Systematic Review

Toward revising dietary zinc recommendations for children aged 0 to 3 years: a systematic review and meta-analysis of zinc absorption, excretion, and requirements for growth

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Context: *The Food and Agriculture Organization of the United Nations and the World Health Organization are updating their dietary zinc recommendations for children aged 0 to 3 years.* ***Objective:*** *The aim of this review was to retrieve and synthesize evidence regarding zinc needs for growth as well as zinc losses, absorption, and bioavailability from the diet.* ***Data Sources:*** *MEDLINE, Embase, and Cochrane Library databases were searched electronically from inception to August 2020. Studies assessing the above factors in healthy children aged 0 to 9 years were included, with no limits on study design or language.* ***Data Extraction:*** *Ninety-four studies reporting on zinc content in tissue ($n = 27$); zinc absorption ($n = 47$); factors affecting zinc bioavailability ($n = 30$); and endogenous zinc losses via urine, feces, or integument ($n = 40$) met the inclusion criteria. Four reviewers extracted data and two reviewers checked for accuracy.* ***Data Analyses:*** *Studies were synthesized narratively, and meta-analyses of zinc losses and gains as well the subgroups of age, type of feeding, country's income, and molar ratio of phytate to zinc were conducted. Meta-analysis revealed an overall mean (95%CI) urinary and endogenous fecal zinc excretion of 17.48 μg/kg/d (11.80–23.15; $I^2 = 94\%$) and 0.07 mg/kg/d (0.06–0.08; $I^2 = 82\%$), respectively, with a mean fractional zinc absorption of 26.75% (23.69–29.81; $I^2 = 99\%$). Subgrouping by age revealed differences in mean values associated with the transition from milk-based diets to solid food during the first 3 years of life.* ***Conclusion:*** *This review synthesizes data that may be used to formulate zinc requirements in young children. Results should be interpreted with caution because of considerable heterogeneity in the evidence.* ***Systematic Review Registration:*** *PROSPERO registration number CRD42020215236.*

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INTRODUCTION

Zinc is an essential trace element involved in numerous biological processes in the human body that plays a key role in growth and development in the first years of life.[1] Zinc deficiency during infancy and early childhood may result in serious health consequences such as growth retardation,[2] delayed wound healing,[3] increased risk of infectious diseases,[4] and impairment of cognitive function.[5]

Zinc deficiency is a public health problem in almost all low- and middle-income countries, with an estimated prevalence that exceeds 20%.[6] The main causes of deficiency include inadequate intake, increased requirements or losses, malabsorption, and impaired utilization of zinc.[7] In healthy children, zinc requirements can be met by breast milk during the first 6 months of life. Beyond this age, the zinc intake required for optimal growth and development may not be achieved without complementary foods.[8] Zinc is found in a variety of foods, including meat, fish, legumes, and other dietary sources,[9] but different dietary factors may affect its bioavailability.[10] For example, high-phytate diets common in many low- and middle-income countries may increase the risk of zinc deficiency in children.[11]

Zinc supplementation and fortification have positive effects on linear growth[2,12] and reduce the incidence of diarrhea and pneumonia.[4] Additionally, motor development may be positively associated with zinc intake.[5] Accurate estimates of the zinc requirements of young children enable criteria to be established for defining the severity of public health micronutrient malnutrition and for developing strategies of prevention. Current dietary zinc recommendations from the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the European Food Safety Authority, the Institute of Medicine, and the International Zinc Nutrition Consultative Group for infants and young children (aged 7–36 months) range from 0.8 to 8.3 mg/d.[13–16] These recommendations vary, depending on the age of the child and the bioavailability of zinc in the diet. Recommendations from expert groups also vary as a result of the different approaches and data used to estimate zinc requirements.[17]

Zinc is classified as a type II nutrient, meaning that it is required for general metabolism, in contrast to type I nutrients which are required for specific functions.[18] Zinc deficiency, therefore, manifests as a myriad of direct and indirect changes to metabolic and biochemical pathways, which makes it difficult to identify a sensitive and specific biomarker of zinc status that can be used to generate a dose-response curve for the identification of optimal dietary intake.[19] The joint FAO-WHO dietary zinc recommendations, as well as those of other expert groups,[13,14,16] are therefore based on a factorial approach for calculating zinc requirements.[17] This method uses estimates of the zinc required for tissue growth (ie, generation of new tissue for organs, muscle, and bones), together with estimates of zinc losses (ie, via urine, feces, skin desquamation of epithelial cells, and sweat), to provide an estimated zinc physiological requirement that can then be adjusted for zinc bioavailability from the habitual diet to determine the dietary intake needed to meet the physiological requirement.[17] The FAO-WHO dietary recommendations for vitamins and minerals were last updated in 2004.[15] These estimates were based on total endogenous losses, extrapolated from adult data based on a metabolic rate of 0.57 mg of zinc per basal kilocalorie, plus the additional amount of absorbed zinc that is incorporated into newly synthesized tissues for growth. New evidence has become available since these requirements were published,[20] and FAO and WHO have convened a group of experts tasked with updating the recommended intakes of nutrients, including zinc, for children aged 0 to 3 years.[21]

The aim of this systematic review was to synthesize the available evidence to answer clearly defined questions posed by the FAO-WHO expert group, described below, regarding zinc intake in relation to needs for growth (tissue composition), excretion, absorption, and factors affecting bioavailability in children aged 0 to 3 years. This information will be used to inform the updating of the FAO-WHO nutrient intake values for zinc in this age range. This review does not attempt to preempt subsequent expert group discussions regarding the inclusion or exclusion of individual studies or data sets from the final calculations.

METHODS

Registration and research questions

This systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020[22] guidelines (see Appendix S1 in the Supporting Information online) and has been registered with the International Prospective Register of Systematic Reviews (PROSPERO registration no. CRD42020215236), where the review protocol can be accessed.

To estimate the zinc needs of children aged 0 to 3 years, data from studies conducted in this age group are required in response to the following 4 questions: (1) What are the zinc needs for growth? To address this

question, studies that reported the zinc content of tissues were sought. (2) What are the routes for endogenous losses and the amounts of zinc lost through these various routes? To address this question, papers that assessed zinc loss through fecal and urinary excretion, sweat, and skin (integument) were identified. Fecal zinc excretion is comprised of two components: unabsorbed zinc that passes through the gastrointestinal tract, and endogenous fecal zinc (EFZ), which is zinc that has been absorbed then re-enters the gut as a component of the gastrointestinal tract secretions and digestive enzymes plus zinc that is present in cells that are sloughed off from the lining of the gastrointestinal tract.[23] Therefore, data relating to both total fecal zinc and EFZ was retrieved. (3) What is the efficiency of absorption of zinc (ie, what percentage of zinc consumed is absorbed by the body) in children aged 0 to 3 years? (4) What factors affect zinc absorption from meals and whole diets, and what is the quantitative effect of these factors on absorption of zinc in children aged 0 to 3 years?

Information sources and search strategy

An information specialist (C.H.) designed two electronic search strategies in consultation with members of the review team (N.M.L., V.H.M., M.C-R., J.M.): one to answer the above questions 1, 3, and 4, and a separate search strategy to address question 2. The searches were conducted in MEDLINE (OVID), Embase (OVID), Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library, from date of inception to August 7, 2020, and were not limited by language or study design. Two separate searches were undertaken, one for zinc gains (absorption, tissue zinc concentrations, etc) and one for zinc losses. Search 1 comprised terms for zinc, terms relating to nutritional requirements, and terms for children and infants. Search 2 included terms for zinc combined with terms relating to endogenous zinc losses. As it was anticipated that there would be limited data available for zinc losses in young children, and data for older children and adults may be required, this search was not limited to children and infants. Full details of the search strategy for each database are provided in Appendix S2 in the Supporting Information online.

Hand searches were conducted by examining the reference lists of the retrieved articles and relevant systematic reviews.[24–26] Following consultation with the FAO-WHO expert group, 4 additional studies not identified by the search were recommended for consideration.[27–30]

Eligibility criteria

The PEO (Population, Exposure/Risk, Outcomes) framework (Table 1) was used to structure the search terms and study selection criteria.[31] Some adaptations were made, particularly around factors considered as exposures or risks.

Population. For Search 1, healthy children aged 0 to 9 years were included. Although children aged 0 to 3 years were the primary interest, the preceding scoping review indicated that there may be limited data available in children aged 0 to 3 years. Therefore, the literature search was expanded to include studies that included prepubertal children up to the age of 9 years to allow for the possibility for data extrapolation from older children, if required. For the same reason, no upper limits on age were placed in Search 2. In both searches, studies were excluded if participants were preterm and low birthweight children, children older than 9 years if data were not disaggregated by age, and sick children without a healthy control group. Studies conducted in areas where children may be at risk of environmental enteric dysfunction were included unless the study specifically identified the condition. Where environmental enteric dysfunction was identified by the author, the papers were flagged for further discussion and reported within this review. Since stunting is also endemic within the context of some of the included studies, studies that included stunted children were also flagged for further discussion and reported within this review. For studies assessing tissue zinc concentrations, studies were included if they reported the zinc content of tissues taken from children deceased from causes nonrelated to a disease. Given the small number of studies assessing zinc in tissues of young children, studies reporting zinc in tissues of fetuses $\geq$ 38 weeks of gestational age were also included.

Exposure/risk. All primary studies addressing issues of zinc in tissue, zinc excretion, and zinc absorption (including balance studies, isotope tracer studies) and in which outcome data were presented by age, intake of zinc, type of diet, or tissue type were included. Studies conducted in vitro and animal studies were excluded.

Outcome. Studies were included if they objectively measured zinc content of tissue (bone, muscle, or organs), losses of zinc through urine, feces, sweat, and skin (losses through hair and nails were excluded), and zinc absorption (total or fractional).

Study selection and data extraction

The search results were downloaded into EndNote software for automatic and manual deduplication by the information specialist (C.H.). The deduplicated titles

Table 1 **PEO (Population, Exposure/Risk, Outcomes) criteria for inclusion of studies**

Parameter	Criteria
Population	Healthy children aged 0–9 years, with children aged 0–3 years being of primary interest, with no regard to ethnicity, sex, or region.
Exposure/risk	All studies in which outcome data of zinc in tissue, zinc excretion, and zinc absorption were presented by age, intake of zinc, type of diet, or tissue type.
Outcomes	Zinc content of tissue; loss of zinc through urine, feces, sweat, and skin; and zinc absorption (total or fractional).

and abstracts were exported into the Rayyan web app,[32] after which one reviewer (M.C-R.) screened by title and abstract. In the event of uncertainty about inclusion, articles were flagged for discussion with senior members of the review team (N.M.L. and V.H.M.). A randomly selected sample (10%) of the articles was cross-checked by two senior members of the review team (N.M.L., V.H.M.). The discrepancies were explored and the screening process adjusted accordingly. Articles were moved forward to the full-text review if discrepancies or uncertainty about their inclusion remained

Members of the review team (M.C.R., A.K.M.B., S.G.) assessed full-text articles for inclusion and exclusion using a predesigned inclusion/exclusion form designed using the PEO framework. Discussions with a senior reviewer (N.M.L.) took place when there was uncertainty about the inclusion of an article. Reasons for excluding papers were recorded.

A data extraction form was designed in Excel by a member of the review team (M.C-R.) and revised and piloted by members of the review team (N.M.L., V.H.M., M.C-R., A.K.M.B., S.G., A.C.). Members of the review team (M.C-R., A.K.M.B., S.G., V.H.M.) extracted the data from studies that met the inclusion criteria, and extractions were checked (M.C.R., N.M.L.).

Data extracted from the studies included the following: study characteristics (first author, publication year, country, study design, and number of participants); participant characteristics (sex, age, and body weight); mean or median values for the outcomes of interest (zinc absorption, content of zinc in tissue, and zinc excretion through sweat, urine, and feces); and confounders (zinc intake and dietary characteristics). For the studies assessing factors affecting zinc absorption, study findings were considered statistically significant where $P < 0.05$.

Risk of bias across studies

All studies included in this review were critically appraised by one member of the review team (V.H.M.) using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist.[33] A second reviewer (M.C-R.) blinded to the first reviewer's (V.H.M.) decisions checked a random 10% of the included articles for risk of bias, and any differences between the reviewers were discussed and resolved. This tool was chosen because it could be used to assess descriptive cross-sectional studies as well as randomized controlled trials (RCTs).[34] Cochrane risk-of-bias assessments for RCTs included in the meta-analyses were also conducted.[35] GRADE (Grading of Recommendations, Assessment, Development and Evaluations) quality-of-evidence assessments were completed for all meta-analyses.[36] GRADE assessments were undertaken with a baseline of high quality for meta-analyses that included only RCTs and low quality for meta-analyses that included non-RCTs, with downgrading undertaken for risk of bias, inconsistency, indirectness, and imprecision.[36] These further risk-of-bias and quality assessments were performed by one reviewer (S.M.) and checked blindly by a second reviewer (V.H.M.), with discrepancies discussed and resolved.

Synthesis of results and data analyses

All studies identified by the search strategy are reported in this review, but only studies reporting data from the target age range, 0 to 3 years, are included in the synthesis and analyses. Data syntheses were structured around zinc losses through fecal, urinary, and integumental routes; zinc gained via absorption and the factors that affect bioavailability; and the zinc content of body tissue. For meta-analysis, studies were subgrouped by age for analysis according to the following ranges: less than 6 months, 6 to 11 months, and 1 to 3 years. Age groups were based on the reported mean age for the study participants. Where participants' ages straddled these categories, if disaggregation of the data was not possible, then the category that represented most of the participants at baseline was chosen. For studies in children aged up to 9 years that were not included in the meta-analyses, the extracted data are presented in the Supporting Information (see Table S1 and Table S2 in the Supporting Information online).

For zinc losses, values were expressed in units per day (μg/d or mg/d) and, when reported, normalized by body weight (μg/kg/d or mg/kg/d). In situations where only one of these was reported, values were calculated accordingly, using the mean body weight for the

participant group. Urinary zinc excretion was subgrouped by age; total fecal zinc excretion was ranked by zinc intake and mean body weight; and endogenous fecal zinc (EFZ) loss was subgrouped by age, feeding type, and phytate-to-zinc (Phy:Zn) molar ratio. When dietary zinc intake was given as an amount of zinc per kilogram of body weight, but body weight was not reported, the median value of body weight for the age group was imputed using WHO weight-for-age data[37] to estimate a mean zinc intake in milligrams per day for the group. No meta-analyses were conducted for integumental losses (sweat and skin), since only 3 studies were identified and none reported data from children in the target age range.

For zinc gains, studies were categorized as single-meal studies, studies measuring zinc absorption over a 24-hour period, or studies measuring zinc absorption using a balance method conducted over several days. Within each category, data for fractional zinc absorption (FZA) were subgrouped or ranked by age group, feeding type, country's income category (based on the World Bank's defined categories of High-Income Countries and Low- and Middle-Income Countries),[38] and the Phy:Zn molar ratio. For one study,[39] data for FZA was calculated from total absorption of zinc and dietary data.

Data were synthesized using the generic inverse-variance method to obtain pooled estimates and 95% confidence intervals for the different outcome measures. Given the likelihood of variability among the studies, random-effects models were estimated. Heterogeneity was assessed through visual inspection of forest plots and through the X^2 and I^2 statistics, with possible causes investigated through subgroup and sensitivity analyses. Prespecified subgroups included age, food type, country's income, tissue type, and Phy:Zn molar ratio. Meta-analyses were conducted using RevMan, version 5.4 (The Cochrane Collaboration, 2014).

RESULTS

Description of studies

The systematic database search for studies reporting zinc needs and gains resulted in the retrieval of 12 692 titles and abstracts, from which 299 potentially relevant full-text articles were collected for additional assessment. Of these, 83 met the inclusion criteria. Additional articles were identified through hand searches (n = 8)[40–47] or FAO-WHO expert group recommendation (n = 4).[27–30] One study identified from the hand searches was excluded because it assessed bone tissue in fetuses of 30 weeks' gestational age.[47] The systematic database search for studies reporting zinc losses resulted in the retrieval of 2689 titles and abstracts (see Figure S1 in the Supporting Information online for PRISMA flow chart of zinc losses). After deduplication with the zinc needs and gains search, only two studies from this search were added to the total of included studies.[48,49] The 94 included studies reported one or more outcomes of interest. Reasons for exclusion and number of studies assessing each outcome of interest are presented in Figure 1.

Data synthesis

Zinc losses. A total of 40 studies from 13 countries reported endogenous zinc losses via urine, feces, or integument in children aged 0 to 9 years.[48–87] Research designs comprised RCTs (n = 4), cross-sectional studies (n = 28), quasi-experimental studies (n = 6), and cohort studies (n = 2). Generally, the studies were of good or adequate quality (see Appendix S3 in the Supporting Information online). The stated primary purpose of the studies was to measure, evaluate, or compare zinc absorption, zinc balance or homeostasis, zinc status, zinc excretion, renal function, or zinc utilization or to conduct a detailed investigation of zinc absorption and excretion.

A total of 26 studies measured urinary zinc in infants and children aged 0 to 9 years.[48–51,54,57,60,62–64,67–71,74,76–79,82–87] Of these, 14 provided data from children aged 0 to 3 years[48–50,54,57,60,67,71,73,74,77,84,86,87] and 13 provided data from children aged 4.5 to 9 years.[48,51,62–64,68–70,76,79,82,83,85] In one study, Manary et al,[63] the mean height-for-age *z* score approached the level of stunting. Tables S1 and S3 in the Supporting Information provide descriptions of these studies, with Table S1 detailing studies excluded from the meta-analyses and Table S3 detailing studies included in the meta-analyses. Urinary zinc was measured by atomic absorption spectrometry,[48,49,51,54,60,62–64,68,69,71,74,76–79,82,84,86,87] a colorimetric method,[50,67] inductively coupled plasma-atomic emission spectrophotometry,[85] inductively coupled plasma mass spectrometry,[70] and liquid chromatography.[83] Three studies assessed zinc excretion from spot urine samples.[57,71,87]

Seven studies conducted in the target age range (0–3 years) were excluded from the meta-analyses because they reported urinary zinc as either zinc per milligrams of creatinine[49,57,77] or the zinc concentration from spot urine samples,[71,87] neither of which could be accurately converted to micrograms of zinc per day. One study was excluded from the meta-analysis because it included data of children ranging from 2 months up to 14 years.[48] Two studies were excluded from the final

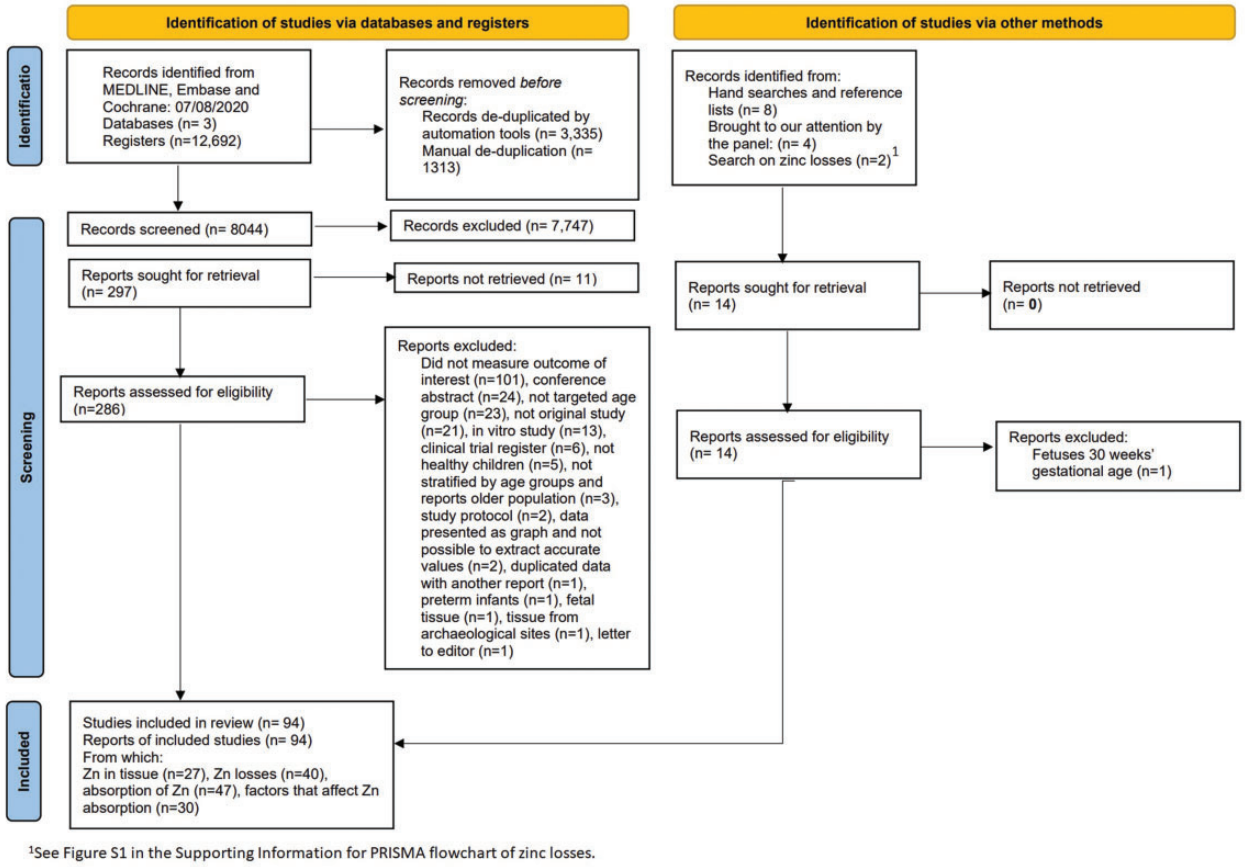

Figure 1 **Flow diagram of the literature search process. *Abbreviation*: Zn, zinc.**

forest plot after sensitivity analysis because the data were outliers,[74,86] based on two criteria: (1) the point estimate and confidence intervals for the specific study did not overlap with the confidence intervals for any other study or with the confidence interval for the overall pooled estimate; and (2) the sequential removal of the individual studies from the meta-analysis showed that a particular study (or studies) had a marked influence on the overall pooled estimate. A summary of the studies included in the meta-analysis is provided in Table 2,[39,48,50,53,56,58–61,65–67,73,75,78,80,84,86,88–106] with details in Table S3 in the Supporting Information online.

The analyses of the data are presented in Figure 2 (μg/kg/d) and in Figure S2 in the Supporting Information online) (μg/d). Results show an overall mean urinary zinc excretion of 147.06 μg/d (95%CI, 103.98–190.14; $I^2 = 95\%$) and 17.48 μg/kg/d (95%CI, 11.80–23.15; $I^2 = 94\%$), in children aged 0 to 3 years. Subgroup analyses performed by age group—less than 6 months,[50,78] 6 to 11 months,[60,84] and 1 to 3 years[48,54]—suggest an inverse relationship between age and urinary zinc excretion, when normalized by body weight (Figure 2). The quality of evidence for urinary zinc excretion assessed using GRADE was very low (see Appendix S3 in the Supporting Information online).

Fecal zinc losses. Twelve studies reported total fecal zinc excretion[50–53,56,68,69,72,73,78,81,86] and 11 reported EFZ loss [54,58,59,61,63,65,66,75,78,80,81] in children aged 0 to 9 years.

Zinc in feces was measured by atomic absorption spectrometry[51–53,56,68,69,72,73,78,81,86] and a colorimetric method,[50] and EFZ loss was measured using stable isotope techniques that included isotope dilution following an oral isotope dose,[59,61] isotope dilution following an intravenous isotope dose,[54,58,65,66,75,80] and measurement of the difference between total fecal zinc and total zinc intake, adjusted for zinc absorption with isotope ratios measured by inductively coupled plasma mass spectrometry.[78] Details of the studies not included in the meta-analysis, including those conducted in children aged > 3 years to 9 years, are provided in Table S3 in the Supporting Information online.

Of the 12 studies that reported total fecal zinc excretion,[50–53,56,68,69,72,73,78,81,86] 7 were conducted in the target age range of 0 to 3 years.[50,53,56,72,73,78,86] One was excluded at this stage from the subsequent meta-

Table 2 **Summary information of studies included in the meta-analyses**

Reference and study location	Study design	Type of zinc loss	Type of zinc absorption study	Zinc absorption assessed	Type of food
Abrams et al (1997)[60] United States	CSS	Urinary[a]	24 h[a]		Breast milk + solid food[b]
Abrams et al (2002)[88] United States	RCT		Single meal[a]	✓	Formula[b]
Ariff et al (2014)[89] Pakistan	RCT		Single meal[a]		Solid food[b]
Assadi & Ziai (1986)[84] United States	CSS	Urinary[a]			NR
Brnić et al (2017)[90] Burkina Faso	Q-NRCT		Single meal[a]	✓	Solid food[b]
Carr & Wilkinson (1975)[48] United Kingdom	CSS	Urinary			NR
Castillo-Duran et al (1988)[86] Chile	CSS	Urinary, TFZ[a]	Several days		Formula[b]
Cavell & Widdowson (1964)[50] United States	CSS	Urinary[a], TFZ[a]	Several days		Breast milk[b]
Chomba et al (2015)[91] Zambia	Q-NRCT		24 h[a]	✓	Solid food[b]
Davidsson et al (1996)[92] Scotland	CSS		Single meal[a]	✓	Solid food[b]
Davidsson et al (1996)[93] Scotland	CSS		Single meal[a]		Solid food[b]
Davidsson et al (2005)[94] United States	Q-NRCT		Single meal[a]	✓	Solid food[b]
Devizia et al (1985)[53] United States	Q-NRCT	TFZ[a]	Several days	✓	Formula[b]
Domellöf et al (2009)[95] Sweden	RCT		Single meal[a]	✓	Breast milk[b]
Esamai et al (2014)[96] Kenya	RCT		24 h[a]	✓	Solid food[b]
Fairweather-Tait et al (1995)[97] United Kingdom	CSS		Single meal[a]	✓	Solid food[b]
Griffin et al (2007)[54] United States	CSS	Urinary[a], EFZ[a]	Single meal[a]		Solid food[b]
Haschke et al (1986)[56] United States	CSS	TFZ[a]	Several days	✓	Formula[b]
Islam et al (2013)[98] Bangladesh	Q-NRCT		24 h[a]	✓	Solid food[b]
Jalla et al (2002)[99] United States	CSS		Single meal[a]	✓	Solid food[b]
Johnson & Canfield (1989)[100] United States	CSS		Single meal[a]	✓	Breast milk[b]
Kennedy et al (2010)[58] Malawi	PCS	EFZ[a]			Solid food[b]
Kodkany et al (2013)[101] India	RCT		24 h[a]		Solid food[b]
Krebs et al (1996)[59] United States	CSS	EFZ[a]	24 h[a]		Breast milk[b]
Krebs et al (2000)[61] United States	RCT	EFZ[a]	24 h[a]		Formula[b]
Krebs et al (2012)[102] United States	RCT		24 h[a]		Solid food[b]
Long et al (2019)[39] Bangladesh	RCT		Single meal[a], 24 h[a]	✓	Solid food[b]
Manary et al (2002)[80] Malawi	CSS	EFZ[a]	24 h[a]	✓	Solid food[b]
May et al (2015)[65] Malawi	Q-NRCT	EFZ[a]	24 h[a]	✓	Solid food[b]
Mondal et al (2019)[66] Bangladesh	Q-NRCT	EFZ[a]	No meal	✓	Solid food[b]

(continued)

Table 2 **Continued**

Reference and study location	Study design	Type of zinc loss	Type of zinc absorption study	Zinc absorption assessed	Type of food
Perrone et al (1990)[67] Unclear	CSS	Urinary[a]			Solid food[b]
Serfass et al (1989)[73] United States	CSS	TFZ[a]	24 h[a]		Formula[b]
Sheng et al (2006)[75] China	CSS	EFZ[a]	24 h[a]		Solid food[b]
Szymlek-Gay et al (2016)[103] Sweden	RCT		24 h[a]	✓	Formula[b]
Thacher et al (2009)[104] Nigeria	CC		Single meal[a], no meal	✓	Solid food[b]
Ziegler et al (1989)[78] United States	Q-NRCT	Urinary[a], TFZ[a], EFZ[a]	24 h[a]		Formula[b]
Zyba et al (2019)[105] Gambia	RCT		Single meal[a]	✓	Solid food[b]

Abbreviations: CC, case control; CSS, analytical cross-sectional study; EFZ, endogenous fecal zinc; NR, not reported; PCS, prospective cohort study; Q-NRCT, quasi-experimental nonrandomized experimental study; RCT, randomized controlled trial; TFZ, total fecal zinc.
[a]Included in the meta-analyses.
[b]Zinc intake measured.

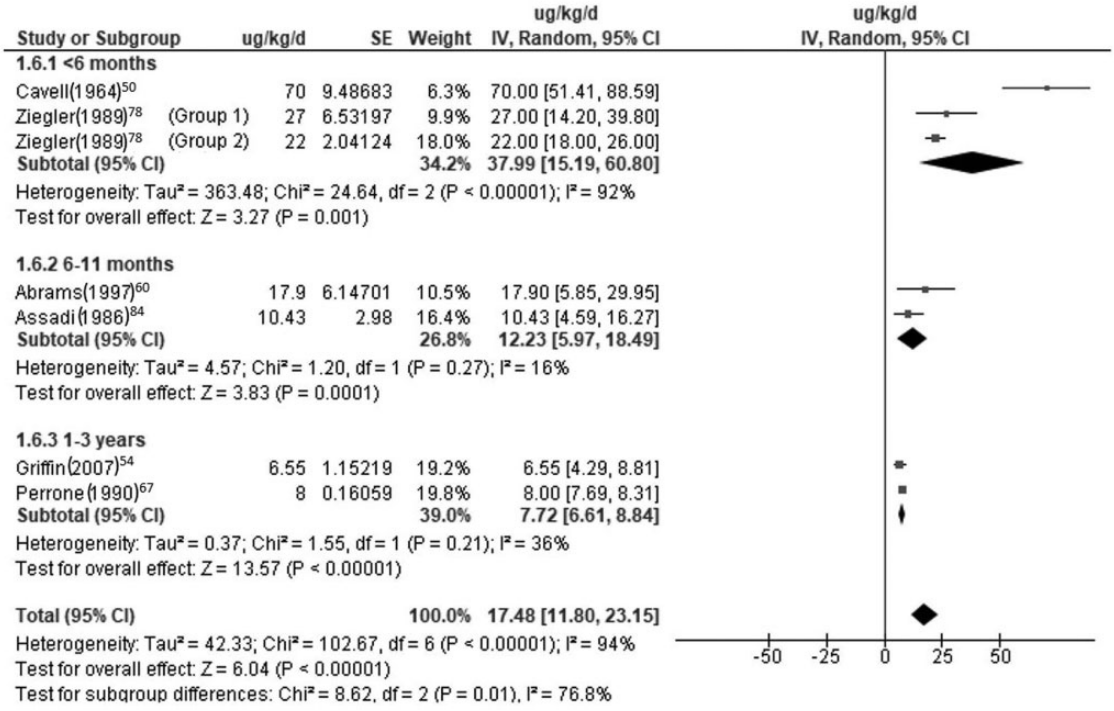

Figure 2 **Forest plot of urinary zinc excretion (μg/kg/d), by age group.**

analysis because the children were recovering from dehydration due to acute diarrhea.[72] A summary of studies included in the meta-analysis is provided in Table 1, with details provided in Table S3 in the Supporting Information online.

All participants were aged 0 to 12 months, and none received solid food in any of the studies. All studies except one[86] were conducted in the United States.

Based on the forest plot and criteria for outliers described above, one study was identified as an outlier (possibly because children identified as controls were recovering from respiratory disease) and removed from the final analysis.[86] In Serfass et al,[73] protocol 3 did not report a standard deviation for total excretion in mg/kg/d or mg/d, and therefore data from protocol 3 were excluded from this analysis. The forest plot of total fecal zinc excretion is presented in Figure 3 (with adjustment for body weight) and in Figure S3 in the Supporting Information online (without adjustment for body weight). The studies are arranged by increasing zinc

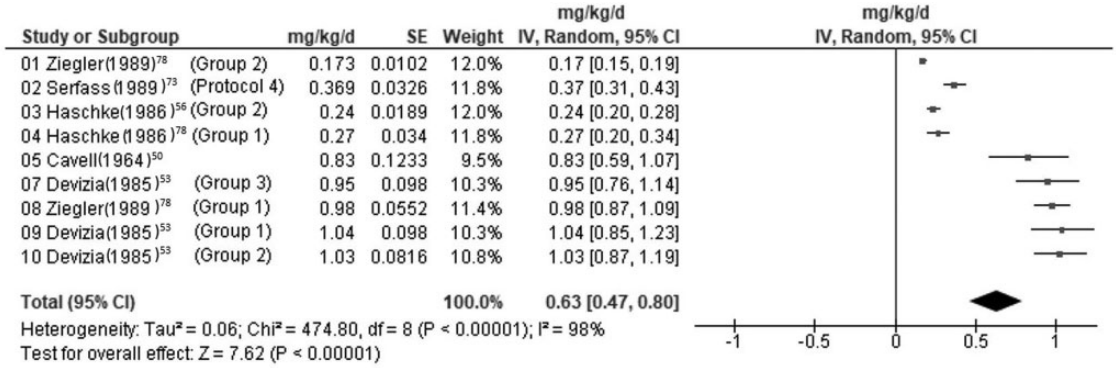

Figure 3 **Forest plot of total fecal zinc excretion (mg/kg/d), with studies ranked by zinc intake.**

intake, which ranged from 1.5 to 7.5 mg/d. This revealed a positive association between zinc intake and total fecal zinc excretion, with an overall mean total fecal zinc excretion of 3.94 mg/d (95%CI, 2.93–4.96; $I^2 = 98\%$). When fecal excretion was normalized by body weight, this association remained, with an overall mean of 0.63 mg/kg/d (95%CI, 0.47–0.80; $I^2 = 98\%$). GRADE quality-of-evidence assessments rated fecal zinc excretion meta-analyses as low or very low (see Appendix S3 in the Supporting Information online).

Endogenous fecal zinc loss. Of the 11 studies identified by the search,[54,58,59,61,63,65,66,75,78,80,81] 9 were conducted in children in the target range of 0 to 36 months[54,59,61,66,75,78] and 3 in children aged 3 years to 4.5 years[58,65,80]; all 9 of these studies were included in the meta-analysis.[54,58,59,61,65,66,75,78,80] In 3 studies, more than 30% of the children included were stunted.[65,75,80] A summary of these studies is provided in Table 2, with details shown in Table S3 in the Supporting Information online.

The forest plot of EFZ loss by age group is shown in Figure 4 (with adjustment for body weight) and in Figure S4 in the Supporting Information online (without adjustment for body weight). Overall, mean EFZ loss across all ages was 0.75 mg/d (95%CI, 0.60–0.89; $I^2 = 94\%$) and, when adjusted for body weight, was 0.07 mg/kg/d (95%CI, 0.06–0.08; $I^2 = 82\%$). When subgrouped by age category, EFZ loss (mg/d) was lower in infants aged less than 6 months than in young children aged 1 to 3 years. When expressed by body weight, EFZ loss (mg/kg/d) was comparable across the two age groups.

Additional analyses were undertaken to examine the effect of both feeding type (breastfed or solid food) and the Phy:Zn molar ratio of the solid food on EFZ loss.

One study (1 data set) reported EFZ loss from breastfed infants,[59] 2 studies (4 data sets) reported EFZ loss from formula-fed infants,[61,78] and 6 studies (12 data sets) reported EFZ loss from infants fed solid-food diets.[54,58,65,66,75,80] Endogenous fecal zinc loss was lowest in infants fed breast milk and highest in infants fed solid food. When normalized by body weight, EFZ loss was similar in all 3 groups (see Figure S5 and Figure S6 in the Supporting Information online).

Three studies reported the Phy:Zn molar ratio of the solid food fed to the children.[65,75,80] One study, Sheng et al,[75] had 4 arms, each with a different Phy:Zn molar ratio that ranged from 2.1:1 to 3.3:1. The remaining two studies fed food with a Phy:Zn ratio of 23:1[80] and 30:1 and 32:1.[65] The studies were divided into two groups for analysis: those that used a diet with a low Phy:Zn molar ratio (2:1 to 3.5:1), and those that used a diet with a high Phy:Zn molar ratio (23:1 to 32:1). The mean EFZ loss (mg/d) was higher with diets that had a high Phy:Zn molar ratio (mean EFZ loss = 1.03 mg/d; 95%CI, 0.59–1.46 mg/d; $I^2 = 94\%$) compared with diets that had a low Phy:Zn molar ratio (mean EFZ loss = 0.66 mg/d; 95%CI, 0.58–0.75 mg/d; $I^2 = 42\%$), but this difference was not present when EFZ loss was normalized by body weight (diet with high Phy:Zn ratio: mean EFZ loss = 0.07 mg/kg/d; 95%CI, 0.06–0.08; $I^2 = 67\%$ vs diet with low Phy:Zn ratio: mean EFZ loss = 0.06 mg/kg/d; 95%CI, 0.05–0.07; $I^2 = 52\%$, see Figure S7 and Figure S8 in the Supporting Information online). When assessed using the GRADE criteria, the quality of evidence was rated as very low (see Appendix S3 in the Supporting Information online).

The impact of environmental enteric dysfunction on excretion was also considered when examining the data. All studies included in the meta-analysis were reviewed for information related to the health of the participating children. Only one study, Mondal et al,[66] reported the presence of environmental enteric dysfunction in the study cohort, with group 1 having high intestinal permeability and group 2 having low intestinal permeability. There were no other health issues reported in the included studies. It is worth noting that

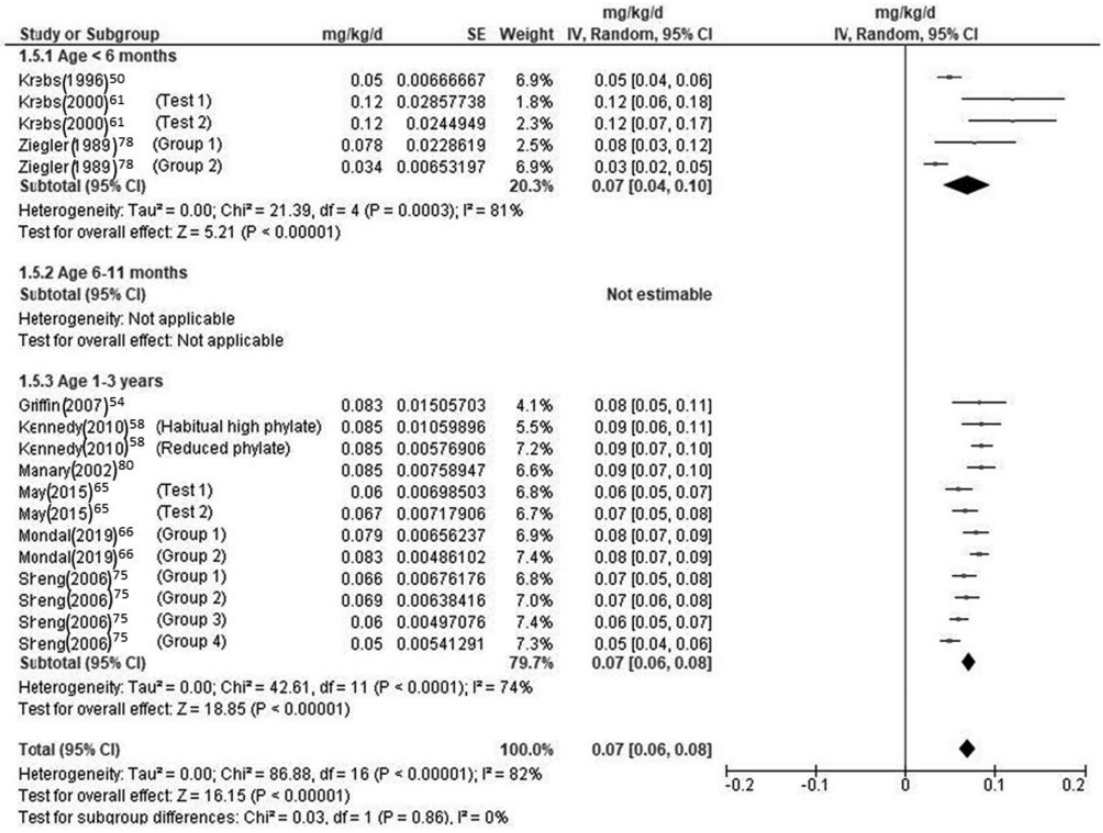

Figure 4 **Forest plot of endogenous fecal zinc excretion (mg/d/kg), by age group.**

4 studies were conducted in the United States,[54,59,61,78] 3 in Malawi,[58,65,80] 1 in China,[75] and 1 in Bangladesh.[66] Removing the data of Mondal et al[66] (group 1 only or groups 1 and 2) from the analysis did not affect the overall mean EFZ loss.

Integumental zinc loss. There were no identified studies assessing zinc losses in sweat and skin in children younger than 3 years. Three studies assessed zinc losses through sweat in children between the ages of 5.5 and 9 years.[51,55,69] Two of these studies assessed zinc loss in sweat using an arm-bag method and calculated the total body loss of zinc from the ratio of mineral and nitrogen in forearm sweat to nitrogen in whole-body loss.[55,69] Chujian et al[51] used a modified procedure of Chen et al[106] and Milne et al[107] to determine whole-body surface zinc loss. The whole-body integumental zinc losses determined from these 3 studies ranged from 0.25 to 1.78 mg/d. Details of these 3 studies are summarized in Table S1 in the Supporting Information online.

Zinc needs for growth. A total of 27 studies assessed zinc in the tissue of donors ranging in age from > 38 gestational weeks to 9 years.[27–30,40–46,71,108–122] Studies were from 16 countries, including low- and middle-income countries[27,28,71,112] and high-income countries.[29,40–46,108–111,113–122] Most studies were cross-sectional in design (n = 25), but there was also one RCT and one case-control study included in the review. Quality, as assessed by the JBI Critical Appraisal Checklist, varied across the studies. The RCT was rated as good quality, the case-control study was rated as poor quality, and the cross-sectional studies were generally of reasonable quality (see Appendix S3 in the Supporting Information online). Tissue samples comprised bone (n = 4) (ie, rib, vertebrae, femur),[29,111,116,118] muscle (n = 4) (ie, diaphragm, abdominal wall, and gluteal area),[27,28,30,43] organs (n = 23) (ie, aorta, brain, eye, glands, gastrointestinal tract, heart, kidney, liver, lung, and skin),[28,40–46,108–115,117,119–122] and whole blood (n = 1).[71] Details of these studies are provided in Table S4 in the Supporting Information online, and the forest plot, including data from all ages, is presented in Figure S9 in the Supporting Information online.

Zinc absorption. A total of 47 studies reported data related to zinc absorption.[39,50–54,56,59–61,63,65,66,68,69,73,75,78,80,86,88–105,123–131] There were 13 RCTs, 21 cross-sectional studies, 11 quasi-experimental studies, 1 case-control study, and 1 cohort study. Studies, regardless of design, were generally of good

quality (see Appendix S3 in the Supporting Information online). Studies reported total zinc absorption (with or without adjustment for endogenous zinc secretion into the gut) or FZA (total absorption/dietary intake) or both.

Studies that included children up to 4.5 years of age were included in the meta-analysis.[58,80,98] The meta-analysis included 15 studies that reported FZA from 24-hour intake studies[39,59–61,65,73,75,78,80,91,96,98,101–103] and 14 that reported FZA from single-meal studies.[39,54,88–90,92–95,97,99,100,104,105] One study assessing absorption from a single meal was excluded because it did not report the standard deviation of the mean.[131] All included studies used a dual-isotope method to calculate FZA. Studies that examined absorption from diets over several days were not included in the meta-analysis because those studies used a different methodological approach involving zinc balance measurements.[50,52,53,56,68,69,86,124,129] Six studies reported that more than 20% of the children included were stunted,[39,65,75,90,105,129] and two studies reported including children with environmental enteric dysfunction.[39,66]

Table 2 summarizes the studies included in the meta-analysis. Details of the included studies can be found in Table S5 (studies assessing absorption from a single meal) and Table S6 (studies assessing absorption from a 24-hour intake) in the Supporting Information online. Details of all other studies, including those conducted in children in the older age ranges, can be found in Table S2 in the Supporting Information online.

Zinc absorption in single-meal studies. Analysis by age group revealed a decline in mean FZA with age, from a mean of 34.24% (95%CI, 27.84–40.63; $I^2 = 90\%$) in the youngest group (< 6 mo) to a mean of 14.54% (95%CI, 11.48–17.61; $I^2 = 99\%$) in the oldest group (≥ 12 mo) (see Figure S10 in the Supporting Information online). The overall mean FZA was 25.44% (95%CI, 22.54–28.34; $I^2 = 99\%$). The GRADE quality-of-evidence assessment was rated as very low for these meta-analyses (see Appendix S3 in the Supporting Information online).

Further analysis was undertaken to explore the impact of feeding type (breast milk, breast milk plus complementary foods, formula, or solid food) on FZA (see Figure S11 in the Supporting Information online). Analysis by feeding type revealed that the mean FZA from breast milk was higher than that from formula milk, with the lowest mean FZA reported in children fed solid food. The mean FZA in children fed breast milk was 51.46% (95%CI, 45.07–57.86; $I^2 = 0\%$), but these data were from just one study, Domellöf et al[95]. In children fed breast milk plus complementary foods, the mean FZA was 31.50% (95%CI, 22.68–40.32; $I^2 = 93\%$) (data from 1 study, Jalla et al[99]). The mean FZA in children fed formula was 32.10% (95%CI, 28.13–36.08; $I^2 = 0\%$) (data from 1 study, Abrams et al[88]). In children fed solid food, the mean FZA was 21.33% (95%CI, 18.36–24.29; $I^2 = 99\%$).

The effect of the dietary Phy:Zn molar ratio on FZA was also explored (see Figure S12 in the Supporting Information online). Studies of infants less than 6 months of age were excluded from the Phy:Zn ratio analysis because infants under 6 months are unlikely to be consuming solid food alone. Datasets were ranked by the Phy:Zn molar ratio, which ranged from < 1 to 26:1. The details of the test meals consumed are presented in Table S5 in the Supporting Information online. Note that Brnić et al[90] provided a meal with and without added phytase in groups 1 and 2, respectively, although the original Phy:Zn molar ratio was the same in both groups. The forest plot was created with and without the added phytase group. Studies in Figure S12 in the Supporting Information online have been ranked by the Phy:Zn molar ratio of the test meal. There is no evidence of an inhibitory effect of increasing phytate on FZA from these single-meal studies.

Zinc absorption in 24-hour studies. Analysis by age group revealed a decline in mean FZA with age, from 32.76% in the youngest group to 23.78% in the oldest group. The overall mean FZA was 26.75% (95%CI, 19.51–26.96; $I^2 = 99\%$). Ranking the studies by zinc intake indicates that FZA generally decreases as intake increases in all 3 age ranges (Figure 5) The quality of evidence assessed using GRADE was rated as very low (see Appendix S3 in the Supporting Information online).

Analysis by feeding type revealed that the mean FZA from breast milk was higher than that from formula milk, with the lowest mean FZA reported in children fed solid food (see Figure S13 in the Supporting Information online). Mean FZA values were as follows: from breast milk, 47.41% (95%CI, 42.03–52.79; $I^2 = 95\%$); from formula milk, 28.76% (95%CI, 21.95–35.57; $I^2 = 97\%$); and from solid food, 22.91% (95%CI, 20.98–24.85; $I^2 = 99\%$). Only one study, Abrams et al,[60] reported zinc absorption from a study of breast milk plus complementary feeding.

The Phy:Zn molar ratio of the children older than 6 months who were included in the 24-hour studies ranged from 1.9:1 to 38:1 The data were subgrouped according to the Phy:Zn molar ratio categories adopted by the European Food Safety Authority[13] in their recent dietary zinc recommendations: ≤ 18 or > 18. Analysis revealed a small reduction in the mean FZA from diets with a Phy:Zn molar ratio > 18 compared with those with a ratio of ≤ 18. For diets with a Phy:Zn ratio of

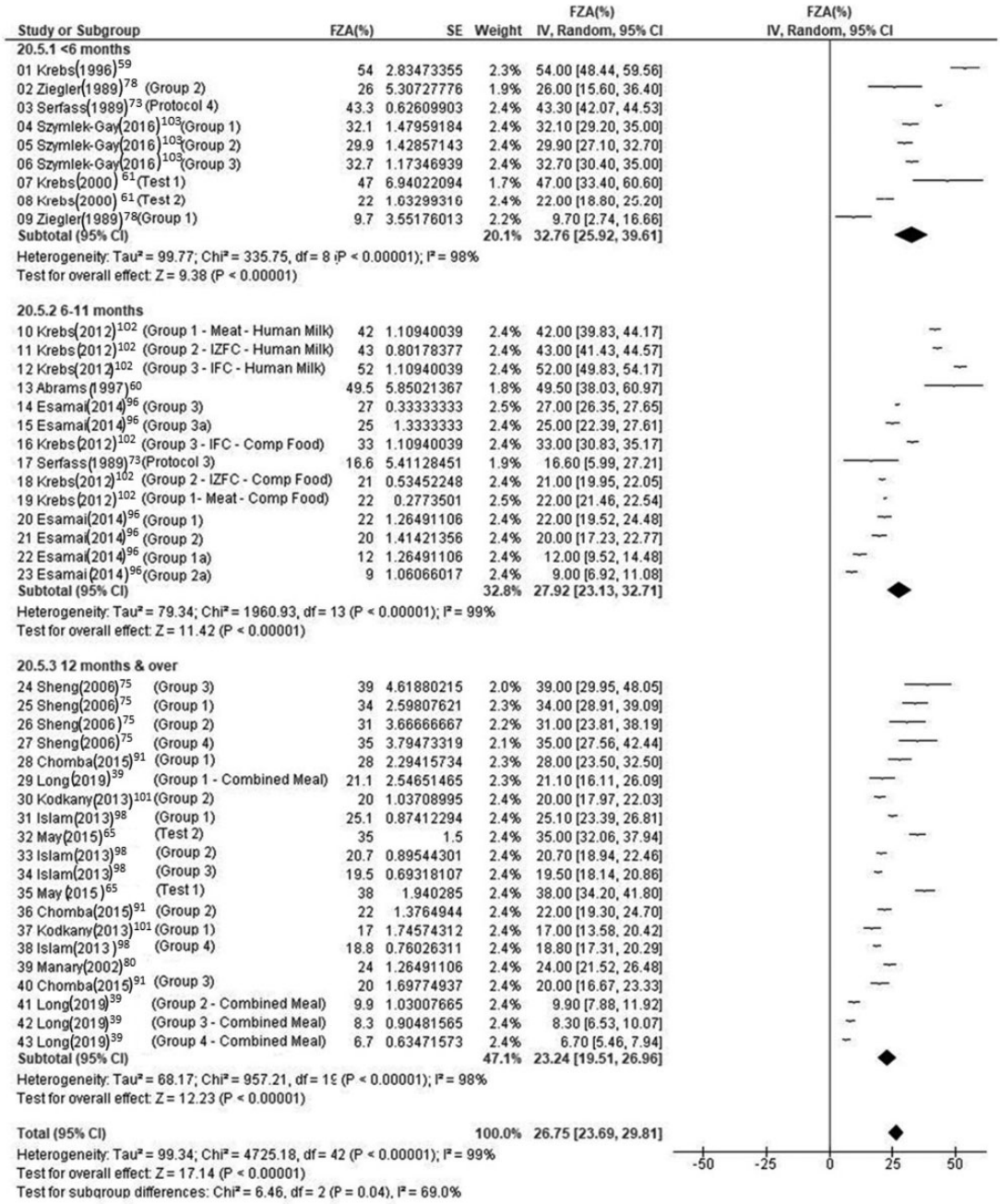

Figure 5 **Forest plot of fractional zinc absorption (%) by age group, ranked by zinc intake from 24- hour studies.**

≤ 18, the mean FZA was 25.15% (95%CI, 21.74–28.56; $I^2 = 91\%$), while for diets with a Phy:Zn ratio of > 18, the mean FZA was 23.93% (95%CI, 20.75–27.12; $I^2 = 95\%$) (see Figure S14 in the Supporting Information online). Details of the foods consumed in each study are provided in Table S6 in the Supporting Information online.

Subgroup analysis by country's income category, ie, low- or middle-income countries (Zambia, Kenya, Bangladesh, India, Malawi, China) and high-income countries (Sweden, United States), was also conducted (see Figure S15 in the Supporting Information online). Results showed that FZA is generally lower in low- and middle-income countries than in high-income countries. The mean FZA in low- and middle-income countries was 22.75% (95% CI, 20.22–25.29; $I^2 = 97\%$) and 34.90% (95%CI, 26.64–43.16; $I^2 = 100\%$) in high-income countries.

Factors affecting zinc bioavailability. A total of 30 studies reported data on the efficacy of zinc absorption

in children aged 0 to 9 years,[39,53,56,63,65,66,68,80,88,90–92,94–100,103–105,123–130] of which 19 were conducted in the target age range of 0 to 3 years.[39,53,56,66,88,90–92,94–97,99,100,103–105,124,129] Results from these studies are summarized in Table 3.

These studies reported on the bioavailability of zinc in the presence of iron,[56,95–97,103,125,128] calcium,[53,68] Na_2EDTA (ethylenediaminetetraacetic acid disodium salt),[94,127] long-term exposure to zinc,[129] phytate/phytase,[63,80,90,104,105,124,130] dietary fiber,[92] resistant starch,[65] lactose,[88] or environmental enteric dysfunction[39,66]; the bioavailability of zinc from fortified vs biofortified foods,[91,98] plant- vs animal-source foods with and without human milk,[99,126] human milk vs formula milk,[100]; and the bioavailability of zinc in the presence or absence of a meal.[104,123]

Overall, there were no statistically significant changes in the bioavailability of zinc in the presence of iron,[56,95–97,103,125,128] lactose,[88] calcium,[53,68] resistant starch,[65] or dietary fiber.[92] Results from one study suggested that zinc absorption in formula-fed children was lower than that in children fed breast milk.[100] One study reported that the addition of human milk to beef and cereal meals decreased FZA and absorbed zinc.[99] Two studies found that zinc absorption in children with environmental enteric dysfunction was low, regardless of whether their lactulose-to-mannitol ratio was low or high.[39,66]

The evidence relating to the effect of phytate/phytase was less clear. In some studies, consumption of dephytinized products seemed to result in higher absorption of zinc,[90,104,105,124] whereas in other studies the consumption of low-phytate products did not seem to have an effect on zinc absorption.[63,130] Similarly, one study found that the EFZ loss of high-phytate diets was high in comparison with results of previous studies.[80]

Several studies examined factors that might affect the bioavailability of zinc, including the presence or absence of a meal,[104,123] Na_2EDTA,[94,127] fortification vs biofortification,[91,98] and plant-source vs animal-source foods,[99,126] but results were conflicting.

DISCUSSION

Using a systematic search strategy and review process, this review has collated and synthesized all available data relevant to the 4 questions posed by the FAO-WHO expert group convened to update dietary zinc recommendations for children aged 0 to 3 years.

The first question was designed to gather data related to zinc content in tissue in children within the target age range in order to facilitate an estimation of the zinc required to synthesize new tissue for growth. Current FAO-WHO values for zinc requirements in young children hypothesize that new tissue contains 30 µg of zinc per gram of wet weight tissue (zinc concentration, 0.46 mmol/g),[15,132] values that were extrapolated from adult data from a study by Widdowson[133] in 1964. Other expert groups have considered 20 µg of zinc per gram of wet weight to be needed for tissue accretion.[13,14,16] The present systematic review revealed wide variation in the reported zinc content of organ tissues of children, both within and between tissue types. For example the mean wet weight zinc content ranged from 7.8 µg/g (95%CI, 5.14–10.46) in the brain to 92.4 µg/g (95%CI, 68.56–116.24) in the liver. On a whole-body basis, muscle and bone are two of the largest contributors to the total body zinc content in young children, yet findings showed a lack of new evidence reporting zinc concentrations in these tissues, Moreover, the evidence available came from a very small number of cadaver studies conducted between 1968 and 1984.[27–30,43,111,116,118] Zinc concentrations in muscle and bone were commonly reported per gram of dry weight and per gram of ash, respectively, requiring conversion to wet weight. If these conversions can be made based on assumptions of the water and organic content of the tissues, then an estimate of tissue accrual across the age range is required to arrive at the amount of zinc needed for growth. Modeling techniques to achieve this are currently being considered by the FAO-WHO expert group.

The purpose of the second question was to identify the routes of zinc loss and the quantity of zinc lost through endogenous excretion. Zinc is lost through urine, EFZ loss, and the integument. Results of the meta-analysis revealed that urinary zinc excretion in children aged 0 to 3 years, expressed on a per kilogram of body weight basis, was negatively correlated with age. Unsurprisingly, total fecal zinc excretion was highly dependent upon dietary zinc intake (irrespective of body weight) and ranged from 1.5 to 7.5 mg/d. The contribution to zinc loss through was lowest in infants fed breast milk and highest in infants fed solid food. When normalized by body weight, EFZ loss was consistent across the age ranges at 70 µg/kg/d and did not appear to be affected by the Phy:Zn molar ratio of the diet. Integumental losses through sweat and skin made a small contribution to daily zinc losses.[15] On the basis of a study in adult men,[15] the WHO estimated an integumental loss of 0.5 to 0.7 mg/d in adults. Although no studies included in this systematic review reported integumental loss in children aged 0 to 3 years, studies in older children estimated losses to be between 0.06 and 0.12 mg/d or between 0.003 and 0.120 mg/kg/d.

The final two questions considered the efficiency of zinc absorption and the factors that affect this in the target age range. Meta-analysis of FZA determined

Table 3 **Summary of studies assessing factors that influence zinc absorption**

Factors that affect zinc absorption in each age group (no. of studies)	Reference	Conclusion
Iron intake		
0–3 years (n = 5)	Domellöf et al (2009)[95]	Iron intake at 1 mg/kg/d did not significantly alter zinc absorption.
	Esamai et al (2014)[96]	Iron at 12 mg/d in micronutrient powder did not significantly alter zinc absorption.
	Fairweather-Tait et al (1995)[97]	No significant difference in absorption from fortified meal (iron, 263 mg/kg) and unfortified product (iron, 39.2 mg mg/kg).
	Haschke et al (1986)[56]	No significant difference in absorption between fortified (iron, 10.2 mg/L) and unfortified product (iron, 2.5 mg/L)
	Szymlek-Gay et al (2016)[103]	Neither supplemental iron (iron, 1.2 mg/d) or iron fortification (iron, 6.4 mg/d) nor the amount of iron habitually consumed (5.7 mg/d) altered zinc absorption
4.5–9 years (n = 2)	Etcheverry et al (2007)[125]	Iron intake at different levels (3.85 mg/d, 5.85 mg/d, 9.85 mg/d, 3.85 mg/d, 5.85 mg/d, or 9.85 mg/d) did not affect absolute zinc absorption.
	Hettiarachchi et al (2010)[128]	No significant difference in zinc absorption between high (iron, 9 mg) and low (iron, 4.5 mg) supplementation.
Phytate/phytase intake		
0–3 years (n = 3)	Brnić et al (2017)[90]	Adding 20.5 phytase units in meals (phytic acid content, 108.2 mg) can increase zinc absorption in young children. $P < 0.0001$
	Davidsson et al (2004)[124]	Apparent zinc absorption measured by a stable isotope technique was greater from the dephytinized formula (6 mg of phytic acid per kilogram of liquid formula) than from the regular soya formula (300 mg of phytic acid per kilogram of liquid formula) ($P = 0.03$), but this was not statistically significant in the 72-h chemical balance study.
	Zyba et al (2019)[105]	TAZ from meals containing phytase (588 phytase units) was more than double that of TAZ from test meals without phytase.
3–4.5 years (n = 1)	Thacher et al (2009)[104]	Enzymatic dephytinization increased zinc absorption ($P < 0.001$). Fermentation of the meal did not modify zinc absorption. Rickets was not associated with impaired zinc absorption.
	Manary et al (2002)[80]	Endogenous fecal zinc associated with high-phytate diets (phytate:zinc molar ratio of 23:1) was high in comparison with results for this measure in previous studies.
4.5–9 years (n = 2)	Manary et al (2000)[63]	No effect of phytate reduction (phytase enzyme, 5000 U/g) was seen in well children (phytate:zinc molar ratio: low phytic acid, 7, vs high phytic acid, 30).
	Mazariegos et al (2006)[130]	Zinc absorption was not increased by the long-term use of low-phytate maize in children whose major dietary staple is maize.
Dietary fiber intake		
0–3 years (n = 1)	Davidsson et al (1996)[92]	Dietary fiber from weaning cereals in a low (1.8% dietary fiber) or higher (8% dietary fiber) level did not have a negative effect on the apparent absorption of zinc in healthy formula-fed infants. Absorption of zinc was relatively high and was comparable to earlier reported values for a wheat/milk-based weaning cereal.
Lactose intake		
0–3 years (n = 1)	Abrams et al (2002)[88]	No difference in FAZ and TAZ between lactose (70% lactose) and lactose-free (70% corn maltodextrin) formula.
Calcium intake		
0–3 years (n = 1)	Devizia et al (1985)[53]	Dietary calcium (389 mg/L vs 659 mg/L vs 1024 mg/L) had no significant effect on absorption of zinc.
4.5–9 years (n = 1)	Price et al (1970)[68]	The form of calcium used did not affect zinc retention at either protein level.
Long-term zinc intake		
0–3 years (n = 1)	López de Romaña et al (2005)[129]	FAZ was inversely related to zinc intake from zinc-fortified meals ($P < 0.001$), but TAZ was progressively greater in children who received higher amounts of zinc ($P < 0.001$). The mean FAZ from the zinc-fortified meals did not change significantly during approximately 7 weeks, and the inverse relation between mean

(continued)

Table 3 **Continued**

Factors that affect zinc absorption in each age group (no. of studies)	Reference	Conclusion
		zinc intake and FAZ remained significant in the final studies. TAZ, expressed as mg/d, decreased slightly, but not significantly, from baseline, although these changes from the initial values were significant when expressed in relation to body weight ($P < 0.007$).
Zinc fortification and biofortification of foods		
0–3 years (n = 1)	Chomba et al (2015)[91]	FAZ from control maize (zinc, 2.3 mg/d) did not differ from FAZ from the biofortified maize (zinc, 5.0 mg/d). TAZ from the biofortified maize was higher than TAZ from the control maize ($P = 0.0001$) but did not differ from TAZ from the fortified maize (zinc, 6.3 mg/d).
3–4.5 years (n = 1)	Islam et al (2013)[98]	Absorption of zinc from conventional rice and high-zinc rice variety was not different, but TAZ from conventional rice fortified with zinc was higher compared with conventional rice and high-zinc rice variety ($P < 0.001$).
Plant-sourced vs animal-sourced zinc (for age group 0–3 years, with and without intake of human milk)		
0–3 years (n = 1)	Jalla et al (2002)[99]	FAZ did not differ between beef and cereal. The higher intake of zinc from beef (zinc, 1.5 mg/d) vs cereal (zinc, 0.2 mg/d) resulted in a greater amount of TAZ ($P < 0.05$). The addition of human milk decreased both FAZ ($P < 0.05$) and TAZ ($P < 0.05$).
4.5–9 years (n = 1)	Etcheverry et al (2006)[126]	Intake of beef protein (2.75 mg of endogenous zinc and 2 mg of extrinsic zinc as zinc-67) contributed to greater zinc absorption than equivalent intake of soy protein meal (1.22 mg of endogenous zinc and 3.53 mg of extrinsic zinc, of which 2 mg was zinc-67) ($P = 0.047$).
Human milk intake vs formula milk intake		
0–3 years (n = 1)	Johnson & Canfield (1989)[100]	Zinc absorption in formula-fed infants was lower than absorption in breastfed infants, despite a higher zinc intake (7.91 ± 0.08 mg/L vs 1.56 ± 0.46 mg/L) ($P < 0.01$).
Environmental enteric dysfunction		
0–3 years (n = 2)	Long et al (2019)[39]	Zinc absorption did not differ between groups with low lactulose-to-mannitol ratio (< 0.09) and high lactulose-to-mannitol ratio (≥ 0.09). Regardless of lactulose-to-mannitol ratio, only groups exposed to higher zinc intake (10–15 mg) met the recommended values for daily zinc absorption ($P = 0.002$).
	Mondal et al (2019)[66]	No differences in zinc homeostasis between groups of low lactulose-to-mannitol ratio (< 0.09) and high lactulose-to-mannitol ratio (≥ 0.09).
Resistant starch intake		
3–4.5 years (n = 1)	May et al (2015)[65]	Consumption of resistant starch (8.5 g/d) did not improve zinc homeostasis.
Na_2EDTA		
0–3 years (n = 1)	Davidsson et al (2005)[94]	Absorption from NaFeEDTA (3.7%) was not different than absorption from ferrous sulfate plus ascorbic acid (4.9%).
4.5–9 years (n = 1)	Hettiarachchi et al (2004)[127]	Zinc absorption was higher in the group with Na_2EDTA (Na_2EDTA 4.7%) than in the group without Na_2EDTA (Na_2EDTA 2.2%) ($P = 0.04$).
Zinc absorption from fortified juice, with or without a meal		
4.5–9 years (n = 1)	Avalos Mishaan et al (2004)[123]	No difference in zinc absorption with or without a meal.
3–4.5 years (n = 1)	Thacher et al (2009)[104]	Zinc absorption was lower with a meal than without a meal ($P < 0.001$).

Abbreviations: FAZ, fractional absorption of zinc; NaFeEDTA, ferric sodium ethylenediaminetetraacetate; Na_2EDTA, ethylenediaminetetraacetic acid disodium salt; TAZ, total absorption of zinc.

from 24-hour studies revealed that the mean FZA declined with age: 32.76% in the youngest age category (< 6 months) and 23.87% in the oldest age category (> 12 months). This is, at least in part, due to the high bioavailability of zinc from human breast milk: the mean FZA from 24-hour studies was 47.41% for breast milk, compared with 28.7% for formula milk and 22.91% for solid food. The data from single-meal studies also reflect this decrease in FZA with age and the high FZA from breast milk (51.46%) compared with formula (32.10%) or solid food (21.33%). The proportion of dietary zinc absorbed from complementary and weaning foods is dependent on the bioavailability of zinc in the food consumed. A number of dietary

components that influence dietary zinc absorption in adults have been reviewed previously.[10] The picture is complex, being largely dependent on the combination of foods consumed together. Phytate is well established as an inhibitor of zinc absorption in adults, but the addition of phytase to food ameliorates this effect.

Current FAO-WHO values assume an FZA of 80% from breast milk and an FZA of 30% for nonexclusively breastfed infants, unless infants consume diets with a high content of phytate, in which case the FZA is 15%.[15] In a previous meta-analysis of data from infants and children, the inhibitory effect of phytate was not demonstrated.[134] Grouping the FZA reported from 24-hour studies of children older than 6 months of age according to a dietary Phy:Zn molar ratio of <18 or ≥ 18 indicated a small reduction in mean FZA in the higher phytate group, but the 95%CIs were broad, reflecting heterogeneity across the studies. Ranking the FZA data from single-meal studies by Phy:Zn molar ratio did not suggest an inhibitory effect of increasing Phy:Zn ratio, which ranged from < 1 to 26. However, grouping the studies by the income category of the country in which they were conducted suggested an overall higher FZA in the high-income countries; however, since the majority of the studies were conducted in low- and middle-income countries and only two were conducted in high-income countries, it is not possible to draw firm conclusions.

A total of 19 studies were identified that explored the impact of 14 different dietary components on zinc bioavailability in children aged 0 to 3 years, including iron, phytate and phytase, calcium, and lactose. While there were insufficient data to conduct a meta-analysis of individual dietary components, most studies investigating enzymatic phytate degradation of meals reported an increased zinc absorption in healthy children.[90,104,105,124] Other studies, however, found either no statistically significant effect of phytate reduction[63] or no effect on zinc absorption with long-term use of low-phytate maize.[130] Previous studies have shown conflicting evidence on the effect of iron on zinc bioavailability.[10,135] The narrative synthesis of the studies included in this review suggests that iron supplementation does not have any adverse effect on zinc absorption in young children.

Table 4 presents a summary of how the results of this systematic review and meta-analysis may be used to update or adapt infant and child nutrition guidelines.

Strengths and limitations

This systematic review presents a comprehensive synthesis of studies published in high-, middle-, and low-income countries that assessed zinc losses, zinc absorption, and zinc content of different tissues in children (0–9 years) but does not attempt to preempt the subsequent FAO-WHO expert group discussions regarding the inclusion or exclusion of individual studies or data sets from the final calculations. Meta-analyses were focused primarily on studies conducted in children aged 0 to 3 years that evaluated zinc losses and gains and that explored the influence of age, zinc intake, feeding type, Phy:Zn of diets, and country's income category. A key strength of this systematic review is that it has generated a comprehensive database that will be made available through open access. A limitation is that many of the studies included in the review were conducted in low- and middle-income countries, where environmental enteric dysfunction is likely to be prevalent. Most studies failed to report the presence of environmental enteric dysfunction among their sample, which could lead to lower absorbance and higher zinc excretion values compared with those from samples without environmental enteric dysfunction. The substantial or considerable heterogeneity evident in most meta-analyses, including subgroup analyses, reflects the variability in the evidence base (ie, clinical and methodological heterogeneity). Likewise, the low to very low GRADE quality-of-evidence ratings reflect this heterogeneity and are also a result of the largely nonrandomized data set. Consequently, the results must be interpreted with caution. It is not yet known how the current evidence on zinc gains and losses will impact the resulting dietary recommendations, which are still under discussion, but it will be evaluated and the impact disclosed when the report of new recommendations is published.

CONCLUSION

This review presents a synthesis of the published data on levels of zinc in tissue, zinc losses and gains, and factors affecting the bioavailability of zinc in children aged 0 to 3 years. The pooled analysis highlights the inverse relationship between urinary zinc excretion and age in this age range, a relationship that persists when zinc excretion is adjusted for body weight. Similarly, FZA varies considerably within this age range, depending on whether breast milk, formula, or complementary food is consumed. In contrast, EFZ loss (per kilogram of body weight) appears to be relatively constant across dietary types and Phy:Zn ratios. There is some conflicting evidence about the impact of phytate and enzymatic reduction of the phytate content of foods on zinc bioavailability in this age range. There is a paucity of data regarding tissue zinc concentrations in the target age range, and therefore alternative approaches must be adopted to estimate the zinc needs for the synthesis of new tissue for growth. This systematic review may be a

Table 4 **Contributions of this systematic review and meta-analysis to the body of knowledge, and how the data might be used to update or adapt dietary guidelines for infants and children**

	Age range	FAO-WHO 2004	EFSA 2014	IOM 2001	Contribution of this systematic review to new FAO-WHO recommendations
Approach	0–6 mo	Factorial	Breast-fed, no DRVs	Breast-fed, no DRVs	FAO-WHO is exploring the use of the factorial approach in updating dietary zinc recommendations for the age range 0–3 years. This systematic review is the first step in the process to gather relevant data from published literature.
	7–11 mo	Factorial	Factorial	Factorial	
	1–3 y	Factorial	Factorial	Factorial	
Zinc required for growth	0–6 mo	0.120 mg/kg/d (females); 0.140 mg/kg/d (males)	NA	NA	EFSA[13] and IOM[14] used a tissue zinc concentration of 20 μg/g for children aged 7 mo to 3 years. IOM[14] increased this to 30 μg/g for the age range 1–3 years. FAO-WHO[15] used 30 μg/g for the entire age range. These values were based on Widdowson & Spray[40] estimates for the synthesis of new tissue and multiplied by the estimated daily lean tissue accretion rate. Tissue zinc concentration data retrieved by this systematic review will be used to estimate needs for growth using a new theoretical modeling approach.
	7–11 mo	0.033 mg/kg/d	0.230 mg/d	0.260 mg/d	
	1–3 y	0.030 mg per gram of tissue	0.131 mg/d	0.120 mg/d	
Zinc losses					For infants aged 0–11 months, FAO-WHO[15] estimated total zinc loss values from direct measurements. For children aged 1–3 years, they used a value for total endogenous losses that was extrapolated from estimates in adults and based on a metabolic rate of 0.57 μg of zinc per basal kcal. For infants aged 7–11 months, EFSA[13] and IOM[14] estimated EFZ from empirical data from breastfed infants aged 2–4 months and adjusted by reference body weights. This systematic review presents data, primarily in the age range 1–3 years, which may be used to estimate EFZ directly.
Endogenous fecal	0–6 mo	0.020 mg/kg/d (breastfed); 0.040 mg/kg/d (formula-fed or weaning foods) These are total endogenous losses.	NA	NA	
	7–11 mo		0.343 mg/d	0.450 mg/d	
	1–3 y	0.57 μg/basal kcal	0.738 mg/d	0.442 mg/d	
Urinary	0–6 mo	Total endogenous losses above	NA	NA	
	7–11 mo		0.054 mg/d	0.066 mg/d	EFSA[13] and IOM[14] extrapolated values from adults or younger children using isometric scaling (linear, based on body weight). This systematic review presents data in the age ranges 7–11 months and 1–3 years that may be used to estimate urinary losses directly.
	1–3 years		0.075 mg/d	0.097 mg/d	
Integumental	0–6 mo	Total endogenous losses above	NA	NA	EFSA[13] and IOM[14] extrapolated values from adults. Although this systematic review did not identify any data for children 0–3 years of age, 3 studies in older children were identified that may be relevant.
	7–11 mo		0.105 mg/d	0.060 mg/d	
	1–3 y		0.130 mg/d	0.085 mg/d	
Fractional absorption	0–6 mo	50% (high bioavailability) 30% (medium bioavailability) 15% (low bioavailability)	NA	NA	This systematic review provides an updated analysis of factors that impact FZA in the target age range. These will be considered when setting the new FZA value, which will be based on bioavailability from different types of diets in each age subgroup.
	7–11 mo		30%	30%	
	1–3 y		30%	30%	

Abbreviations: DRV, Dietary Reference Value; EFSA, European Food Safety Authority; EFZ, endogenous fecal zinc; FAO-WHO, Food and Agriculture Organization of the United Nations–World Health Organization; FZA, fractional zinc absorption; IOM, Institute of Medicine; NA, not applicable.

useful resource for future national and international organizations looking to update or adapt dietary guidelines for infants and children.

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Availability of data and material. Template data collection forms; data extracted from included studies; data used for all analyses; and any other materials used in the review are available upon request from the corresponding author.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA 2020 checklist.**

Appendix S2 **Search strategies.**

Appendix S3 **Quality assessment and risk of bias.**

Figure S1 **PRISMA flow chart of the search for zinc losses.**

Figure S2 **Forest plot of zinc excretion through urine (μg/d), by age group.**

Figure S3 **Forest plot of total fecal zinc excretion (mg/d), with studies ranked by zinc intake.**

Figure S4 **Forest plot of endogenous excretion (mg/d), by age group.**

Figure S5 **Endogenous fecal excretion of zinc (mg/d), by feeding type.**

Figure S6 **Endogenous fecal excretion of zinc (mg/kg/d), by feeding type.**

Figure S7 **Endogenous fecal excretion of zinc (mg/d), by phytate:zinc molar ratio.**

Figure S8 **Endogenous fecal excretion of zinc (mg/kg/d), by phytate:zinc molar ratio.**

Figure S9 **Forest plot of zinc in tissues (μg/g) in infants and children aged 0–3 years.**

Figure S10 **Forest plot of fractional zinc absorption (FZA [%]) by age group, stratified by intake from single-meal studies.**

Figure S11 **Fractional zinc absorption (FZA [%]) by feeding type (excludes Johnson 1989) from single-meal studies.**

Figure S12 **Forest plot of fractional zinc absorption (FZA [%]), ordered by phytate:zinc molar ratio from single-meal studies.**

Figure S13 **Fractional zinc absorption (FZA [%]) by feeding type from 24-hour studies.**

Figure S14 **Forest plot of fractional zinc absorption (%) subgrouped by phytate:zinc molar ratio from 24- hour studies.**

Figure S15 **Fractional zinc absorption (FZA [%]) by country's income category in 24-hour studies.**

Table S1 **Studies assessing zinc losses excluded from the meta-analyses.**

Table S2 **Studies assessing zinc absorption excluded from the meta-analyses.**

Table S3 **Studies assessing zinc losses included in the meta-analyses.**

Table S4 **Studies assessing zinc needs for growth.**

Table S5 **Studies included in the meta-analysis assessing zinc absorption from single-meal studies.**

Table S6 **Studies included in the meta-analysis assessing zinc absorption from 24-hour studies.**

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