supplementation to be tested for improving growth or plasma zinc concentrations.

The dynamic model predictions of plasma zinc, weight, length, weight-for-age z score (WAZ), LAZ, and weight-for-length z score (WLZ), calculated by using equations posted on the WHO website (www.who.int/childgrowth/en), were compared with literature results of infants receiving supplemental zinc (i.e., the model prediction was compared with the mean result of each study). We note that the model was not fitted to the observed data, because the published data were from heterogeneous populations.

RESULTS

Development of a steady state model of zinc metabolism for infants aged 9 mo

Published kinetic models of zinc metabolism in adults (25) and neonates (13, 14) (**Figure 1**), together with literature data on tissue zinc concentration and tissue weights for infants (**Table 1**), were used to create a kinetic model (with time-invariant parameters) for zinc metabolism in 9-mo-old healthy infants (Figure 1). Inputs for the kinetic model were infant sex, age, weight, length, and plasma zinc concentration (**Table 2**).

We initially tested the model with the adult transfer coefficient values (25). A transfer coefficient, $L(i,j)$ is the fraction moving into compartment i from compartment j per day. (The value of a transfer coefficient can be >1 if the source pool turns over >1 time/d.) The adult kinetic parameter values fit the infant zinc tissue masses, except that to fit the red blood cell (RBC) zinc mass, it was necessary to reduce the zinc uptake into RBCs from plasma [$L(5,1)$; Figure 1] to 1/d in infants compared with

TABLE 1
Tissue zinc in male infants aged 9 mo[1]

	Tissue[2]		Zinc	
	Wet weight, g	Body weight, %	Wet weight,[3] μg/g	mg/organ
Bone	636	7.1	43	27
Brain	178	2.0	13.94[4]	2.5
Heart	39	0.4	30.8[4]	1.2
Kidney	39	0.4	19	0.7
Liver	229	2.6	34	7.8
Lung	127	1.4	12	1.5
Muscle[5]	2225	25.0	108	240
Plasma	369	4.1	1.1	0.4
RBCs[6]	280	3.1	2	0.6
Other	4778	53.7	—	—
Whole body	8900	100	31.7	282

[1]WHO median body weight (27).
[2]Tissue weights are percentages of body weight for adults (28).
[3]Tissue zinc (microgram per gram of dry weight) for bone, kidney, liver, and lung were from Erickson et al. (29) and tissue percentage wet weight was from Casey and Robinson (30).
[4]For brain and heart, value is the assumed adult concentration based on White et al. (31).
[5]Muscle mass and zinc concentrations were from Cheek et al. (32) for a 72-cm infant; White et al. (31) reported muscle at 25% of body weight at birth, and they assumed adult composition after 6 mo.
[6]RBC, red blood cell.

TABLE 2
Baseline characteristics, zinc metabolism, and growth assumptions for 9-mo-old infants[1]

	IOM[2]	Model[3]
Weight, kg	8.9	8.9 (7.9)
Length, cm	72	72 (67.5)
Plasma zinc, μg/dL	80–100[4]	90 (60)
Zinc absorption, fraction	0.3[5]	0.3
Zinc, mg/d		
Intake	2.5[6]	2.2[7]
Intestinal losses	0.45	0.38[7]
Urinary losses	0.13	0.10[7]
Weight gain, g/d	6–13	7.1 (6.6)[8]
Linear growth, cm/kg	—	5 (4)[9]
Zinc required for weight gain, μg/g	20	30[10]
Fraction of absorbed zinc used for growth	0.3[11]	0.3

[1]EAR, Estimated Average Requirement; IOM, Institute of Medicine; LAZ, length-for-age *z* score; WLZ, weight-for-length *z* score.
[2]Values used are IOM values for normal boys (33).
[3]Values assumed for the model in Figure 1 for infants who are normal or, in parentheses, zinc-deficient and stunted (i.e., –2 SDs of LAZ, but normal WLZ and plasma zinc <65 μg/dL).
[4]See Hess et al. (23).
[5]The value used by the IOM (33) for complementary foods.
[6]EAR used by the IOM for infants aged 7 mo to 3 y (33).
[7]Calculated by the model shown in Figure 1.
[8]Weight gain over 12 mo was 2.6 kg for normal WLZ infants but 2.45 kg for stunted infants, based on WHO values (34).
[9]Change in length was calculated by assuming a 5-cm/kg increase in weight for normal infants (34); on the basis of the WHO growth charts for stunted children, a lower value (4 cm/kg) was used for calculating change in length in stunted infants. Specifically, for infants <−2 SDs of LAZ but with appropriate weight [i.e., SD0 WLZ (i.e., stunted but not wasted) (34)], we used the change in length over change in weight between 6 and 9 mo (i.e., in the period before age of 9 mo).
[10]Weight increased at a rate of 1 g weight/30-μg increase in absorbed zinc, which was calculated from the average zinc concentration per gram of body weight (Table 1) and used for infants by Krebs and Hambidge (35). [We note that the IOM (33) used 20 μg Zn/g as the average concentration in infant tissues.]
[11]With zinc supplementation, it was assumed that 30% of the increase in absorbed zinc was used for infant growth (calculated from reference 34).

2.4/d in adults. In addition, the infant model had only one RBC zinc pool, whereas there are 2 RBC pools in adults (25). To fit the muscle zinc mass in infants, uptake by muscle from plasma [$L(3,1)$; Figure 1] was set at 2.375/d, which is higher than in adults (1.51/d) but lower than in neonates (26/d) (13, 14). The incorporation of zinc into all tissues by growth, represented in the kinetic model by a single loss pathway out of plasma was lower (0.6/d) in 9-mo-old infants than in neonates (20/d) (13, 14). (This pathway was divided between tissue pools in the dynamic model, described below.)

The value calculated for zinc intake by the model to support growth and overall zinc metabolism was 88% of the Institute of Medicine's Estimated Average Requirement for infants aged 7–12 mo (36) (Table 2). Values calculated for gastrointestinal and urinary losses were 84% and 76%, respectively, of those used by the Institute of Medicine (36) (Table 2).

Conversion to a dynamic model by the addition of functions for zinc absorption, endogenous excretion, and growth

To enable changes in zinc pools to be modeled over time in response to changes in zinc intake, a tracee model was set up with the use of the same parameter values as the kinetic model described above, but with initial conditions equated to the pool masses calculated by the baseline kinetic model.

Absorption was modeled by using a saturable function for absorbed compared with ingested zinc (19):

$$\text{Absorbed Zn (mg/d)} = (A_{max} \times ZI/\,(IA_{50} + ZI) \qquad (1)$$

where A_{max} is maximal absorption of zinc (1.9 mg/d for infants), ZI is zinc intake (milligrams per day), and IA_{50} is the quantity of zinc (milligrams per day) required for half-maximal absorption (2.8 mg/d in infants aged <6 mo and 8 mg/d in adults) (19). With the use of Equation *1*, we determined with 30% absorption at 2.2 mg of intake (described above) an IA_{50} of 3.5 mg/d for 9-mo-old infants.

Endogenous excretion was modeled by fitting a linear equation to endogenous fecal zinc data and absorbed zinc for term infants (19):

$$\text{Endogenous excretion (mg/d)} = 0.98156 + 0.30479 \times \text{absorbed Zn (mg/d)} \qquad (2)$$

Urinary zinc excretion was set to increase as a function of zinc absorbed with the use of data in Alexander et al. (37);

$$\text{Urine Zn excreted (mg/d)} = 0.1463 \times \text{Zn absorbed (mg/d)} \qquad (3)$$

Two other pathways (RBC exchange and release from muscle) have been identified as sites of zinc regulation in adults (25). However, they were not included in the infant model due to lack

TABLE 3
Baseline characteristics of published studies of zinc supplementation[1]

Infant age,[2] mo	Zinc, mg	Frequency, d/wk	Duration, d	Plasma zinc, μg/dL	Weight, kg	Length, cm	WAZ	LAZ	WLZ	Growth g/d	Growth cm/kg	Ref
13 (6–23)	0, 5	7	21	63	8.2	72.3	−1.4	−1.5	−0.8	NA	NA	(38)
20 (12–30)	0, 3, 7, 10	7	180	69.2	9.7	77.6	−1.3	−2.3	−0.2	5.6	4.9	(39)
9 (6–12)	0, 10	6	180	94.8[3]	7.4	69.9	−1.5	−0.6	−1.3	5.7	4.9	(40)
9 (6–12)[4]	0, 10	6	180	71.9[3]	6.4	64.4	−2.7	−2.9	−0.7	5.3	3.1	(40)
13 (6–24)	0, 5, (17)	5 (1)	90[5]	84.5	8.3	71.9	−1.6	−1.7	−0.7	6.7	4.3	(41)
18 (4–36)	0, 10	7	150	NA	7.9	71.5	−2.6	−2.9	−1.1	4.0	6.0	(42)

[1]LAZ, length-for-age *z* score; NA, not available; Ref, reference; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.
[2]Values in parentheses are ranges.
[3]Measured at end of study only.
[4]Stunted infants.
[5]Weight and length measured 90 d after the end of supplementation, at 180 d.

of data. In addition, it is unlikely that RBC exchange would affect whole-body zinc metabolism and growth in infants.

Functions were added to the model to account for the increases in the size of zinc pools occurring with growth. Growth was represented by increasing the fractional zinc uptake by tissues from plasma, in proportion to the zinc mass of each tissue pool. A function was included that limited growth during supplementation, such that once a stunted infant attained the length of a normal infant, growth then continued at the rate of a healthy infant. WAZ, WLZ, and LAZ were calculated with the use of published methods (27, 34).

Evaluation of model: simulation of published studies

To test the model, 5 published studies of zinc supplementation in infants aged ∼9 mo old (38–42) were simulated (**Table 3**). Baseline weight, length, and plasma zinc concentration (if measured) were entered as starting values. The amount of zinc administered, the frequency of dosing, and duration of supplementation varied between studies. The actual dosing regimen (e.g., if it was given 5 d/wk) was simulated. The model calculated changes in plasma zinc concentration, weight and length, WAZ, LAZ, and growth rates over time. WLZ was calculated at baseline and at the end of supplementation. The model predictions were compared with the observed values and are reported as differences from baseline. The comparison for plasma zinc is given for 2 studies in **Figure 2**. In a study by Wessells et al. (38), infants aged 6–23 mo in Burkina Faso were supplemented for 3 wk with 5 mg Zn/d. Initial plasma zinc concentrations indicated that the infants were zinc deficient (Table 3). Plasma zinc increases with zinc supplementation were within 22% of observed values (Figure 2). In a study by Weuhler et al. (39), for Ecuadorian infants who were older (≤30 mo of age) and supplemented for 6 mo with 1 of 4 amounts of zinc daily (Table 3), the model-predicted net increases in plasma zinc compared with placebo with each dose were within 16% of the observed values (Figure 2).

Umeta et al. (40) supplemented Ethiopian infants aged 9 mo of age who were either stunted (LAZ <−2) or not stunted with 0 or 10 mg Zn/d for 6 d/wk for 6 mo. Plasma zinc concentrations, measured only at the end of the study, were normal for both groups (Table 3). The observed smaller increases in weight and length in the stunted compared with the nonstunted infants at the end of dosing were also predicted by the model (**Table 4**). The model-predicted increases in weight and length for the normal and stunted infants in the placebo group were within 30% of measured values (Table 4), but weight and length changes were overpredicted in the zinc-supplemented groups (e.g., the model predicted an increase in length of 11 cm in the stunted infants compared with the observed increase of 7 cm). One explanation could be that absorption was lower in the stunted infants. To test this, we reduced absorption by 50% and found that the predicted increases in weight and length in the stunted, supplemented group then matched the observed values (Table 4).

A study observed Vietnamese infants ranging in age from 6 to 24 mo who were not stunted and were supplemented for 3 mo with either 17 mg Zn 1 time/wk or 5 mg Zn/d for 5 d/wk (41). The model predicted the observed increases in plasma zinc concentration after supplementation (Table 4). In addition, the small changes in weight and length predicted by the model were similar to the reported values measured 3 mo after the end of supplementation (Table 4). In another study in stunted infants given 10 mg Zn/d for 5 mo (42), the supplement significantly increased weight and length, as was predicted by the model (Table 4).

Model predictions: simulations of various zinc supplementation regimens

The model was used to simulate changes in weight, length, and plasma zinc concentrations in stunted, zinc-deficient infants,

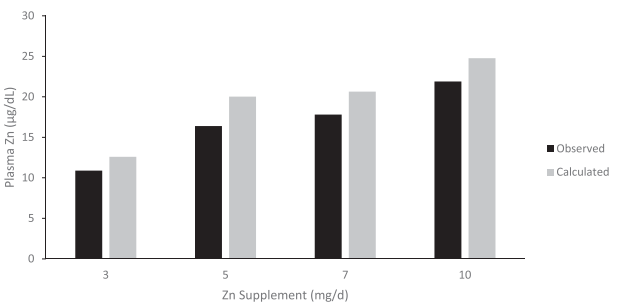

FIGURE 2 Increases in plasma zinc concentration with zinc supplementation at 5 mg/d (38) and at 3, 7, and 10 mg/d (39) comparing observed values (black bars) with values calculated by the model (gray bars).

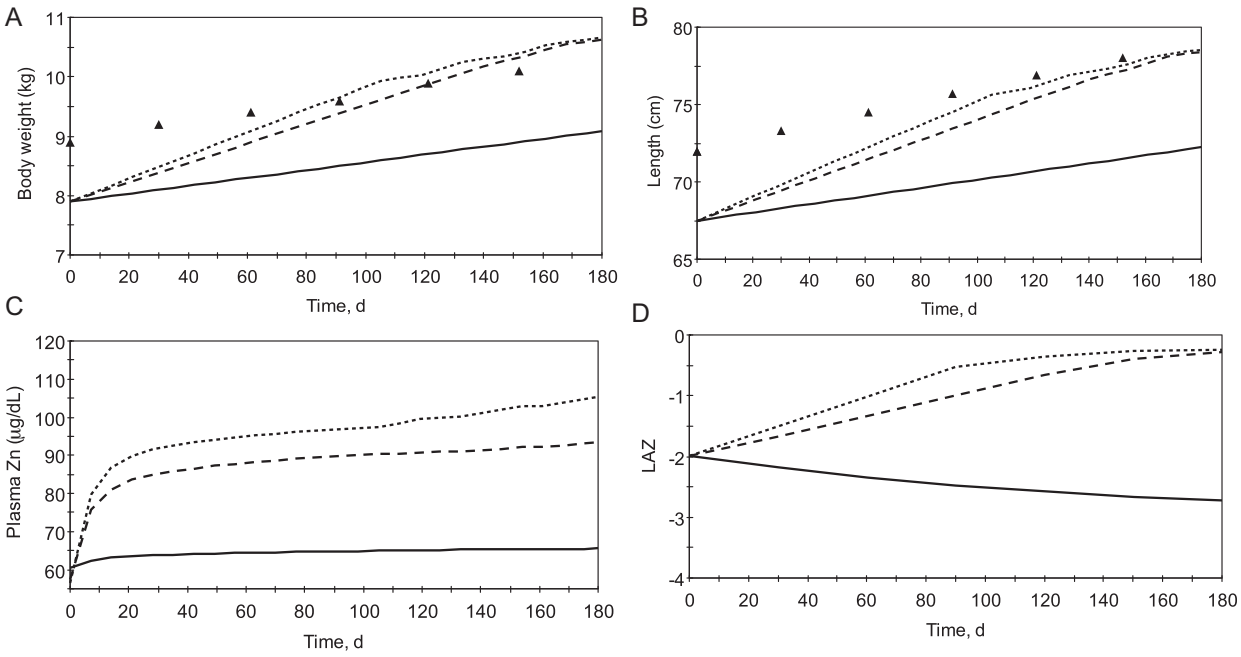

FIGURE 3 Effect of dose amount on changes in metabolism simulated (by using the model in Figure 1) in a normal infant (triangles) or an infant who is stunted and zinc-deficient (solid lines) and supplemented with either 5 (long-dashed lines) or 10 mg Zn/d (short-dashed lines) for body weight (A), length (B), plasma zinc (C), and LAZ (D). LAZ, length-for-age *z* score.

compared with normal infants, in which the amount, frequency, and duration of zinc dosing were varied, by using the characteristics and assumptions in Table 2. When the zinc doses were compared, weight reached normal levels at 90 d with 10 mg Zn/d compared with at 120 d with 5 mg Zn/d. Plasma zinc reached the normal range (80–100 µg/dL) after ~3 wk with both doses; both doses also improved LAZ (**Figure 3**). The model also predicted that normal weight would be achieved after 60 d with 20 mg/d (data not shown).

To assess the effect of frequency of dosing, weekly doses of 10, 15, and 20 mg Zn were compared with daily dosing of 10 mg Zn (**Figure 4**). None of the weekly doses resulted in weight or length reaching normal values. All of the doses increased plasma zinc, but only the daily dose increased plasma zinc concentration to the normal range.

To test the effect of duration of the supplementation period, 10 mg Zn/d was given for 6 mo or for 3 mo (**Figure 5**). When the dose was stopped at 3 mo, normal weight was achieved but length remained below normal. LAZ remained >2 SDs below the median for both durations. Plasma zinc concentration increased during dosing but decreased rapidly when dosing stopped after 3 mo. After 6 mo, the plasma zinc concentration remained at ~80 µg/dL when the dosing stopped, but if dosing stopped after 3 mo, plasma concentration would decrease to <80 µg/dL within 10 d.

In summary, the simulations showed that 10 mg supplemental Zn given daily may be effective for treating zinc deficiency in 9- to 15-mo-old infants if the supplement is given daily and for ≥6 mo.

General parameters of the model are listed in **Supplemental Table 1**, and the model equations are shown in **Supplemental Table 2**. The complete model, in WinSAAM format, with the equations and all parameter values, including the fractional transfer coefficients between compartments, is given in **Supplemental Table 3**.

DISCUSSION

A model for predicting changes in plasma zinc concentrations, weight, and length was developed for stunted, zinc-deficient 9-mo-old infants who receive various amounts of supplemental zinc. The model predictions of changes in plasma zinc, linear growth, and weight gain were compared with 5 published studies of the changes observed when supplemental zinc was provided (38–42); 2 of the studies only reported changes in plasma concentration. The model predictions matched changes in plasma zinc with the reported values as well as changes in weight and length with the published growth responses (41, 42). In one of those studies (41), infants were supplemented with minerals and multivitamins to ensure adequate micronutrient intakes. In a study in which other vitamin and minerals were not given (40), the effects of zinc on growth were more modest than when multiple micronutrient supplements were provided (42), suggesting that other nutrient deficiencies may have limited the growth response to zinc. For that study (40), the model predicted a gain of 2.88 kg; the observed gain was 1.73 kg over the 6-mo supplementation with 10 mg Zn/d. On the basis of the assumption that 30% of the absorbed zinc was used for a gain in tissue containing 30 µg Zn/kg, this would suggest that only 8% (152 mg of the 1800-mg intake) was absorbed. If zinc absorption is reduced in the model, the observed gain is predicted. Zinc absorption was reduced by ~50% in infants aged 18–24 mo who are at risk of environmental enteric dysfunction (43). The model is designed to increase growth when zinc intake is increased. Therefore, it is

TABLE 4
Comparison of published and model-simulated values[1]

Supplement	Plasma zinc, μg/dL	Weight, kg	Length, cm	WAZ	LAZ	WLZ	Growth		Ref
							g/d	cm/mo	
Difference from baseline									(40)
Normal									
0 mg Zn/d	—	1.02	5.0	−0.5	−0.7	−0.2	5.7	0.8	
Model-calculated	—	1.03	5.1	0.0	−0.3	0.1	5.7	0.9	
10 mg Zn/d	—	1.19	6.6	−0.3	−0.2	−0.4	11.7	1.1	
Model-calculated	—	1.81	9.1	0.8	1.3	0.2	10.1	1.5	
Stunted									
0 mg Zn/d	—	0.95	2.9	−0.3	−1.2	−0.3	5.3	0.5	
Model-calculated	—	0.95	3.8	0.1	−0.6	0.0	5.3	0.6	
10 mg Zn/d	—	1.73	7.0	0.4	0.1	−0.2	9.6	1.2	
Model-calculated	—	2.88	11.5	2.1	2.5	0.0	16.0	1.9	
Alternate[2]	—	1.92	7.7	1.2	0.9	−0.1	10.6	1.3	
Difference from nonstunted									
Stunted									
0 mg Zn/d	—	−1.07	−7.6	−1.1	−2.8	0.5	−0.4	−0.4	
Model-calculated	—	−1.07	−6.8	−1.2	−2.8	0.2	−0.4	−0.2	
10 mg Zn/d	—	−0.36	−4.6	−0.4	−1.7	0.8	−2.1	0.1	
Model-calculated	—	0.17	−2.5	0.2	−1.0	0.1	5.9	0.4	
Difference from baseline									(41)
0 mg Zn/d	−0.6	1.20	5.1	0.0	−0.2	0.0	—	—	
Model-calculated	6.5	1.30	6.5	0.2	0.2	0.0	—	—	
17 mg Zn 1 d/wk	26.7	1.30	5.7	0.1	0.2	−0.1	—	—	
Model-calculated	23.3	1.30	6.5	0.2	0.1	0.2	—	—	
5 mg Zn/d	22.2	1.30	5.4	0.1	−0.1	0.1	—	—	
Model-calculated	26.4	1.42	7.1	0.3	0.4	0.1	—	—	
Difference from unsupplemented									
17 mg Zn 1 d/wk	27.0	0.10	1.00	−0.1	0.0	−0.2	0.6	0.1	
Model-calculated	16.5	0.00	0.40	0.0	0.2	−0.1	0.0	0.0	
5 mg Zn/d	21.0	0.10	−0.10	0.2	0.1	0.2	0.6	0.1	
Model-calculated	18.1	0.13	0.23	0.1	0.1	0.1	0.7	0.1	
Difference from baseline									(42)
0 mg Zn/d	—	0.6	3.6	−0.2	−0.1	−0.2	—	—	
Model-calculated	—	1.1	4.2	0.2	−0.3	0.3	—	—	
10 mg Zn/d	—	1.1	5.1	0.2	0.3	−0.1	—	—	
Model-calculated	—	1.6	6.4	0.8	0.6	0.5	—	—	
Difference from unsupplemented									
10 mg Zn/d	—	0.40	1.1	0.3	0.3	0.1	3.3	0.3	
Model-calculated	—	0.45	1.8	0.4	0.7	0.3	3.7	0.4	

[1]LAZ, length-for-age *z* score; Ref, reference; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.
[2]Simulation with absorption function multiplied by 0.5.

not able to reproduce the results of studies in which zinc had no effect on growth (44, 45). However, it can be used to test theories as to why no growth response occurred, such as low absorption.

The model predicts the metabolic responses to zinc supplementation on the basis of the amount, frequency, and duration of zinc dosing. Initial predictions suggest that frequent doses are more effective at promoting growth than are large doses given less frequently. A higher daily dose (e.g., 10 mg/d) normalized length and weight 3 mo earlier than did 5 mg/d. In addition, smaller amounts (5 mg) given daily were more effective than a larger amount (10 mg) given weekly. Thus, the model supports quantitatively the recommendation to give smaller, more frequent doses (46). Possibly, larger doses given less frequently trigger homeostatic responses, such as increased excretion rather than increasing the amount of zinc absorbed for deposition in body tissues and increasing growth. The model also showed the importance of the length of time that the zinc dose is provided; if the period is too short (e.g., 3 mo compared with 6 mo), the plasma zinc concentrations do not reach normal values.

The model is based on the assumption that the metabolic regulation of zinc in stunted, zinc-deficient infants is similar to that of normal infants. When normal infants aged 1–8 mo were fed 2 amounts of zinc in a crossover design, zinc balance was achieved with a lower zinc intake by increasing fractional absorption and decreasing endogenous excretion (47). Kinetic studies in young piglets also showed that a reduction in zinc intake to 15% of the recommended amount caused an increase in fractional absorption to maintain weight gain similar to that of an adequate-zinc group (48). Studies in healthy, breastfed 5-mo-old infants fed complementary foods with varying zinc intakes showed that fractional zinc absorption increased with lower zinc intakes, but the amount absorbed from the low-zinc diet was insufficient to meet physiologic requirements, suggesting that absorption may not compensate for low zinc intakes in healthy infants (46). However, other

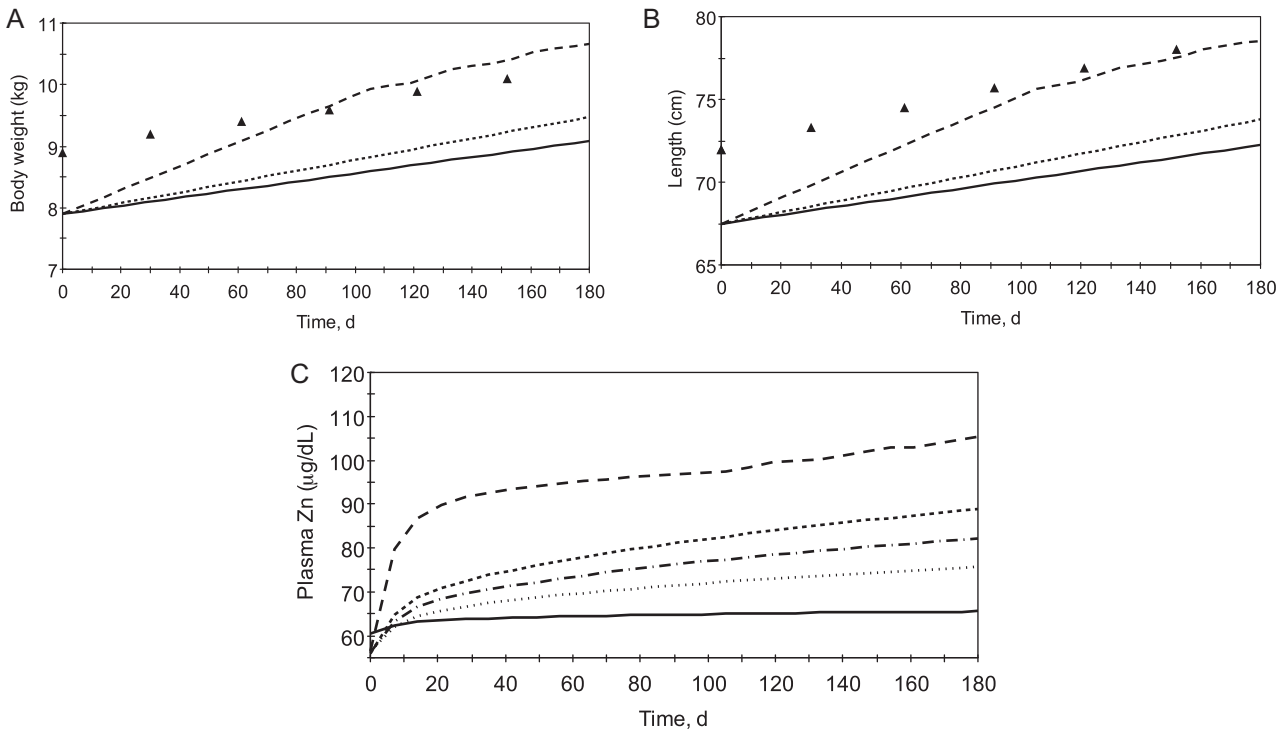

FIGURE 4 Effect of dose frequency on changes in metabolism simulated (by using the model in Figure 1) in a normal infant (triangles) or an infant who is stunted and zinc deficient (solid lines) and supplemented with 10 mg Zn/d (long-dashed lines), 10 mg Zn/wk (dotted lines), 15 mg Zn/wk (dotted-dashed lines), or 20 mg Zn/wk (short-dashed lines) for body weight (A), length (B), and plasma zinc (C). Note that lines for 10, 15, and 20 mg/wk are coincident for length and body weight.

homeostatic responses (i.e., endogenous fecal excretion) were not measured.

One limitation of our model is that the calculated increase in length is a constant function of weight gain. However, length and mass may change differently over time (e.g., LAZ declines more sharply from birth to age 24 mo than does WAZ) (49). Some studies report that there is a lag between linear growth and weight gain in infants, and that during catch-up growth weight gain takes precedence over length gain (50). Thus, it appears that after repletion of infants who experience growth-faltering, muscle recovery occurs before bone growth. Our adult tracer studies are consistent with that conclusion (i.e., that muscle zinc release is regulated and conserved when zinc intake is low) (25). This is not seen in bone. Both animal and human studies show that muscle zinc is retained with zinc deficiency, whereas bone loses zinc (12). On the basis of these observations, when zinc-deficient infants who are stunted but not wasted are given zinc we predict that bone growth would be stimulated more than muscle gain. Due to the lack of data, we were unable to model differential rates of bone and muscle growth with zinc repletion. However, the potential effect of incorporating these rates into the model would likely have a small effect of the predicted gain in weight and length. Strengths of the model are that it is physiologically based on human data, it is parameterized for infants, and it includes nonlinear functions for zinc metabolic pathways known to be regulated by zinc intake (absorption and excretion) and for growth of tissues.

The comparison of simulated results with reported values was challenging because the pediatric populations studied varied widely in terms of age, health, and nutritional status (38–42). For example, the ages often varied from 6 to 36 mo. Because our model simulates values for 9-mo-old infants, it was difficult to compare simulated to published values because the growth rates are faster at 9 mo than at 20 mo (27). In addition, the growth rates frequently are not analyzed according to sex, even though boys have different growth rates than girls (27). Finally, the proportion of zinc-deficient infants in a study can vary widely [e.g., 27–40% (39) to >70% (38)]. However, the model can be readily modified to simulate different degrees of stunting and zinc deficiency in both boys and girls by adjusting the initial weight and baseline growth rates (49). It is important to keep in mind that supplements may have a limited effect on growth in infants >24 mo of age (49).

In summary, we developed a dynamic model of zinc metabolism for infants. The model predicts growth on the basis of the amount, frequency, and length of zinc supplementation for 9-mo-old stunted infants who are zinc-deficient based on their diet and plasma zinc concentrations. The predicted response can be applied to infants who receive supplemental zinc administered alone or as a part of multiple micronutrient powder. Because weight and length changes are approximately linear between 9 and 24 mo of age (27), the model can be used for infants in that age range. In the future, our model predictions of zinc supplementation need to be evaluated in homogenous groups of stunted infants with respect to age, sex, and zinc status. That information will improve predicted amounts of supplemental zinc, and it will identify the population subgroups (e.g., infants with plasma zinc and LAZ below defined thresholds) most likely to respond to zinc supplementation. Eventually, this dynamic model can be used to

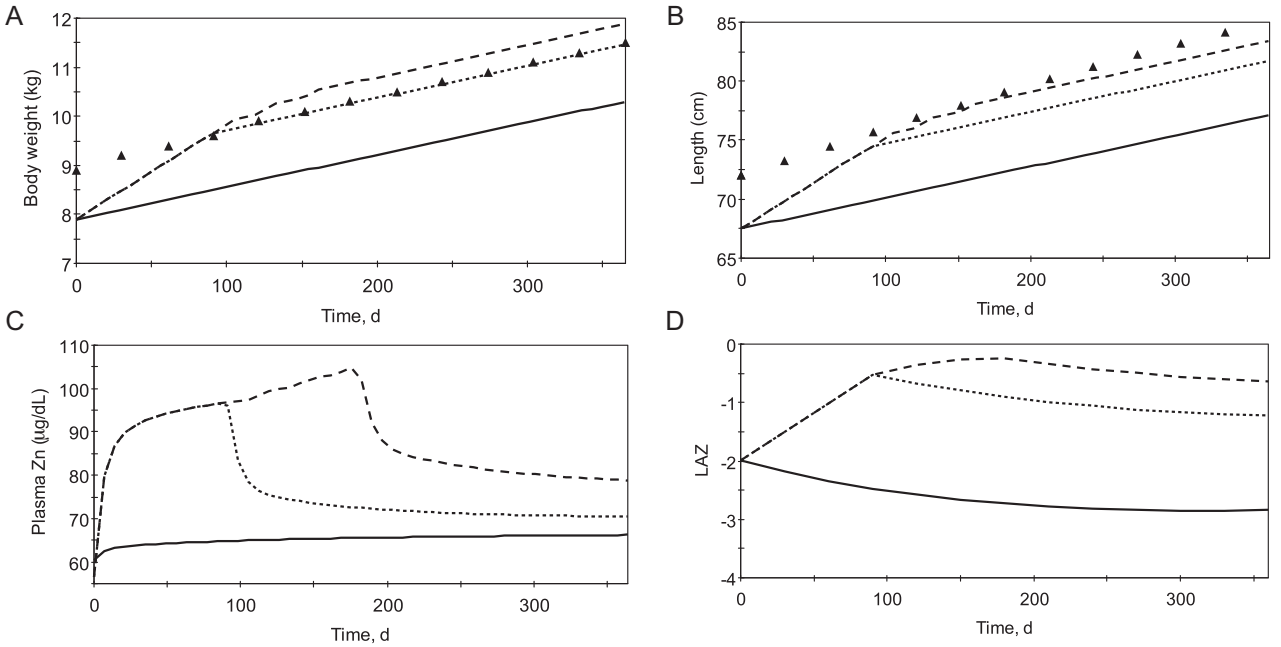

FIGURE 5 Effect of dosing duration on changes in metabolism simulated (by using the model in Figure 1) in a normal infant (triangles) or an infant who is stunted and zinc-deficient (solid lines) and supplemented with 10 mg Zn/d for 6 mo (long-dashed lines) or 3 mo (short-dashed lines) for body weight (A), length (B), plasma zinc (C), and LAZ (D). LAZ, length-for-age *z* score.

develop evidence-based clinical protocols for zinc supplementation trials to treat zinc deficiency in various pediatric populations around the world.

We thank Nancy Krebs for her insightful suggestions on the manuscript and Ray Boston for supplying the WinSAAM equation to limit growth.

The authors' responsibilities were as follows—MEW and JCK: designed and conducted the research; MEW: analyzed the data and had primary responsibility for final content; and all authors: wrote the manuscript, and read and approved the final manuscript. None of the authors declared a conflict of interest.

REFERENCES

1. Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lonnerdal B, Ruel MT, Sandtrom B, Wasantwisut E, Hotz C, et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25:S99–203.
2. Krebs NF, Miller LV, Hambidge KM. Zinc deficiency in infants and children: a review of its complex and synergistic interactions. Paediatr Int Child Health 2014;34:279–88.
3. Lindenmayer GW, Stoltzfus RJ, Prendergast AJ. Interactions between zinc deficiency and environmental enteropathy in developing countries. Adv Nutr 2014;5:1–6.
4. Brown KH, Peerson JM, Baker SK, Hess SY. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull 2009;30:S12–40.
5. World Health Organization, World Food Program and United Nations Children's Fund. Preventing and controlling micronutrient deficiencies in populations affected by an emergency. 2007. http://www.who.int/nutrition/publications/WHO_WFP_UNICEFstatement.pdf.
6. WHO. WHO Guideline: use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6–23 months of age. Geneva (Switzerland); WHO; 2011.
7. Tran CD, Gopalsamy GL, Mortimer EK, Young GP. The potential for zinc stable isotope techniques and modelling to determine optimal zinc supplementation. Nutrients 2015;7:4271–95.
8. de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. Food Nutr Bull 2007;28:S480–4.
9. King JC. Zinc: an essential but elusive nutrient. Am J Clin Nutr 2011;94:679S–84S.
10. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. Br J Nutr 2008;99(Suppl 3):S14–23.
11. International Atomic Energy Agency. Analysis of stable isotope data to estimate vitamin A body stores. Austria: IAEA Vienna; 2008.
12. King J, Shames D, Woodhouse L. Zinc homeostasis in humans. J Nutr 2000;130:1360S–6S.
13. Wastney ME, Angelus P, Barnes RM, Siva Subramanian KN. Zinc kinetics in preterm infants: a compartmental model based on stable isotope data. Am J Physiol 1996;271:R1452–9.
14. Wastney ME, Angelus PA, Barnes RM, Subramanian KN. Zinc absorption, distribution, excretion, and retention by healthy preterm infants. Pediatr Res 1999;45:191–6.
15. Griffin IJ, King JC, Abrams SA. Body weight-specific zinc compartmental masses in girls significantly exceed those reported in adults: a stable isotope study using a kinetic model. J Nutr 2000;130:2607–12.
16. King JC, Shames DM, Lowe NM, Woodhouse LR, Sutherland B, Abrams SA, Turnlund JR, Jackson MJ. Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. Am J Clin Nutr 2001;74:116–24.
17. Miller LV, Krebs NF, Hambidge KM. Development of a compartmental model of human zinc metabolism: identifiability and multiple studies analyses. Am J Physiol 2000;279:R1671–84.
18. Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol 1986;251:R398–408.
19. Hambidge KM, Krebs NF, Westcott JE, Miller LV. Changes in zinc absorption during development. J Pediatr 2006;149:S64–8.
20. Wastney ME, Patterson BH, Linares OA, Greif PC, Boston RC. Investigating biological systems using modeling: strategies and software. New York: Academic Press; 1998.
21. Stefanovski D, Moate PJ, Boston RC. WinSAAM: a Windows-based compartmental modeling system. Metabolism 2003;52:1153–66.

22. WHO. Nutrition Landscape Information System (NLIS). Country profile indicators: interpretation guide. Geneva (Switzerland): WHO; 2010.
23. Hess SY, Peerson JM, King JC, Brown KH. Use of serum zinc concentration as an indicator of population zinc status. Food Nutr Bull 2007;28:S403–29.
24. Jackson AJ, Zech LA. Easy and practical utilization of CONSAM for simulation, analysis, and optimization of complex dosing regimens. J Pharm Sci 1991;80:317–20.
25. Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol 1986;251:R398–408.
26. House WA, Wastney ME. Compartmental analysis of zinc kinetics in mature male rats. Am J Physiol 1997;273:R1117–25.
27. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85.
28. International Life Sciences Institute. Physiological parameter values for PBPK models Washington (DC): US Environmental Protection Agency; 1994.
29. Erickson MM, Poklis A, Gantner GE, Dickinson AW, Hillman LS. Tissue mineral levels in victims of sudden infant death syndrome. II. Essential minerals: copper, zinc, calcium, and magnesium. Pediatr Res 1983;17:784–7.
30. Casey CE, Robinson MF. Copper, manganese, zinc, nickel, cadmium and lead in human foetal tissues. Br J Nutr 1978;39:639–46.
31. White DR, Widdowson EM, Woodard HQ, Dickerson JW. The composition of body tissues. II. Fetus to young adult. Br J Radiol 1991;64:149–59.
32. Cheek DB, Hill DE, Cordano A, Graham GG. Malnutrition in infancy: changes in muscle and adipose tissue before and after rehabilitation. Pediatr Res 1970;4:135–44.
33. Institute of Medicine Food and Nutrition Board. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc Washington (DC): National Academies Press; 2001.
34. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva (Switzerland): WHO; 2006.
35. Krebs NF, Hambidge KM. Zinc requirements and zinc intakes of breast-fed infants. Am J Clin Nutr 1986;43:288–92.
36. Institute of Medicine (US) Panel on Micronutrients. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): The National Academies Press (US); 2001.
37. Alexander FW, Clayton BE, Delves HT. Mineral and trace-metal balances in children receiving normal and synthetic diets. Q J Med 1974;43:89–111.
38. Wessells KR, Ouedraogo ZP, Rouamba N, Hess SY, Ouedraogo JB, Brown KH. Short-term zinc supplementation with dispersible tablets or zinc sulfate solution yields similar positive effects on plasma zinc concentration of young children in Burkina Faso: a randomized controlled trial. J Pediatr 2012;160:129–35 e3.
39. Wuehler SE, Sempertegui F, Brown KH. Dose-response trial of prophylactic zinc supplements, with or without copper, in young Ecuadorian children at risk of zinc deficiency. Am J Clin Nutr 2008;87:723–33.
40. Umeta M, West CE, Haidar J, Deurenberg P, Hautvast JG. Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. Lancet 2000;355:2021–6.
41. Thu BD, Schultink W, Dillon D, Gross R, Leswara ND, Khoi HH. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. Am J Clin Nutr 1999;69:80–6.
42. Ninh NX, Thissen JP, Collette L, Gerard G, Khoi HH, Ketelslegers JM. Zinc supplementation increases growth and circulating insulin-like growth factor I (IGF-I) in growth-retarded Vietnamese children. Am J Clin Nutr 1996;63:514–9.
43. Krebs NF, Long J, Mondal P, Westcott J, Miller L, Islam M, Ahmed M, Mahfuz M, Ahmed T. Zinc (Zn) absorption from micronutrient powders (MNP) is low in Bangladeshi toddlers with environmental enteric dysfunction (EED). FASEB J 2017;31:786 4. http://www.fasebj.org/doi/10.1096/fasebj.31.1_supplement.786.4.
44. Bates CJ, Evans PH, Dardenne M, Prentice A, Lunn PG, Northrop-Clewes CA, Hoare S, Cole TJ, Horan SJ, Longman SC, et al. A trial of zinc supplementation in young rural Gambian children. Br J Nutr 1993;69:243–55.
45. Hess SY, Abbeddou S, Jimenez EY, Some JW, Vosti SA, Ouedraogo ZP, Guissou RM, Ouedraogo JB, Brown KH. Small-quantity lipid-based nutrient supplements, regardless of their zinc content, increase growth and reduce the prevalence of stunting and wasting in young Burkinabe children: a cluster-randomized trial. PLoS One 2015;10:e0122242.
46. Gibson RS, Ferguson EL. Nutrition intervention strategies to combat zinc deficiency in developing countries. Nutr Res Rev 1998;11:115–31.
47. Ziegler EE, Serfass RE, Nelson SE, Figueroa-Colφn R, Edwards BB, Houk RS, Thompson JJ. Effect of low zinc intake on absorption and excretion of zinc by infants studied with ^{70}Zn as extrinsic tag. J Nutr 1989;119:1647–53.
48. Serfass RE, Fang Y, Wastney ME. Zinc kinetics in weaned piglets fed marginal zinc intake: compartmental analysis of stable isotopic data. J Trace Elem Exp Med 1996;9:73–86.
49. Victora CG, de Onis M, Hallal PC, Blossner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. Pediatrics 2010;125:e473–80.
50. Dewey KG, Hawck MG, Brown KH, Lartey A, Cohen RJ, Peerson JM. Infant weight-for-length is positively associated with subsequent linear growth across four different populations. Matern Child Nutr 2005;1:11–20.