Adverse effects of excessive zinc intake in infants and children aged 0-3 years: A systematic review and meta-analysis

Marena Ceballos-Rasgado[a], Nicola M Lowe [a]*, Simonette Mallard [b] , Andrew Clegg [c] , Victoria H Moran [a], Catherine Harris [c] , Jason Montez [d], Maria Xipsiti [e].

a Centre for Global Development, University of Central Lancashire, Preston, United Kingdom

b Independent contractor, Dunedin, New Zealand

c Synthesis, Economic Evaluation and Decision Science (SEEDS) Group, Applied Health Research Hub, University of Central Lancashire, Preston, United Kingdom.

d Department of Nutrition and Food Safety, World Health Organization (WHO), Geneva, Switzerland

e Food and Nutrition Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

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*Professor Nicola M Lowe, Director of the UClan Research Centre for Global Development

Faculty of Allied Health and Wellbeing, University of Central Lancashire, Preston UK.

Email: nmlowe@UClan.ac.uk

Abstract

Zinc supplementation reduces morbidity, but evidence suggests that excessive intakes may have negative health consequences. Current guidelines of upper limits (ULs) of zinc intake for young children are extrapolated from adult data. This systematic review (PROSPERO; registration no. CRD42020215187) aimed to determine the levels of zinc intake at which adverse effects are observed in young children. Studies reporting potential adverse effects of zinc intake in children aged 0-3 years were identified (from inception to August 2020) in MEDLINE, EMBASE and the Cochrane Library, with no limits on study design. Adverse clinical and physical effects of zinc intake were synthesized narratively, and meta-analyses of biochemical outcomes were conducted. Random effects models, forest plots were generated to examine the evidence by age category, dose, dose duration, chemical formula of zinc, and zinc vs placebo. The Joanna Briggs Institute Critical Appraisal Checklist, Cochrane Risk of Bias 2, and Grading of Recommendations Assessment, Development and Evaluation guideline (GRADE) were employed to assess risk of bias and to appraise the certainty of evidence. Fifty-eight studies assessed possible adverse effects of zinc doses ranging from 3 to 70 mg/d. Data from 39 studies contributed to meta-analyses. Zinc supplementation had an adverse effect on serum ferritin, plasma/serum copper concentration, sTfR, hemoglobin, hematocrit, and the odds of anemia in at least one of the subgroups investigated. Lactulose:mannitol ratio was improved with zinc supplementation, and no significant effect was observed on CRP, eSOD, ZPP and blood cholesterol and iron deficiency anemia. The certainty of the evidence, as assessed using GRADE, was very low to moderate. Although possible adverse effects of zinc supplementation were observed in some subgroups, it is unclear whether these findings are clinically important. The synthesized data can be used to undertake a dose-response analysis to update current guidelines of ULs of zinc intake for young children.

Key words: Zinc, dietary requirements, children, upper limits, systematic review, meta-analysis

Statement of significance

This systematic review and meta-analysis was commissioned by the FAO-WHO, and aimed to synthesize the available evidence to answer clearly defined questions posed by the FAO-WHO expert group, regarding zinc intake in relation to needs for growth (tissue composition), excretion, absorption, and factors affecting bioavailability in children aged 0-3 years Findings of this review may be a valuable resource for future national and international organizations looking to update or adapt dietary guidelines for infants and children.

Introduction

The Upper Limits (ULs) of nutrient intakes have been defined as the maximum intake from food, water and supplements that is unlikely to pose risk of adverse health effects to most individuals in the general population (1). This information is particularly valuable when designing large-scale supplementation or fortification programs to ensure that the resulting nutrient intakes do not exceed a value that is considered safe for human health. ULs are determined through a risk assessment process which assesses the probability of the occurrence of an adverse health effect from an excess exposure to the nutrient (2). This process requires the collection of information of known or potential adverse effects attributed to the nutrient, followed by a dose-response analysis to determine the relationship between the dose of the nutrient and adverse effect on key outcome measures (3). For most nutrients no adverse effects are anticipated when they are consumed as foods because their absorption and/or excretion are regulated through homeostatic mechanisms (1). This is the case for zinc, where absorption and excretion are adjusted over a wide range of dietary intakes (4). In

addition, zinc is not stored in body tissues, thus the potential for zinc to reach toxic levels is limited. However, if zinc is ingested in excessive amounts or in smaller amounts but on a chronic basis through supplementation, it is associated with deleterious alterations in iron, copper lipoprotein and cholesterol metabolism (3), and adverse physiological effects including nausea, vomiting and general gastrointestinal disturbances (3,5).

The current Food and Agriculture Organization (FAO) - World Health Organization (WHO) values for zinc ULs are 35-50 mg/day (690 mmol/day) for adults, and 23–28 mg/day (350-430 mmol/day) for children depending on their age (1). In setting these ULs, a dose response analysis for children was not possible due to a lack of data, therefore the ULs for children in various age categories were extrapolated from adult data based on basal metabolic rate (1,3). An alternative strategy was adopted when considering zinc ULs by expert groups convened by the Institute of Medicine (IOM)(6) and International Zinc Nutrition Consultative Group (IZiNCG)(7). Both groups used data from a small number of studies conducted in children relating to the impact of zinc intake on copper status. IZiNCG concluded that there were insufficient data to define ULs for children and instead published a "No Observed Adverse Effect Level" (NOAEL) value of 6-26 mg/day depending on the age of the child. The IOM identified a NOAEL value and divided it by an uncertainty factor which considered the length of exposure and the number of infants included in the one study (8). After obtaining values for young infants, the IOM adjusted the ULs for older infants and children on the basis of relative body weight to produce a recommendation of 4 mg/day for infants 0-6 months, 5- mg/day for infants 7-12 months and 7 mg/day for children 1-3 years (6).

FAO-WHO has convened an expert group to update their vitamin and mineral requirements and ULs of intake for micronutrients in children aged 0-3 years (9), and commissioned this review to inform the work of updating the ULs for zinc in this age group. The aim of this

review was to determine the levels of zinc intake at which adverse effects are observed in children aged 0-3 years

Methods

Protocol and registration

This systematic review was registered with the international Prospective Register of Systematic Reviews (PROSPERO; registration no. CRD42020215187) and was conducted following the PRISMA-2020 statement for reporting systematic reviews and meta-analyses (10).

Eligibility Criteria

Eligibility criteria are based on the Population, Intervention, Comparison, Outcomes and Study (PICOS) elements. Criteria were identified through discussion with the expert group and shown in **Table 1**.

Search Strategy, Study Selection and Data Extraction

The search was carried out using Medline (OVID), Embase (OVID), Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Trials (CENTRAL) (Cochrane Library) from date of inception to 7th August 2020. The searches had no date nor language restriction. The initial search strategy is presented in **Supplementary file 1** and comprised terms related to zinc, adverse effects toxicity and outcomes known to be potentially affected by excess zinc. As a preceding scoping review indicated a lack of studies investigating excess zinc intake among children aged 0-3 years, the search was not limited by age. However, for the purposes of this manuscript, only studies that included children aged 0-3 years will be reported.

The search results were downloaded into Endnote software for automatic and manual deduplication and then exported into the Rayyan web app (11) where one reviewer (MCR) screened for inclusion and exclusion by title and abstract. The articles were flagged when there was uncertainty, and discussions for their inclusion or exclusion took place with senior members of the review team (NML, VHM). Hand-searches were conducted by examining the reference lists of the retrieved articles, and relevant systematic reviews. Articles potentially meeting inclusion criteria and those that remained uncertain were moved forward to the next stage, where one reviewer (SM) screened the full text. At each stage of screening, a randomly selected 10% of articles were cross-checked by a second member of the review team (NML, VHM or MCR). Any disagreement was resolved by discussion and changes made accordingly.

Due to the broad range of outcome measures reported by a small number of studies, the search strategy was adjusted to include studies that did not have terms related to toxicity and adverse effects in the title and abstract. The expanded search strategy is shown in **Supplementary file 1**. The purpose of this expanded search was to capture studies that measured relevant outcomes without the presupposition of toxicity, thus increasing the number of included studies, particularly at the lower zinc dose/exposure rates. The expanded search results were de-duplicated against the original search and screened for inclusion and exclusion as described above **(Supplementary file 1)**.

Data extraction and synthesis

One reviewer (SM) extracted the data from the included articles into a specifically designed Excel form, and a randomly selected sample (10%) of extracted articles was cross-checked by a member of the review team (MCR). Data extracted included bibliographic information, location, aim, study methods, population characteristics, type of exposures to zinc (the type of exposure, duration of exposure, amount of zinc exposed to), outcomes (adverse event or

status measures), and background dietary zinc intake. If dietary zinc intake was not reported, data obtained from external studies conducted in areas and with demographics close to the study were used, with preference given to data nearest in age range to that of the study population. Where such data were not available, data from national surveys were obtained. Efforts were also taken to directly contact authors for dietary intake data, if available. For studies including only infants <6 months of age at baseline (n=2) (12,13), estimated dietary zinc intake from exclusive breastfeeding was used, obtained from a systematic review on breast milk intake commissioned by FAO-WHO to inform the work of updating requirements and ULs (14). For studies in which zinc intake was only assessed from complementary foods, breast milk zinc intake was added to the complementary food zinc intake. These age-matched values were taken from the FAO-WHO breast milk review , to provide a total dietary zinc intake estimate (14).

Studies were considered for meta-analyses if they included children 0-3 years and where the effect of zinc intake could be isolated (including a comparable arm without zinc). Where possible, forest plots were generated for the following: age category [0-90 days, 91-180 days, 6-12 months, >12 months], dose of zinc given [<5mg/d, 5-10 mg/d, 10.1-20mg/d, >20 mg/d, bolus], duration of intervention [0-3 months, > 3 months), chemical formula of zinc [gluconate, sulphate, acetate, not stated, other], zinc vs placebo, and high vs low dose of zinc.

A narrative synthesis was undertaken incorporating information about the population characteristics, zinc exposure and physical and clinical descriptive outcomes proposed by the FAO-WHO expert group (i.e., vomiting, regurgitation, nausea, constipation, abdominal pain, drowsiness, mouth irritation, taste aversion, diarrhea, and dysentery). Because there was no standard way of reporting these outcomes, they were not considered for meta-analyses.

Statistical Analysis

Meta-analyses were conducted using RevMan (Version 5.4. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration 2014). Where outcomes were presented as continuous data, they were synthesized as weighted mean differences with 95% confidence intervals (CI) using the generic inverse-variance method. Dichotomous data were pooled as odds ratios (OR) with 95% CI through the Mantel-Haenszel method. Given the likelihood of variability among the studies, we estimated random-effects models. Heterogeneity was assessed through visual inspection of forest plots and through the Chi^2 and I^2 statistic, with possible causes investigated through sub-group and sensitivity analyses. Publication bias was assessed using funnel plots for comparisons of 10 or more studies (15), with the causes of asymmetry judged in relation to non-reporting bias, methodological quality, heterogeneity and artefactual reasons. Where appropriate, GRADE assessment was adjusted to reflect any bias identified.

Assessment of Risk of Bias

Cochrane Risk of Bias 2 (RoB) was used to assess the quality of all randomized controlled trials (RCTs) (16) Four trials were classified as 'quasi-experimental' rather than RCT (8,17–19) because details of the randomization process were not made explicit, although all had elements of random allocation and all were double blind. In two studies participants were allocated into groups based on successive age sequence (8,18) and in two studies methods of allocation were not adequately described (17,19). Therefore these studies were deemed acceptable for inclusion in meta-analyses by consensus and were assessed using Cochrane RoB. Two case studies (20,21) were assessed using the Joanna Briggs Institute (JBI) Appraisal checklist (22). The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (23) system was used to evaluate the certainty of evidence of all outcomes included in meta-analyses. As all studies included in meta-analyses had been assessed using Cochrane RoB, the initial level of certainty for GRADE was high (24) .

Reasons for considering lowering the level of certainty included risk of bias, inconsistency, indirectness (such as the population having an underlying health condition), imprecision and publication bias (25). Raising the level of certainty was not considered as this is generally used for observational studies only (24).

Results

Description of studies

From the studies retrieved by the initial electronic search (N=7,158), hand searches (N=90), and the expanded search (N=15), 316 potentially relevant studies were assessed for inclusion once the full-text reports had been obtained. From these, 180 papers were excluded on a full-text basis and the reasons for exclusion are shown in the PRISMA diagram in **Figure 1**. A total of 136 articles were considered relevant across all population groups (N=58 infants and children aged 0-3 years, N= 18 children aged >3 years, and N=53 adults). As the evidence available on infants and children aged 0-3 years was sufficient to avoid extrapolation from data on older children and adults, this paper focusses only on 58 articles from 55 studies (8,12,13,17–21,26–75) that investigated possible adverse effects of excess of zinc intake in infants and children aged 0-3 years. A complete list of biochemical outcome measures and physical and clinical descriptive outcomes included in these studies are given in **Table 2**. (Detailed information on study design can be found in **Table 1 of the supplementary file 2**).

Two articles, Hess et al. (43) and Sazawal et al. (63), were the same study of Abbeddou et al. (74) and Olney et al. (53) respectively, but reported additional data, therefore both studies were included in this review. Two papers that reported on the same study but measured outcomes at different timepoints following zinc supplementation (Muñoz et al. (52) at 6 months and Rosado et al. (59) at 12 months) were included in the review, but only data from Rosado et al. (59) was considered for meta-analyses.

Almost all included studies were RCTs (N=53) (12,13,18,26–75) and three were quasi-experimental (8,17,19).

Daily doses of zinc ranged from 3 mg/d to 45 mg/d, from zinc supplementation or fortification. Six studies (8,19,34,48,50,72) administered doses below 5 mg/d, 34 studies (in 36 articles) (12,13,17,26,29–31,35–43,47,51,53–56,60–65,68–75) reported administering doses of 5 to 10 mg/d, ten studies (in 11 articles) (28,44,46,49,52,56–59,67,69) reported the effects of doses between 10.1 and 20 mg/d, and in six studies children consumed doses of between 21 and 45 mg/d (20,21,33,45,49,67).

In one study children received a dose of 50 mg weekly (42) and two studies gave a 70 mg bolus either weekly (66) or twice weekly (18).

Only 10 (13,17,19,34,37,40,47,48,60,75) studies included in the meta-analyses included dietary zinc intake data. Estimated dietary zinc intake from the studies included in the meta-analyses can be found in **Table 2 of the supplementary file 2.**

A total of 39 studies from 41 articles (8,12,13,17–19,26,28–40,43,47,48,51,53–55,58–60,62–65,67,70–75) were included in meta-analyses. The outcomes that were examined through meta-analyses, were hemoglobin, anemia, serum ferritin, serum copper, iron deficiency, serum transferrin receptor (sTfR), hematocrit, c-reactive protein (CRP), Erythrocyte SOD (eSOD), zinc Protoporphyrin (ZPP), serum total cholesterol, lactulose:mannitol molar ratio, and serum iron concentration.

A total of 32 studies (8,13,18,26,27,29–34,36,41–46,49,50,55,56,58,59,61,63,67,69,72,74–76) included physical and clinical outcomes (i.e., vomiting, regurgitation, nausea, constipation, abdominal pain, drowsiness, mouth irritation, taste aversion, diarrhea, and dysentery). These studies are summarized narratively.

The GRADE, ROB and JBI quality assessment of the studies can be found in **Supplementary file 3.** Less than 20% of studies were at high risk of bias using Cochrane RoB 2 criteria, with risk of bias in the randomization process being the main contributor. After consideration of risk of bias, inconsistency, indirectness, imprecision, and publication bias, GRADE certainty of evidence assessments for meta-analyses ranged from very low to moderate quality. Certainty of the evidence was primarily downgraded for risk of bias, indirectness (where study populations included some older children and/or had underlying morbidities), and imprecision.

Meta-analyses of the biochemical outcomes

Outcome measures from all studies were mapped and, where reported in 2 or more studies, were considered for meta-analysis. A summary of the biochemical outcome measures considered for meta-analyses is presented in **Table 2**. Where possible, forest plots have been generated for the following: age category [0-90 days, 91-180 days, 6-<12 months, ≥12 months]; dose of zinc given [<5mg/d, 5-10 mg/d, 10.1-20mg/d, >20 mg/d, bolus]; duration of intervention [0-3 months, > 3 months]; chemical formula of zinc [gluconate, sulphate, acetate, not stated, other] ; zinc vs placebo; and high vs low dose of zinc.

Hemoglobin

Thirty-two studies (8,12,13,18,19,26,28,29,31,33–40,43,47,48,51,53,54,58,59,62,64,65,70–73) assessed the effect of zinc on hemoglobin, all of which were included in the meta-analyses. The pooled analyses of the effect of zinc supplementation on hemoglobin concentration (g/L) by age and dose, showed that overall, there was no evidence for an impact of zinc on hemoglobin levels in infants aged between 0 and 90 days when doses of 4-10 mg/d (mg/d) were provided (Figure 2). In children aged 91-180 days, doses of 5-10 mg/d were associated with a significant reduction in hemoglobin concentration (mean difference

(MD) [95% confidence intervals (CI)] = - 2.39 g/L [-3.94, -0.84], I^2= 28%) (**Figure 3**). Pooled analyses of studies with children aged 6-12 months **(Figure 4)** and > 12 months (**Figure 5**) showed that at the doses assessed, there were no significant effects on hemoglobin concentration.

No significant effect of zinc on hemoglobin levels were found in studies grouped by study duration, chemical formula, or comparator group, as summarized in **Table 3**. The quality of evidence for hemoglobin assessed using GRADE ranged from very low to moderate **(Supplementary file 3**).

Anemia and severe anemia

The effect of zinc supplementation on the odds ratio (OR) for anemia (defined as < 10.0 – 11.0 g/dL) was explored in all age categories. Only one study, Carter 2018 (12) included children aged 0-90 days. Doses of 5-10 mg/d of zinc were provided with no significant effect on the odds ratio for anemia OR [95%CI] = 1.20 [0.89, 1.60], I^2=NA). Three studies (29,39,71) (6 data sets) included children aged 91-180 days. All studies administered doses of 5-10 mg/d with and without additional iron, compared with iron alone or placebo, respectively (**Figure 6)**. In children aged 6-12 months, seven studies (34,36,38,43,47,53,73) (14 comparisons) examined doses of zinc with and without combinations of iron, multiple micronutrients (MMN) and vitamin C (**Figure 7).** In children aged >12 months, six studies (19,37,48,54,55,70) (9 comparisons) investigated zinc supplementation with and without combinations of vitamin A, iron and MMN. (**Figure 8).** In all age groups, there was no significant effect of zinc supplementation on the OR for anemia.

Pooled analyses of the OR for anemia by duration of intervention, chemical formula of zinc supplement and comparator (placebo or high vs low zinc dose) are summarized in **Table 4**. Zinc supplementation had no effect on the OR for anemia when data were grouped by

chemical formula and comparator. When grouped by study duration, two studies (48,73) had interventions that lasted for 0-3 months, and fifteen studies (12,19,29,34,36–39,43,47,53–55,70,71) (26 comparisons) had interventions that lasted longer than 3 months. In both duration categories children received doses of 3-10 mg/d. For studies with interventions of a shorter duration, the OR for anemia increased significantly with zinc supplementation (**Table 4**). For studies of longer duration, the overall OR for developing anemia was not significantly changed by zinc supplementation.

The impact of zinc supplementation on the risk for severe anemia was examined by three studies (12,63,64) and defined as hemoglobin level less than 70 g/L in two studies (63,64) and <85 g/L in one study (12). Combining data from these three studies did not reveal a significant impact of zinc supplementation at doses of 5-10 mg/d on the odds ratio for severe anemia OR [95% CI]: 1.00 [0.77, 1.28], I^2= 0%).

The quality of evidence for anemia assessed using GRADE ranged from very low to moderate **(Supplementary file 3).**

Serum ferritin

The effect of zinc supplementation on serum ferritin (µg/L) in children was assessed by age category and dose. Meta-analysis showed that in children aged 91-180 days, a dose of 10 mg/d resulted in a significantly lower serum ferritin concentration than in controls. However, no significant effect was observed in children aged 0-90 days, 6-12 months or >12 months at any of the doses assessed (**Figure 9 - 12)**.

When assessed by dose and duration of intervention (across all ages), no significant difference was found in serum ferritin concentration compared with controls neither in interventions lasting less than three months nor in those lasting more than three months

(**Table 5**). No significant effect associated with the zinc chemical formula could be detected with the data available (**Table 5**).

The quality of evidence for serum ferritin using GRADE ranged from very low to moderate (**Supplementary file 3**).

Serum/plasma copper concentration

Zinc supplementation had no significant effect on serum/plasma copper concentration (μg/dL) at any dose in children aged 0-3 months (data from one study (8) p=0.056) or those aged 3-6 months (**Figure 13**). However, in age groups, 6-12 months and >12 months (**Figure 14 and 15),** doses of 3-20 mg/d have a significant, negative effect on serum/plasma copper concentration.

The effect of zinc supplementation on serum copper concentrations across all age groups was analyzed by duration of intervention, chemical formula of zinc supplement, and comparator group (**Table 6**). From the two studies (32,67) with interventions shorter than three months, only one (32) reported a highly significant impact of a dose of 18 mg/d, given with MMN, compared with a placebo plus MMN in children >12 months of age. For interventions longer than three months, data from one study (30), showed that a dose of 10-20 mg/d was associated with a significant reduction in serum/plasma copper concentration. This association was not shown for interventions with zinc doses up to and including 0-10 mg/d zinc or a bolus of 20-21 mg/wk.

Two studies used zinc gluconate, one at a dose of 10 mg/d of elemental zinc (62) and the other at 10-20 mg/d of elemental zinc depending on participant age (30). The overall effect was a significant reduction in serum/plasma copper concentration, mainly driven by the large effect at the higher dose. The overall effect of zinc sulphate, acetate, zinc-fortified breakfast (17) and non-stated forms of zinc were not significant. However, studies using zinc sulphate

at a elemental zinc dose of 3 mg/d (34) and 18 mg/d zinc with MMN (32) had a deleterious impact on serum/plasma copper concentrations. Combining studies where the comparator arm was a placebo, there was an overall significant effect of zinc in serum/plasma copper concentration **Table 5.**

The quality of evidence for copper using GRADE ranged from very low to moderate (**Supplementary file 3**).

Iron deficiency

Analysis of the effect of zinc supplementation on the OR for iron-deficiency (serum ferritin concentration <12 µg/L) by age showed a significantly increased OR in infants younger than three months (**Figure 16**). However, this was derived from a single study (73), providing a dose of 10 mg/d zinc, with and without MMN. In older children, pooled analysis showed no evidence of a significant effect of zinc supplementation on the OR for iron deficiency at the doses, intervention duration or chemical formula used in the studies**.**

The quality of evidence for iron deficiency using GRADE ranged from very low to moderate (**Supplementary file 3**).

Iron deficiency anemia

No significant association was observed between zinc intake and the odds of iron deficiency anemia (defined as having both anemia and ID) in any of the assessed age categories (**Figure 17**), zinc dose, duration of dose or zinc chemical formula (**Table 8**). No studies were conducted in children under three months or in those older than 12 months.

The quality of evidence for iron deficiency using GRADE ranged from very low to low (**Supplementary file 3**).

Serum transferrin receptor (sTfR)

Five studies examined the impact of zinc (5-20 mg/d) on sTfR (mg/L). Of these, one study (12) analyzed the effect of zinc on sTfR in children 0-3 months (mg/L); results showed no significant effect of a dose of 5-10 mg/d on sTfR **(Figure 18).** Four studies (28,36,47,74) examined the impact of 5-20 mg/d on sTfR (mg/L) in children 6-12 months. Overall, there was a significant impact of zinc on sTfR, indicating worsening iron status with zinc supplementation **(Figure 18**). No data was available for children aged 91-180 days or for those aged over 12 months.

A statistically significant negative impact on sTfR was observed when zinc sulphate was administered. No significant effects were observed when data were combined by other chemical formula, duration of intervention or comparator (**Table 9**).

The quality of evidence for sTfR using GRADE ranged from very low to moderate (**Supplementary file 3**).

Hematocrit

There was no overall effect of zinc supplementation on hematocrit in children aged 0-3 months, 6-12 months or >12 months (**Figure 19**). Zinc doses ranged from 4-12.3 mg/d, with additional supplements including iron, folic acid, and MMN. The duration of the intervention was greater than 3 months in all studies, except Moradveisi et al. (51). When examined by chemical formula, one study reported that zinc gluconate had a significant positive effect in hematocrit MD [95% CI] = 0.02% [0.00, 0.03], p=0.04 (62). No other significant effects were observed. (**Table 10**).

The quality of evidence for hematocrit using GRADE ranged from very low to moderate (**Supplementary file 3**).

C-reactive protein (CRP)

Data assessing the effect of zinc intake on CRP were limited, and there are no data in children under the age of six months, but data suggests there is no association between zinc supplementation and the likelihood of raised CRP in children aged over six months (**Figure 20**). The duration of the intervention for all studies was >3 months. None of the studies stated the chemical formula of the zinc administered. The combined data from all studies showed no significant effect of duration of treatment or chemical formula on the likelihood of raised CRP **(Table 11)**.

The quality of evidence for CRP using GRADE was low (**Supplementary file 3**).

Erythrocyte SOD (eSOD)

Two studies reported the effect of zinc supplementation on eSOD. Wuehler et al. (72) provided a dose of 10 mg/d of elemental zinc (zinc sulphate) and Bates et al. (18) administered two bolus doses of 70 mg of elemental zinc per week (zinc gluconate). Both studies compared zinc against a placebo in children aged over 12 months. Combining the data from the two studies showed no overall significant effect on eSOD (**Figure 21**).

The quality of evidence for eSOD using GRADE was low (**Supplementary file 3**).

Zinc Protoporphyrin (ZPP)

Two studies (53,74) measured ZPP following doses of 5-10 mg/d for a period > 3 months. The pooled analysis revealed that zinc supplementation had no significant effect on ZPP (**Figure 22**).

The quality of evidence for ZPP using GRADE was very low (**Supplementary file 3**).

Serum total cholesterol

Three studies assessed the effect of zinc on serum total cholesterol (mg/dL). One study (8), examined serum total cholesterol in children aged 0-3 months and two studies (17,72) were conducted with children aged over 12 months. There was no significant effect on zinc in any age group (**Figure 23**). All three studies had an intervention period of >3months with doses ranging from 3.75 to 10 mg/d of elemental zinc, either in the form of zinc sulphate (8,72), or in a fortified breakfast cereal (17). Grouping the studies by dose, mode of delivery, duration and zinc vs placebo did not reveal any significant impact of zinc supplementation on total blood cholesterol concentration (**Table 12**).

The quality of evidence for serum total cholesterol using GRADE ranged from very low to low (**Supplementary file 3**).

Lactulose:mannitol molar ratio

Two studies (18,60) reported the effect of zinc supplementation on lactulose:mannitol molar ratio. Both studies were conducted with children older than 12 months. Overall, there was a significant reduction in the ratio following zinc supplementation, indicating reduced (improved) gut permeability (**Figure 24**). Ryan et al. (60) provided a dose of 20 mg/d of elemental zinc in the form of zinc acetate for a period <3months. This dose regimen yielded a significant reduction in the ratio compared with a placebo. Bates et al. (18) provided a dose of 70 mg of elemental zinc, twice a week, in the form of zinc sulphate for a period > 3 months. There was a fall in the ratio compared with placebo, but it failed to reach statistical significance (**Table 13**).

The quality of evidence for lactulose:mannitol molar ratio using GRADE ranged from very low to low (**Supplementary file 3**).

Serum iron concentration

Two studies assessed the effect of zinc on serum iron concentration (µg/dL). Both studies were conducted with children older than two years (51,65). Pooling data from both studies revealed no significant effect on serum iron concentrations (**Figure 25**). Moradveisi et al. (51) provided a dose of 12.3 mg/d of elemental zinc as zinc sulphate with iron (60 mg/d) or iron alone (60 mg/d) for a period <3 months with no significant effect. Silva et al. (65) provided a dose of 10 mg/d of elemental zinc as zinc sulphate or a placebo for >3 months. There was a significant increase in serum iron following zinc supplementation (**Table 14**).

The quality of evidence for serum iron using GRADE was very low (**Supplementary file 3**).

Narrative description of physical and clinical outcomes

Vomiting, regurgitation and nausea

Thirteen studies (8,27,30,36,41,44–46,49,50,67,69,76) reported on the effect of zinc supplementation on the occurrence of vomiting (8,27,30,36,41,44–47,49,50,67,69).

Overall, four (30,46,67,69) of the 13 studies found a higher incidence of vomiting following zinc supplementation, with single daily zinc doses ranging from 10 to 20 mg for infants under 12 months of age and 20 to 30 mg/d for children aged ≥12 months. Three studies were of short duration (10 to 14 days) in children with acute diarrhea (46,67) or pneumonia (69), and one study (30) was of four months duration for the prevention of diarrhea. In Chang et al. (36), zinc provided at the same time as iron resulted in a significant increase in the frequency of vomiting compared with placebo or other intervention groups where zinc alone or zinc with iron provided separately

Five studies (8,33,46,67,69) reported on post-treatment regurgitation. Three studies found an increased frequency of regurgitation in children with acute diarrhea (46,67) or pneumonia (69), who received single daily zinc doses ranging from 10 to 20 mg (infants under 12 months of age) or 20 to 30 mg/d (children aged ≥12 months) for 10-14 days. One study (33) administered weekly zinc doses of 21 mg to children aged 2 to 11 months for 12 months for the prevention of diarrhea and pneumonia cited taste aversion sometimes leading to regurgitation as a potential reason for the higher number of withdrawals amongst the treatment group (n=103 vs n=44 in the placebo group).

Two studies (44,45) reported on the frequency of nausea without specifying its relation to timing of treatment. Neither study found an increase in reports of nausea with zinc supplementation.

Constipation and abdominal pain

Four studies reported on the effect of zinc on constipation (44,45,49,50), none of found a significant difference between treadment groups. (50)

Three trials (41,44,45) reported on the occurrence of abdominal pain without specifying its relation to timing of treatment. No significant differences between treatment groups were found.

Drowsiness

Two studies (44,45) reported on the effect of zinc on drowsiness; neither study found a significant increase in reports of drowsiness with zinc supplementation.

Mouth irritation and taste aversion

Two studies (44,45) reported on the frequency of mouth irritation without specifying its relation to timing of treatment. Both studies used a syrup formulation with 15 mg/d of

elemental zinc as zinc sulphate in 5 mL of syrup; neither study found an increase in mouth irritation with zinc supplementation. Three studies (33,44,45) reported on taste aversion to zinc syrup, provided as 21 mg/d elemental zinc acetate in 10mL syrup (33) or 15 mg/d elemental zinc as zinc sulphate in 5 mL syrup (44,45). Only Brooks et al. (33), reported taste aversion, sometimes leading to regurgitation and was highlighted as a potential reason for the higher number of withdrawals amongst the treatment group (n=103 vs n=44 in the placebo group) (44,45).

Diarrhea and dysentery

Twenty-seven studies (8,13,18,26,27,29–34,36,42–45,49,50,55,56,58,59,61,63,67,72,75) reported on the effect of zinc on diarrhea, with the majority (n=23) investigating zinc supplementation for the prevention or treatment of diarrhea. Four studies (8,44,45,49) suggested that zinc supplementation may have a potential adverse effect on diarrheal incidence (8,44,77). None of the included studies reported a significant increase in or worsening of diarrhea with zinc supplementation.

Three studies (36,42,55) reported on the effect of zinc on dysentery or bloody/mucoid diarrhea in children. None of the studies reported a worsening of the condition as a result of zinc supplementation given alone or in combination with iron. Two of these studies reported an improvement in the dysentery, one when zinc was provided alone (42,55) and one when zinc was provided at the same time as iron (36).

Case studies

Two case studies were identified. Botash et al. (20) reported data from a 6-month-old infant given a dose of 16-24 mg/d zinc prophylactically. Adverse effects were noted for hematological indices, including serum copper, ceruloplasmin, serum iron. Sugiura et al. (21)

reported on an 11- month-old infant with atopic dermatitis who consumed 45 mg/d zinc, and recorded adverse effects on serum copper, ceruloplasmin and hemoglobin.

Discussion

The setting of ULs for zinc has previously been considered by international panels, including those convened by FAO-WHO (2004)(1), IOM (78), IZiNCG (7) and the European Food and Safety Authority (3). An early step in this process is to collate data from published literature that enable the relationship between zinc intake and adverse effects on key outcomes to be described. To date, however, this has been hindered by a lack of data in the 0-3year age range. Our search identified 62 studies that assessed possible adverse effects of zinc intake in children aged 0-3 years with zinc doses ranging from 3 to 70 mg/d. In most studies doses were below 20 mg/d. Data from 39 studies allowed meta-analyses of outcome measures of interest, as identified by the FAO-WHO expert group. Meta-analyses revealed that zinc supplementation had a significant adverse effect on serum ferritin, plasma/serum copper concentration, sTfR, hemoglobin, hematocrit, and the odds of anemia in at least one of the subgroups of pooled data. A significant reduction of lactulose:mannitol ratio was found, indicating improved gut permeability. No significant effect of zinc supplementation on CRP, eSOD, ZPP, blood cholesterol, or iron deficiency anemia were observed in any of the pooled datasets.

Our analyses revealed a significant reduction in serum copper concentration following zinc supplementation in children aged >6-12 months (**Figure 14**) and >12 months (**Figure 15**). Mean reductions were 3.17 and 5.25 µg/dL respectively. Despite this decrease, the mean serum copper concentration reported in each of the studies remained within the reference range post intervention (children <1 year: 71.16-168.11 µg/dL) (79); 0.5-2 years: 72-178 µg/dl (80) and 3-4 years 80-160 (80)), with the exception of one study where children were

recovering from diarrhea and had low baseline serum copper levels (32). This example raise the question about the point at which a change in biochemical outcome measure becomes clinically important, such that the zinc dose that resulted in the change would be considered to pose a risk to health. This can be further explored using dose response modelling to determine the zinc intake required to result in a clinically significant change in either serum copper concentration or serum ferritin concentration, including the contribution from background dietary zinc intake, in children of various age categories (81). Identifying the threshold value that corresponds to this clinically significant change is also a crucial part of this risk assessment process.

A competitive interaction between zinc and iron during intestinal absorption has been long debated (82), and it has been proposed that high zinc intakes could induce a secondary iron deficiency. In addition to serum ferritin, outcome measures relating to iron status that were included in our meta-analyses include hemoglobin, hematocrit, iron deficiency (measured by: plasma ferritin concentration <12 µg/L) iron deficiency anemia (measured by hemoglobin <11 g/dL and plasma ferritin <12 µg/L), serum iron concentration and sTfR. Meta-analysis of pooled data revealed that hemoglobin concentration was significantly reduced in children aged 91-180 days following zinc doses of 5-10 mg/d (**Figure 6**), but there was no significant effect on mean values from data pooled by age in the younger or older age categories. Hematocrit and serum iron concentration data were sparse, but analysis of pooled data provided no evidence for a significant effect of zinc supplementation on these outcome measures. Similarly, analysis of the pooled data from studies reporting the risk of iron deficiency anemia and anemia did not reveal any significant effect of zinc supplementation on the OR in any of the age categories for which there were data (**Figures 6-8, Table 7**). However, combining data from studies with a short duration (< 3 months) did reveal a significant increase in the OR for anemia indicating a possible short-term effect (**Table 4**).

Levels of sTfR are high when iron deficiency in present, and in situations of increased erythropoietic activity (83). Most of the studies included in the meta-analysis were conducted in children aged 6-12 months. Analysis of the pooled data indicated a highly significant, detrimental increase of sTfR levels. In some studies, children in the intervention but also in the comparison groups (36,47,74) had levels of sTfR above the reference values for healthy children proposed by some studies (83–85). However, there is a lack of standardization on the methods used to determine sTfR, which limits the comparability of the results and our understanding of the severity of the effect of zinc on sTfR levels (83).

The urinary lactulose:mannitol ratio is a biomarker for environmental enteropathy, and its reduction indicates a fall in the gut permeability which is a desirable outcome from zinc supplementation. Meta-analysis of the data from the two studies that reported this outcome showed a statistically significant reduction of the lactulose:mannitol ratio following zinc supplementation in children over 12 months old. This concurs with previous systematic reviews that have investigated the effectiveness of zinc supplementation as a treatment for diarrhea in children (86) .

Doses of zinc ranging from 10 to 20 mg/day in infants under 12 months of age and 20 to 30 mg/day for children aged ≥ 12 months increase the risk of vomiting (30,46,67,69) and regurgitation (46,67,69) in some studies. From the studies reporting on taste aversion, only one study (33), reported it as leading to regurgitation and authors highlighted it as a potential reason of withdrawals among the children in the zinc arm. The studies reporting on for nausea, constipation, abdominal pain, mouth irritation or dysentery did not find an increase incidence of these adverse effects as a result of the zinc doses provided.

Overall, the certainty of the evidence, as assessed using GRADE, was very low to moderate. Factors influencing the downgrading of the evidence included the presence of underlying

morbidities and the inclusion of older children in some studies, imprecision in effect estimates due to low numbers and/or heterogeneity, and risk of bias due to randomization processes. Given these limitations in the certainty of the evidence, it is possible that data from further trials may alter the effect estimates summarized here.

Strengths and Limitations

Studies that have collected data on the potential adverse effects of zinc intake in children aged 0-3 years are scarce. As a consequence, it was not possible to conduct meta-analyses by age category, dose, dose duration, and chemical formula of zinc for all the outcomes explored. Additionally, most studies included data from children with relatively low exposures to zinc where adverse effects at such ranges may not be expected. Therefore, it was not possible to identify dose ranges in which zinc may be detrimental for most individuals in the target age group. Nonetheless, data from this review may be used by expert groups to conduct dose response modelling to establish the tolerable upper intake levels of zinc in children aged 0-3 years.

Conclusion

Whilst zinc supplementation at doses of 3 to 20 mg/d had an adverse effect on levels of serum/plasma copper, ferritin, hemoglobin and sTfR in children aged 0-3 years, the change observed may not have a detrimental effect on healthy populations. However, recommended maximum zinc doses may need to be adjusted for children at risk or recovering from iron or copper deficiency. Data from this review may be used to undertake dose response modelling to estimate tolerable upper intake level of zinc in children aged 0-3 years.

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Statement of Authors' contributions to manuscript

NML, JM and MX conceptualized the review. CH and MCR searched the databases, AC conducted the meta-analyses, MCR, SM, VHM and NML assessed the records, SM extracted the data, collated dietary data, conducted GRADE and risk of bias assessments, MX and SM contacted authors for dietary data, MCR, NML, SM and VHM contributed to drafting of the manuscript. All authors contributed to the study design, provided the methodology for the study, and edited and revised the manuscript.

Notes

Abbreviations used: CRP, c-reactive protein; CI, confidence intervals; eSOD , Erythrocyte SOD; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; JBI, Joanna Briggs Institute; ID, Iron deficiency; IDA, Iron deficiency anemia; IOM, Institute of Medicine; IZiNCG , International Zinc Nutrition Consultative Group; FAO, Food and

Agriculture Organization; MD, Mean difference; MMN, multiple micronutrients; NOAEL, No Observed Adverse Effect Level; ULs, upper limits; OR, odds ratios; RCTs , randomized controlled trials; RoB, Cochrane Risk of Bias 2; sTfR , serum transferrin receptor; WHO, World Health Organization; ZPP, zinc Protoporphyrin

Data Sharing plan

Data collection forms: data extracted from included studies; data used for all analyses is available upon request to the corresponding author.

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Tables

Table 1. Eligibility criteria are based on the Population, Intervention, Comparison, Outcomes and Study (PICOS) elements.

Population(s)	Children and adults[1] who are generally healthy or who have symptoms related to excessive zinc intake
Interventions, exposures	• Intake of zinc via supplements or foods (fortified and non-fortified) • Studies where zinc intake was not given were excluded • Studies where the effect of zinc intake could not be isolated were excluded
Comparator(s)	• Higher zinc intake vs lower or no zinc intake
Outcome	Adverse effects include impact on: • Absorption/status of other minerals (e.g. copper, iron, etc.) • Haemoglobin, ferritin • Blood lipids • Immune function • Gastrointestinal function • DNA breaks/damage
Study Designs	• Intervention studies assessing effects of zinc intake. Including but not limited to: RCTs, cross-over RCTs, non RCTs, pre-pots studies. • Observational studies assessing effects of zinc intake. Including but not limited to: case-control, cohort studies, cross-sectional studies • Case reports of excess zinc intake • In vitro and animal studies were **not** included

[1]Based on results of the scoping review and related discussions, the expert group concluded that the limited data available in children aged 0-36 months may not be sufficient to identify ULs and therefore it was decided to expand the literature search to include studies in older children and adults. Data for older children and or adults will be used (i.e. extrapolated) only if data obtained via the literature for children aged 0-36 months is insufficient to identify ULs directly. RCT: randomized control trial

Table 2. Biochemical, physical, and clinical adverse effects of zinc intake in infants and children up to 3 years of age reported on the included studies

Outcome reported	Number of studies[1]	References
Biochemical outcome measures		
Hemoglobin	34 (32[2])	(8,12,13,18,19,26,28,29,31,33–40,43,47,48,51,53,54,58,59,62,64,65,70–73) (66)[3] (52)[4]
Hematocrit	6 (6)	(8,19,31,51,62,65)
Serum Ferritin	21 (20)	(12,18,19,26,28,29,31,34,35,39,40,47,48,51,59,70–72,74,75) (52)[3]
Serum/soluble transferrin receptor (sTfR)	5 (5)	(12,28,36,47,74)
Iron deficiency	9 (9)	(12,31,34,39,47,53,59,70,71,73)
Anemia	18 (18)	(12,19,29,34,36–39,43,47,48,53–55,63,64,70,71,73)
Severe anemia	3 (3)	(12,63,64)
Iron deficiency anemia	4 (4)	(29,34,47,71,74)
Serum iron	2 (2)	(51,65)
Zn protoporphyrin (ZPP)	2 (2)	(53,74)
Plasma/serum copper	13 (13)	(51,65)
Erythrocyte SOD (eSOD)	2 (2)	(18,72)
Elevated C-Reactive Protein (CRP)	3 (3)	(36,43,70)
Lactulose:mannitol	2 (2)	(18,60)
Serum total cholesterol	3 (3)	(8,17,72)
Physical or clinical descriptive outcomes		
Vomiting	13 (0)	(8,27,30,36,41,44–46,49,50,67,69,76)
Regurgitation	5 (0)	(8,33,46,67,69)
Nausea	2 (0)	(44,45)
Constipation	4 (0)	(44,45,49,50)
Abdominal pain	3 (0)	(41,44,45)
Drowsiness	2 (0)	(44,45)
Mouth irritation	2 (0)	(44,45)
Taste aversion	3 (0)	(33,44,45)
Respiratory infection	13 (0)	(13,18,29,33,34,49,50,55,57–59,61,75)
URTI	3 (0)	(33,34,50)
Bronchiolitis	2 (0)	(33,36)
Cough	2 (0)	(29,72)
Diarrhea	27 (0)	(8,13,18,26,27,29–34,36,42–45,49,50,55,56,58,59,61,63,67,72,75)
Dysentery/bloody diarrhea	3 (0)	(36,42,55)
ORS use	2 (0)	(27,56)
Malaria	9 (0)	(18,43,55,58,63,64,70,73,74)
Fever	5 (0)	(29,34,59,72,74)
Death	7 (0)	(31,33,36,43,49,58,63)
Other	23 (0)	(8,18,20,21,31–34,36,41–45,50,51,56,62–64,68,72,74)

[1]Number of studies reporting the outcome in the review (Number of studies included in meta-analysis).
[2]Includes 2 values converted from hematocrit. [3]No SD values reported, [4]Not included in meta-analysis as same study of Rosado et al. (59). ORS, oral rehydration solution; URTI, Upper respiratory tract infection,

Table 3. Summary of forest plot analyses of the effect of zinc supplementation intake in infants and children up to 3 years of age on hemoglobin (g/L), analyzed by duration, chemical formula, and comparator group.

Group/subgroup	References	Mean Difference	95% CI	P	I^2
Children receiving interventions for <3 months by zinc dose					
< 5 mg/d	(48)	-5.40	-24.21, 13.41		NA
5-10 mg/d	(48,73)	-5.26	-8.98, -1.54		0
10.1-20 mg/d	(51)	0.40	-3.67, 4.47		NA
Overall		-3.13	-6.82, 0.57	0.10	24
Children receiving interventions for >3 months by zinc dose					
< 5 mg/d	(8,19,34,72)	0.39	-2.70, 3.48		33
5-10 mg/d	(12,13,26,29,31,35–40,43,47,53,62,64,65,70–72)	-0.21	-1.20, 0.77		63
10.1-20 mg/d	(54,58,59)	-0.13	-1.39, 1.12		0
Zn bolus[1]	(18,28,33)	-1.24	-2.53, 0.06		0
Overall		-0.25	-1.00, 0.50	0.51	54
Children receiving zinc through zinc sulphate by zinc dose					
< 5 mg/d	(8,34,48,72)	1.82	-0.89, 4.53		0
5-10 mg/d	(12,13,26,29,35,39,40,47,48,65,71,72)	-0.97	-2.51, 0.56		66
10.1-20 mg/d	(54,58,59)	-0.03	-1.54, 1.49		0
Overall		-0.46	-1.60, 0.68	0.43	54
Children receiving zinc through zinc acetate by zinc dose					
Zn bolus[1]	(28,33)	-1.30	-2.62, 0.01		0
Overall		-1.30	-2.62, 0.01	0.05	0
Children receiving zinc through an unstated approach by zinc dose					
5-10 mg/d	(31,36,43,53,70)	0.59	-1.01, 2.18		66
10.1-20 mg/d	(54)	-0.80	-4.80, 3.20		NA
Overall		0.47	-1.01, 1.96	0.53	0
Children receiving zinc through an 'other' approach by zinc dose					
< 5 mg/d	(19)	-5.10	-10.24, 0.04		NA
10.1-20 mg/d	(59)	0.00	-2.24, 2.24		0
Overall		-1.06	-3.73, 1.62	0.44	37
Children receiving zinc versus placebo by zinc dose					
< 5 mg/d	(19,72)	-1.12	-9.70, 7.47		73
5-10 mg/d	(12,13,29,36,39,40,43,47,53,64,65,70–72)	0.25	-1.11, 1.61		52
10.1-20 mg/d	(58,59)	-0.18	-2.09, 1.73		0
Zn bolus[1]	(18,28,33)	-1.04	-2.39, 0.31		0
Overall		-0.10	-1.10, 0.90	0.84	43
Children receiving low compared to high dose zinc					
5-10 mg/d	(43,48,72)	0.35	-1.89, 2.58		0
Overall		0.35	-1.89, 2.58	0.76	0

[1]Bolus doses given as follows: Brooks et al. (33) 21/d mg given weekly; Baqui et al. (28) 20 mg/d once weekly; Bates et al. (18) 70 mg/d twice weekly. CI: confidence interval, NA: not applicable

Table 4. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Anemia (odds ratio), analyzed by duration, chemical formula, and comparator group

Group/subgroup	References	Mean Difference	95% CI	P	I^2
Children receiving interventions for <3 months by zinc dose					
< 5 mg/d	(48)	4.33	0.14, 132.32		NA
5-10 mg/d	(48,73)	1.74	1.01, 2.98		0
Overall		1.78	1.04, 3.03	0.03	0
Children receiving interventions for >3 months by zinc dose					
< 5 mg/d	(19,34)	0.85	0.37, 1.95		44
5-10 mg/d	(12,29,36–39,43,47,53–55,70,71)	1.08	0.93, 1.25		30
10.1-20 mg/d	(54)	1.07	0.60, 1.90		NA
Overall		1.06	0.92, 1.22	0.43	28
Children receiving zinc through zinc gluconate by zinc dose					
5-10 mg/d	(37,38,55,73)	1.10	0.71, 1.72		41
Overall		1.10	0.71, 1.72	0.66	41
Children receiving zinc through zinc sulphate by zinc dose					
< 5 mg/d	(34,48)	0.66	0.36, 0.121		0
5-10 mg/d	(12,29,39,47,48,71)	1.28	0.98, 1.67		49
Overall		1.18	0.92, 1.53	0.19	47
Children receiving zinc through an unstated approach by zinc dose					
5-10 mg/d	(36,43,53,70)	0.97	0.81, 1.15		0
10.1-20 mg/d	(54)	1.07	0.60, 1.90		NA
Overall		0.97	0.82, 1.15	0.76	0
Children receiving zinc versus placebo by zinc dose					
< 5 mg/d	(19)	3.75	0.65, 21.74		NA
5-10 mg/d	(29,36,39,43,47,53,55,70,71)	0.89	0.75, 1.05		0
Overall		0.90	0.76, 1.06	0.21	0
Children receiving low compared to high dose zinc					
5-10 mg/d	(43,48)	1.13	0.83, 1.53		0
Overall		1.13	0.83, 1.53	0.44	0

CI: confidence interval, NA: not applicable

Table 5. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Serum ferritin (μg/L), analyzed by duration, chemical formula, and comparator group

Group/subgroup	References	Mean Difference	95% CI	P	I^2
Children receiving interventions for <3 months by zinc dose					
< 5 mg/d	(48)	-3.00	-22.84, 16.84		NA
5-10 mg/d	(48)	-3.79	-16.21, 8.63		0
10.1-20 mg/d	(51)	-0.10	-3.85, 3.65		NA
Overall		-0.49	-4.02, 3.04	0.79	0
Children receiving interventions for >3 months by zinc dose					
< 5 mg/d	(19,34)	-1.85	-9.37, 5.67		0
5-10 mg/d	(12,26,29,31,35,39,40,47,70–72,74,75)	-2.32	-5.18, -0.53		99
10.1-20 mg/d	(59)	-3.23	-8.40, 1.94		0
Zn bolus	(18,28)	3.09	-2.25, 8.43		0
Overall		-1.80	-4.31, -0.72	0.16	98
Children receiving zinc through zinc gluconate by zinc dose					
Zn bolus[1]	(18)	1.30	-5.84, 8		NA
Overall		1.30	-5.84, 8	0.72	NA
Children receiving zinc through zinc sulphate by zinc dose					
< 5 mg/d	(34,48)	-4.11	-11.95, 3.73		0
5-10 mg/d	(12,26,29,35,39,40,47,48,71,72,75)	-2.50	-6.69, 1.70		99
10.1-20 mg/d	(51)	-0.10	-3.85, 3.65		NA
Overall		-2.48	-6.30, 1.34	0.20	99
Children receiving zinc through zinc acetate by zinc dose					
10.1-20 mg/d	(28)	5.35	-2.68, 13.39		0
Overall		5.35	-2.68, 13.39	0.19	0
Children receiving zinc through an unstated approach by zinc dose					
5-10 mg/d	(31,70,74)	-0.93	-2.46, 0.61		78
Overall		-0.93	-2.46, 0.61	0.24	78
Children receiving zinc through an 'other' approach by zinc dose					
< 5 mg/d	(19)	6.65	-9.21, 22.51		NA
10.1-20 mg/d	(59)	-3.23	-8.40, 1.94		0
Overall		-2.28	-7.19, 2.63	0.36	0
Children receiving zinc versus placebo by zinc dose					
< 5 mg/d	(19)	6.65	-9.21, 22.51		NA
5-10 mg/d	(12,29,39,40,47,70–72,74,75)	-0.56	-2.35, 1.23		90
10.1-20 mg/d	(59)	-3.20	-9.33, 2.93		NA
Zn bolus[1]	(18,28)	2.75	-3.30, 8.79		0
Overall		-0.41	-2.08, 1.25	0.63	86
Children receiving low compared to high dose zinc					
5-10 mg/d	(48,74)	-8.10	-18.98, 2.78		45
Overall		-8.10	-18.98, 2.78	0.14	45

[1] Bolus doses given as follows: Baqui et al. (28) 20 mg/d once weekly; Bates et al. (18) 70 mg/d twice weekly.
CI: confidence interval, NA: not applicable

Table 6. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Serum/plasma copper concentration (µg/dL), analyzed by duration, chemical formula, and comparator group

Group/subgroup	References	Mean Difference	95% CI	P	I^2
Children receiving interventions for <3 months by zinc dose					
10.1-20 mg/d	(32)	-16.50	-24.09, -8.91		NA
>20 mg/d	(67)	0.02	-0.41, 0.45		42
Overall		-0.50	-1.90, 0.90	0.48	90
Children receiving interventions for >3 months by zinc dose					
< 5 mg/d	(8,17,34)	-4.62	-15.01, 5.78		50
5-10 mg/d	(13,35,47,62,72)	-1.50	-5.10, 2.11		0
10.1-20 mg/d	(30)	-15.50	-18.21, -12.79		NA
Zn bolus[1]	(28,33)	-1.03	-4.08, 2.01		0
Overall		-4	-8.82, 0.82	0.10	82
Children receiving zinc through zinc gluconate by zinc dose					
5-10 mg/d	(62)	-5.20	-20.16, 9.76		NA
10.1-20 mg/d	(30)	-15.50	-18.21, -12.79		NA
Overall		-13.08	-21.64, -4.53	0.003	43
Children receiving zinc through zinc sulphate by zinc dose					
< 5 mg/d	(34)	-10.25	-20.18, -0.33		0
5-10 mg/d	(8,13,35,47,72)	-0.63	-4.26, 2.99		0
10.1-20 mg/d	(32)	-16.50	-24.09, -8.91		NA
Overall		-4.09	-9.31, 1.12	0.12	61
Children receiving zinc through zinc acetate by zinc dose					
Zn bolus[1]	(28,33)	-1.03	-4.08, 2.01		0
Overall		-1.03	-4.08, 2.01	0.51	0
Children receiving zinc versus placebo by zinc dose					
< 5 mg/d	(17)	-7.52	-21.69, 6.65		NA
5-10 mg/d	(13,47,72)	-1.41	-6.76, 3.93		0
10.1-20 mg/d	(30,32)	-15.61	-18.17, -13.06		0
>20 mg/d	(67)	0.02	-0.41, 0.45		42
Zn bolus[1]	(28,33)	-0.21	-3.52, 3.09		0
Overall		-4.11	-6.48, -1.74	<0.001	94

[1] Bolus doses given as follows: Brooks et al. (33) 21/d mg given weekly; Baqui et al. (28) 20 mg/d once weekly; Bates et al. (18) 70 mg/d twice weekly. CI: confidence interval, NA: not applicable

Table 7. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Iron deficiency (odds ratio), analyzed by duration, chemical formula, and comparator group

		References	Mean Difference	95% CI	P	I^2
By treatment duration	0-3 months	(73)	0.58	0.29, 1.18		NA
	>3 months	(12,29,31,34,47,53,59,70,71)	1.02	0.77, 1.24		29
	Overall		0.98	0.77, 1.24	0.87	32
By chemical formula	Zn gluconate	(73)	0.58	0.29, 1.18		NA
	Zn sulphate	(12,29,34,47,71)	1.14	0.82, 1.60		34
	Not stated	(31,53,70)	1.04	0.80, 1.34		0
	Other	(59)	0.97	0.37, 2.53		NA
	Overall		1.01	0.83, 1.23	0.89	12
Zinc Vs Placebo		(12,29,53,59,70,71)	1.04	0.79, 1.37	0.79	13

CI: confidence interval, NA: not applicable

Table 8. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Iron deficiency anemia (odds ratio), analyzed by duration, chemical formula, and comparator group

		References	Mean Difference	95% CI	P	I^2
By treatment duration	>3 months	(29,34,39,47,71,74)	1.01	0.73, 1.39	0.97	20
By chemical formula	Zn sulphate	(29,34,39,47,71)	0.99	0.68, 1.44		34
	Not stated	(74)	1.46	0.42, 5.06		0
	Overall		1.01	0.73, 1.39	0.97	20
Zinc versus placebo		(29,39,71,74)	0.98	0.67, 1.45	0.93	20
High dose VS Low Dose		(74)	4.93	0.56, 43.27	0.15	NA

CI: confidence interval, NA: not applicable

Table 9. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Serum/soluble transferrin receptor (sTfR) (mg/L), analyzed by duration, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	>3 months	(12,28,36,47,74)	0.19	0.08, 0.29	<0.001	89
By chemical formula	Zn sulphate	(12,47)	0.18	0.07, 0.29		97
	Zn acetate	(28)	0.44	-1.08, 1.96		63
	Not stated	(36,74)	0.26	-0.13, 0.65		0
	Overall		0.19	-0.08, 0.29	<0.001	89
Zinc versus placebo and (ii) high dose versus low dose zinc		(12,28,36,47,74)	0.43	-0.16, 0.93	0.08	76
High dose VS Low Dose		(74)	-0.10	-1.19, 0.99	0.86	NA

CI: confidence interval, NA: not applicable

Table 10. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on hematocrit (proportion), analyzed by duration if intervention, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	0-3 months	(51)	-0.01	-0.04, 0.02		NA
	>3 months	(8,19,31,62,65)	0.00	-0.01, 0.01		28
	Overall		-0.00	-0.01, 0.01	0.97	17
By chemical formula	Zn gluconate	(62)	0.02	0.00, 0.03		NA
	Zn sulphate	(8,51,65)	0.00	-0.0-1, 0.01		0
	Not stated	(31)	-0.00	-0.00, 0.00		NA
	Other	(19)	-0.01	-0.02, 0.01		NA
	Overall		-0.00	-0.01, 0.01	0.97	17
Zinc Vs Placebo		(19,65)	-0.00	-0.01, 0.01	0.51	0

CI: confidence interval, NA: not applicable

Table 11. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on elevated C-Reactive Protein (CRP) (odds ratio), analyzed by duration, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	>3 months	(36,43,70)	1.19	0.91, 1.55	0.21	0
By chemical formula	Not stated	(36,43,70)	1.19	0.91, 1.55	0.21	0
Zinc Vs Placebo		(36,43)	1.05	0.74, 1.48	0.79	0
High dose VS Low Dose		(43)	0.73	0.37, 1.44	0.36	NA

CI: confidence interval, NA: not applicable

Table 12. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on serum total cholesterol (mg/dL), analyzed by duration, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	>3 months	(8,17,72)	-0.79	-6.24, 4.66	0.78	0
By chemical formula	Zn sulphate	(8,72)	-1.73	-7.70, 4.23		0
	Other	(17)	4.00	-9.45, 17.45		NA
	Overall		0.79	-6.24, 4.66	0.78	0
Zinc Vs Placebo		(17,72)	-1.07	-6.82, 4.69	0.72	0

CI: confidence interval, NA: not applicable

Table 13. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on Lactulose:Mannitol molar ratio, analyzed by duration, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	0-3 months	(60)	-0.08	-0.15, -0.01		NA
	>3 months	(18)	-0.10	-0.27, 0.07		NA
	Overall		-0.08	-0.14, -0.02	<0.001	0
By chemical formula	Zn gluconate	(18)	-0.10	-0.27, 0.07		NA
	Zn acetate	(60)	-0.08	-0.15, -0.01		NA
	Overall		-0.08	-0.14, -0.12	<0.001	0
Zinc Vs Placebo		(18,60)	-0.08	-0.14, 0.02	0.009	0

CI: confidence interval, NA: not applicable

Table 14. Summary of forest plot analyses of the effect of zinc supplementation in infants and children up to 3 years of age on serum iron concentration (µg/dL), analyzed by duration, chemical formula, and comparator group.

		References	Mean Difference	95% CI	P	I^2
By treatment duration	0-3 months	(51)	-0.12	-11.16, 10.92		NA
	>3 months	(65)	12.50	10.37, 14.63		NA
	Overall		7.40	-4.74, 19.54	0.23	79
By chemical formula	Zn sulphate	(51,65)	7.40	-4.74, 19.54	0.23	79
Zinc Vs Placebo		(65)	12.50	10.37, 14.63	<0.001	NA

CI: confidence interval, NA: not applicable

Figures

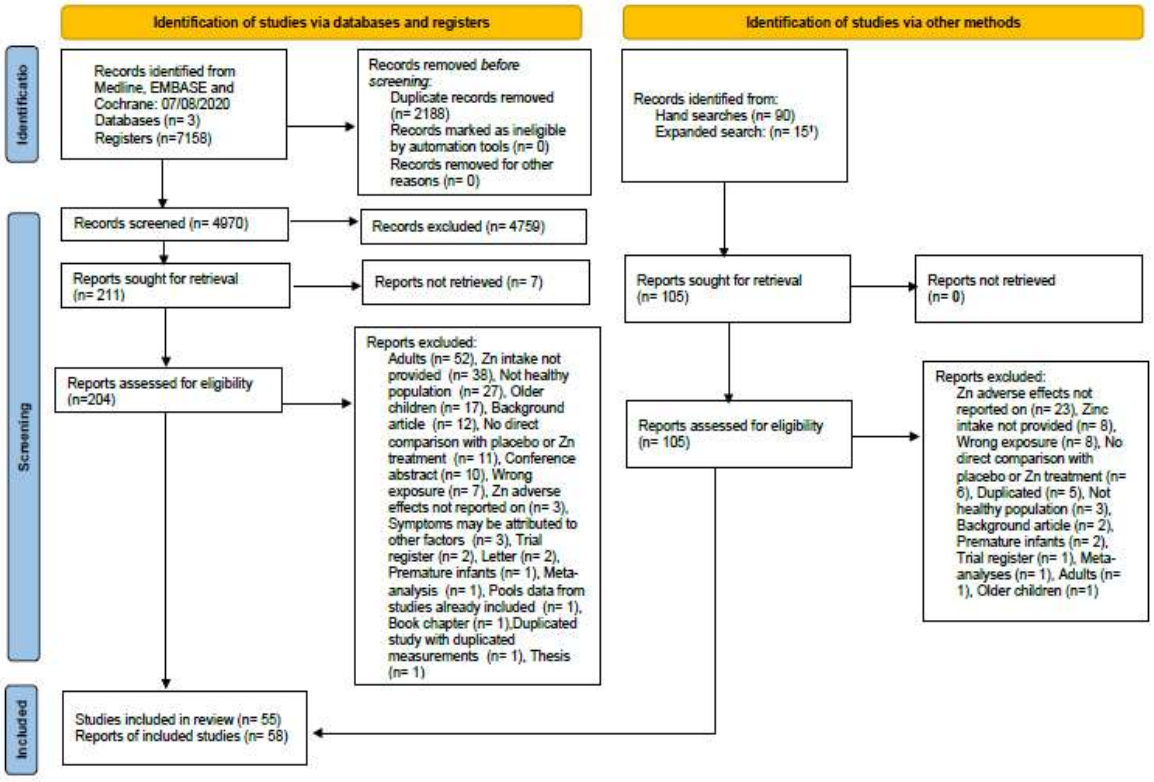

Figure 1. PRISMA-2020 flow diagram of the search procedure. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Note: [1] See supplementary file 2 for further information on how studies were selected.

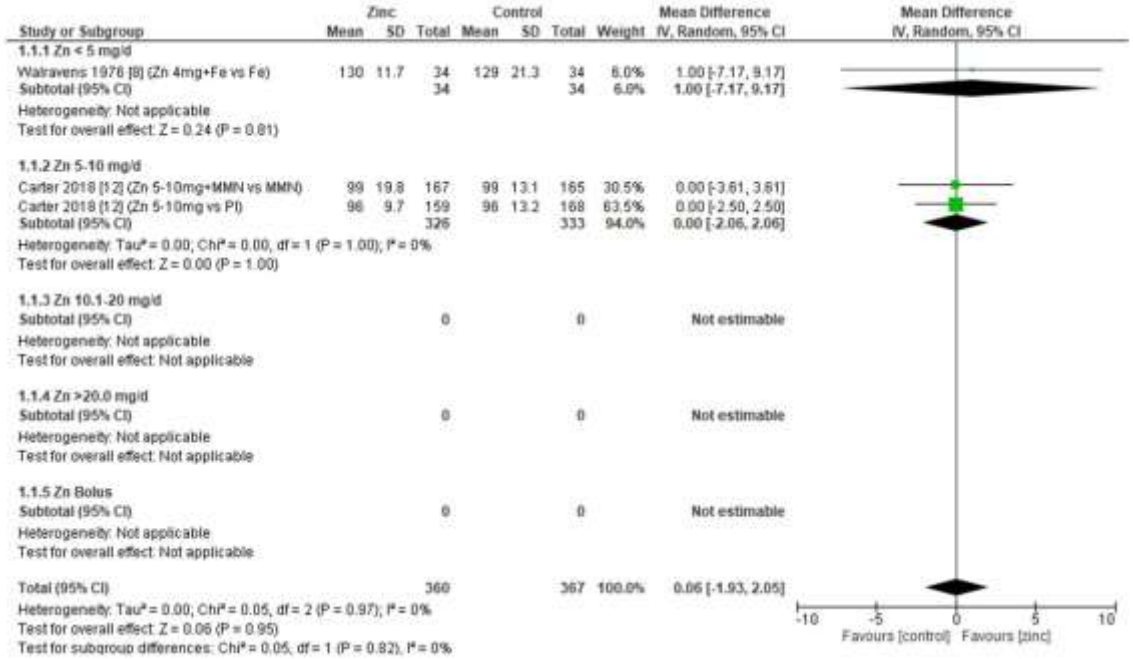

Figure 2. Effect of zinc supplementation on hemoglobin (g/L) in children aged 0-90 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

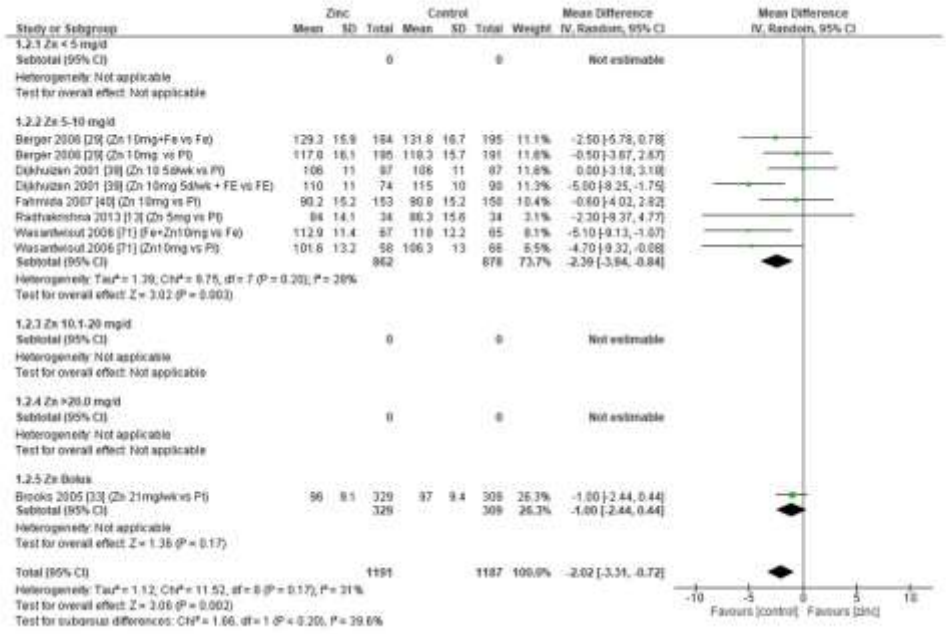

Figure 3. Effect of zinc supplementation on hemoglobin (g/L) in children aged 91-180 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

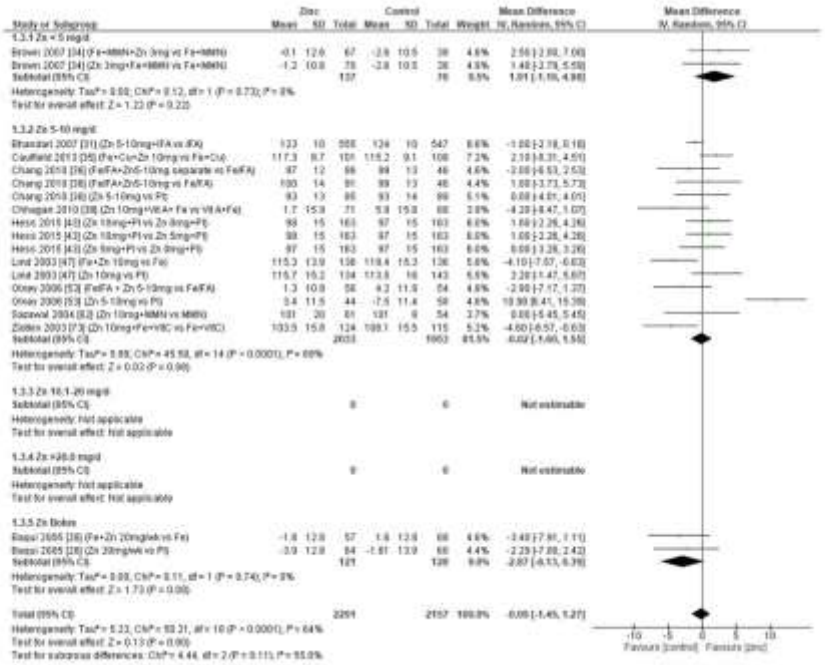

Figure 4. Effect of zinc supplementation on hemoglobin (g/L) in children aged over 6 months to 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

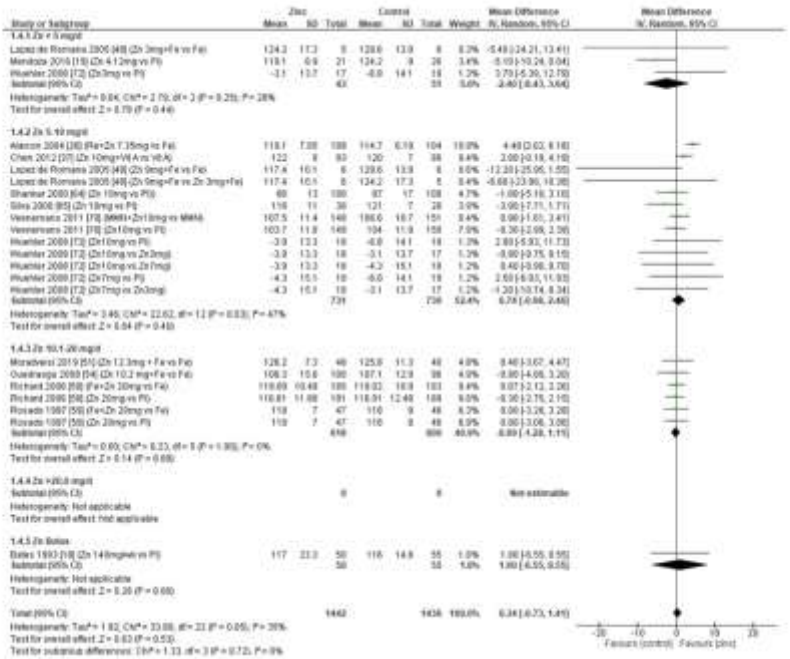

Figure 5. Effect of zinc supplementation on hemoglobin (g/L) in children aged over 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

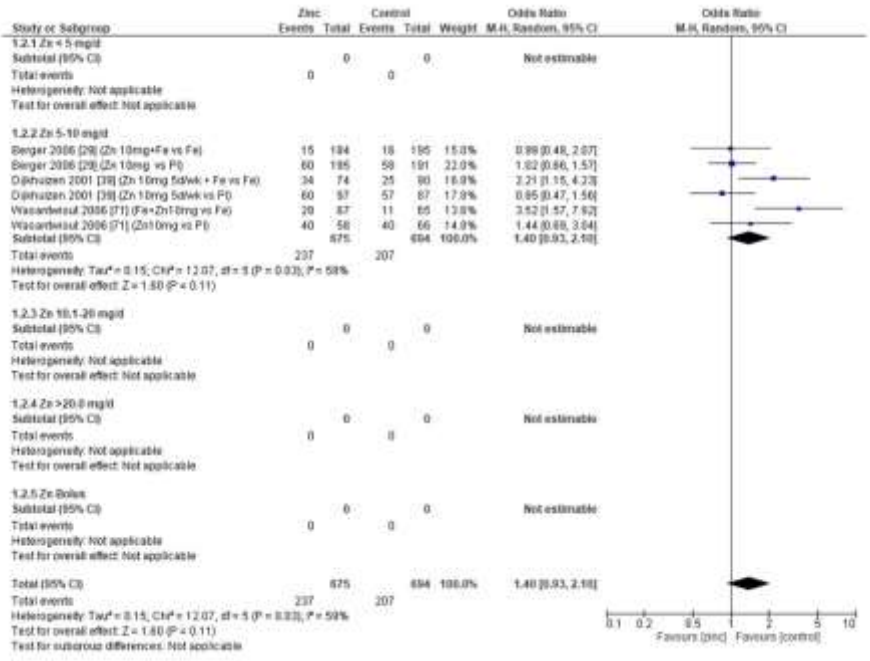

Figure 6. Effect of zinc supplementation on anemia (OR) in children aged 91-180 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

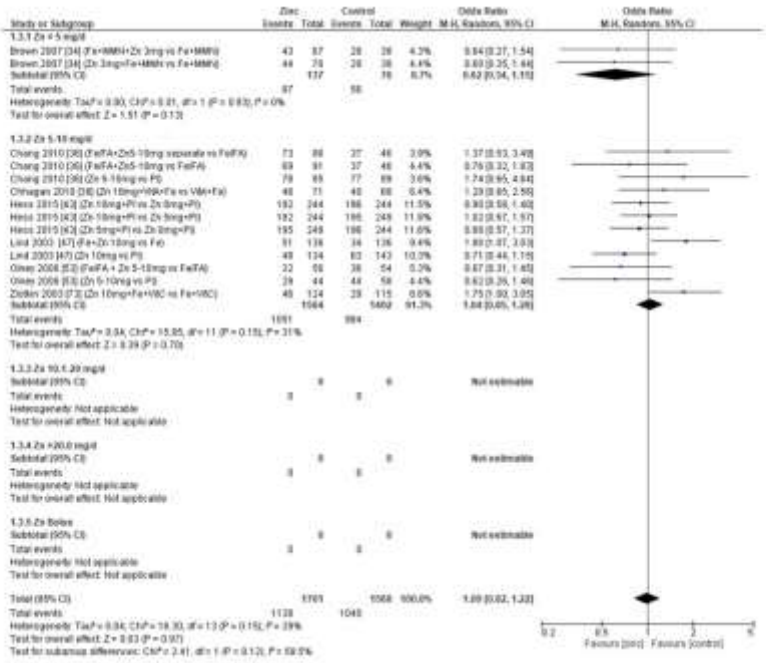

Figure 7. Effect of zinc supplementation on anemia (OR) in children aged over 6 months to 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

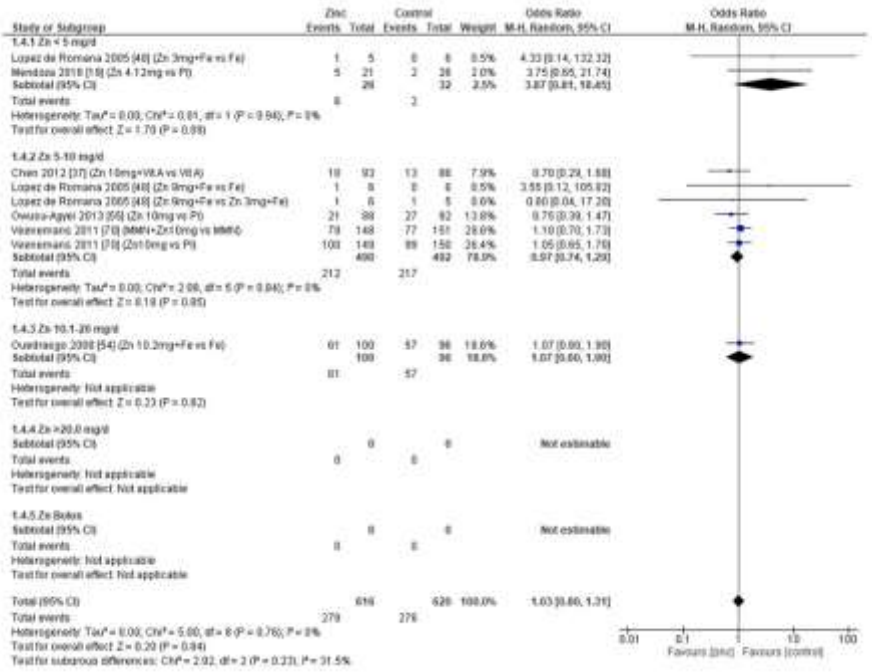

Figure 8. Effect of zinc supplementation on anemia (OR) in children aged over 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

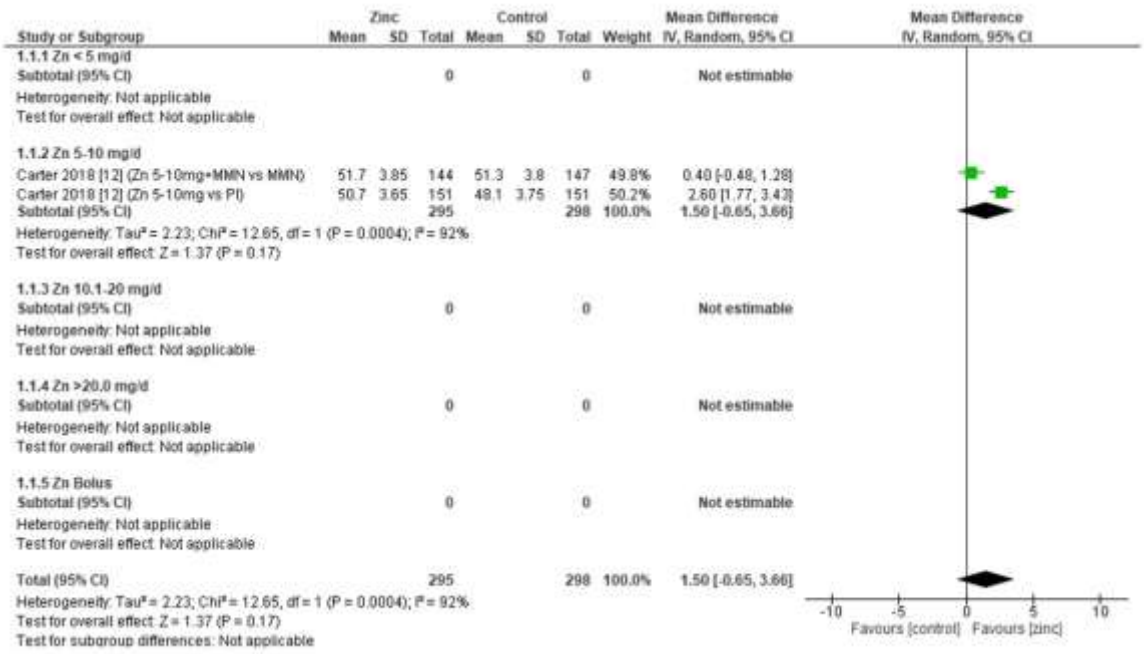

Figure 9. Effect of zinc supplementation on serum ferritin (μg/L) in children aged 0-90 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

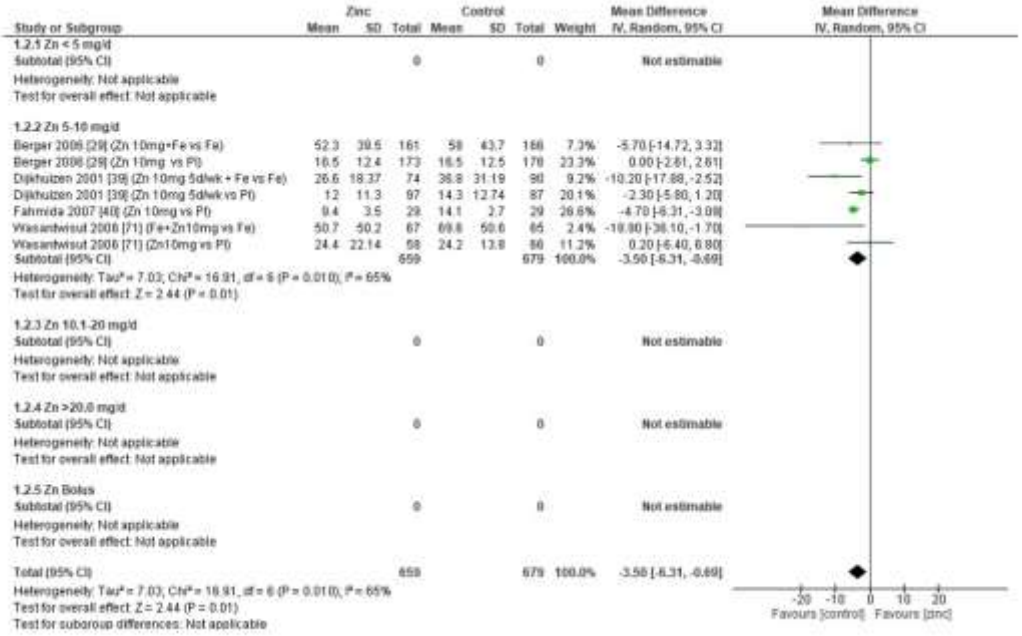

Figure 10. Effect of zinc supplementation on serum ferritin (µg/L) in children aged 91-180 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

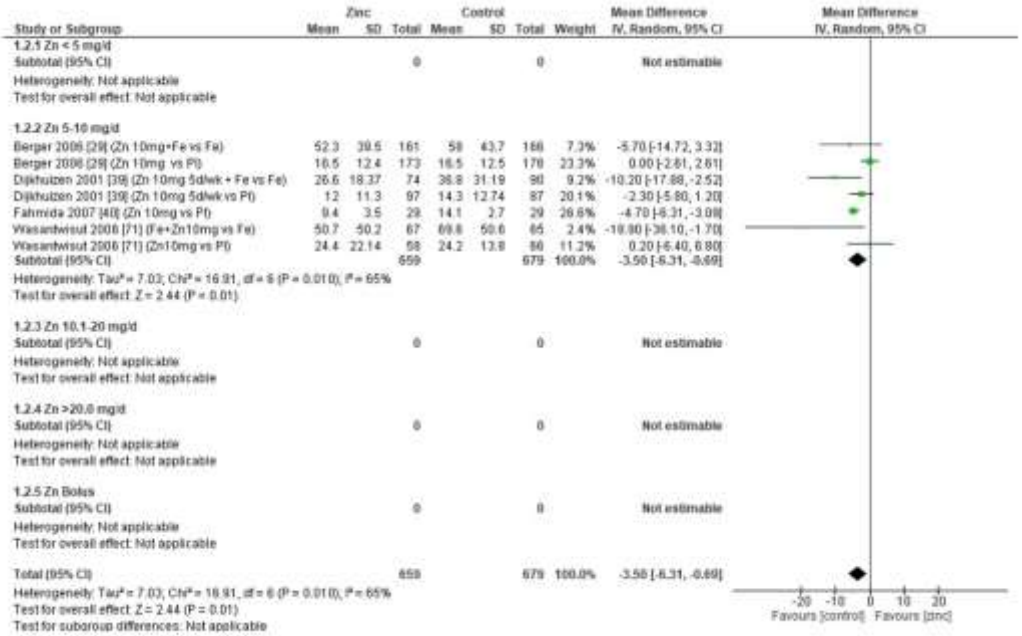

Figure 11. Effect of zinc supplementation on serum ferritin (µg/L) in children aged over 6 months to 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

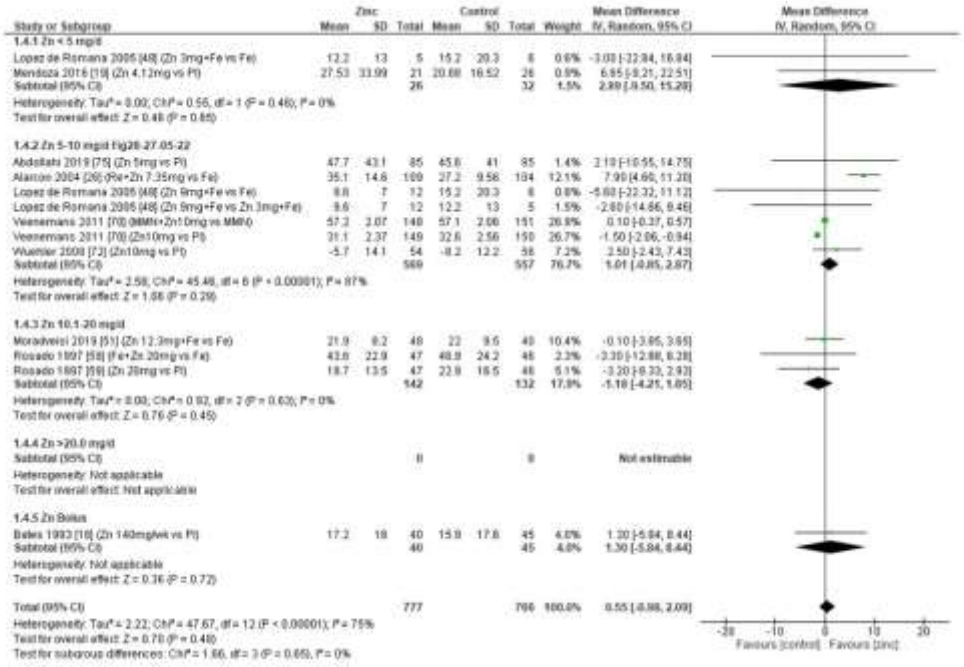

Figure 12. Effect of zinc supplementation on serum ferritin (µg/L) in children aged over 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

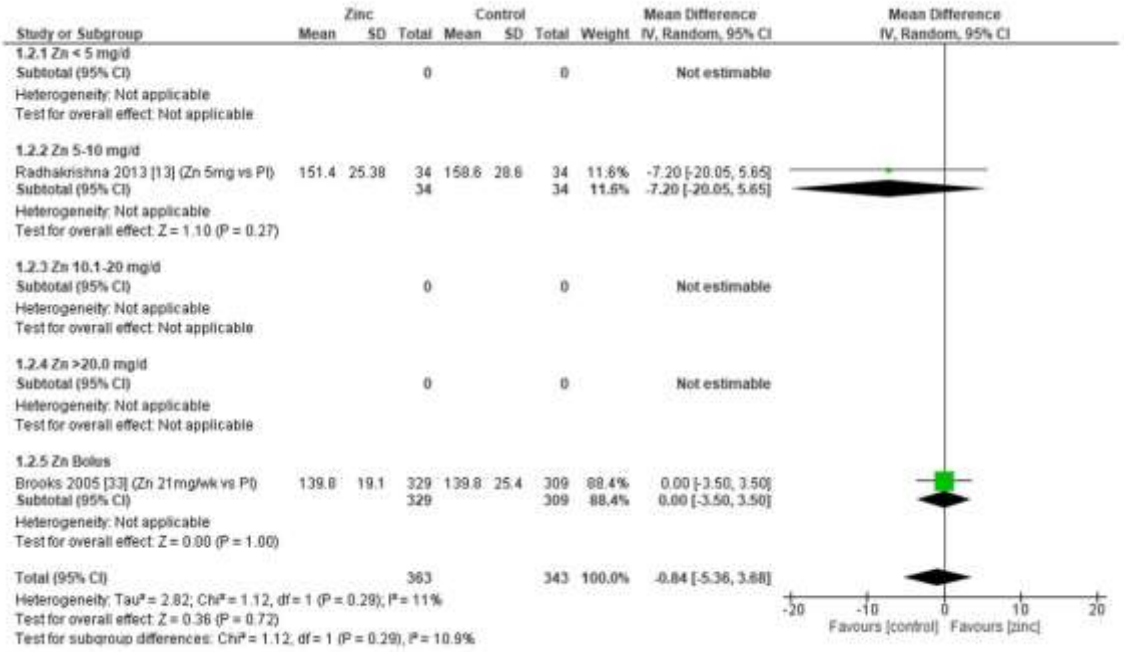

Figure 13. Effect of zinc supplementation on serum/plasma copper (µg/dL) in children aged 91-180 days by zinc dose

Note: CI: confidence interval, SD: standard deviation.

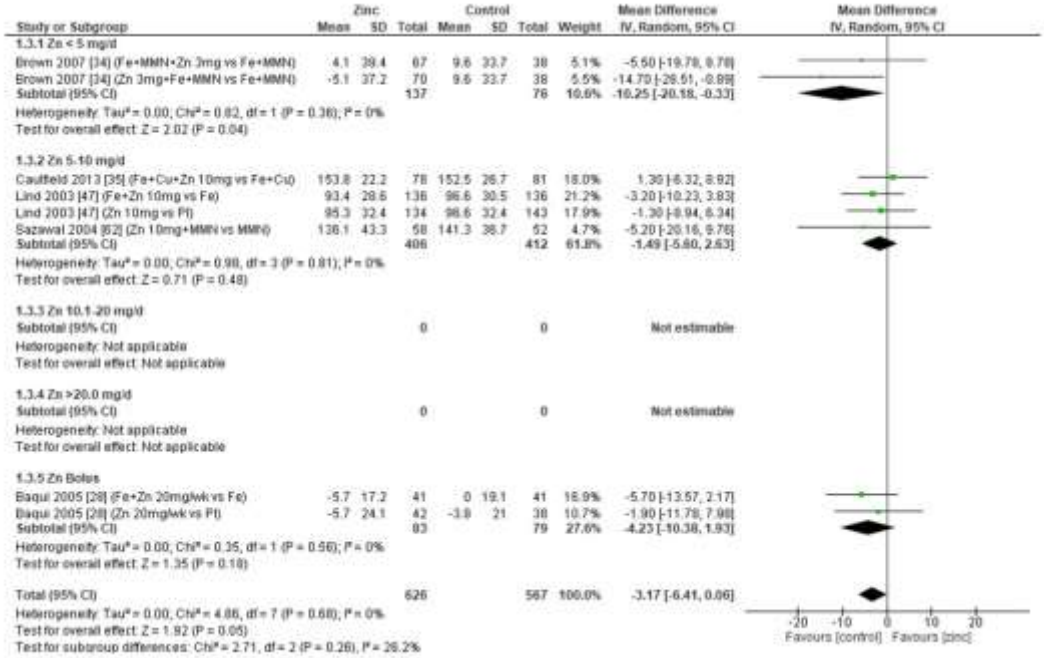

Figure 14. Effect of zinc supplementation on serum/plasma copper (µg/dL) in children aged over 6 months to 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

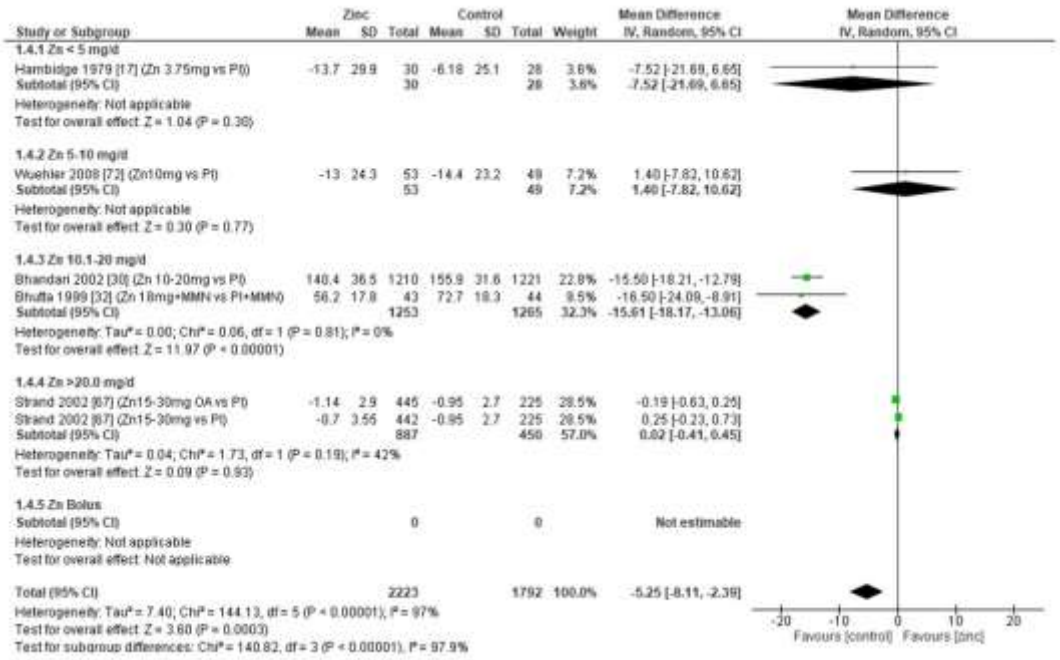

Figure 15. Effect of zinc supplementation on serum/plasma copper (µg/dL) in children aged over 12 months by zinc dose

Note: CI: confidence interval, SD: standard deviation.

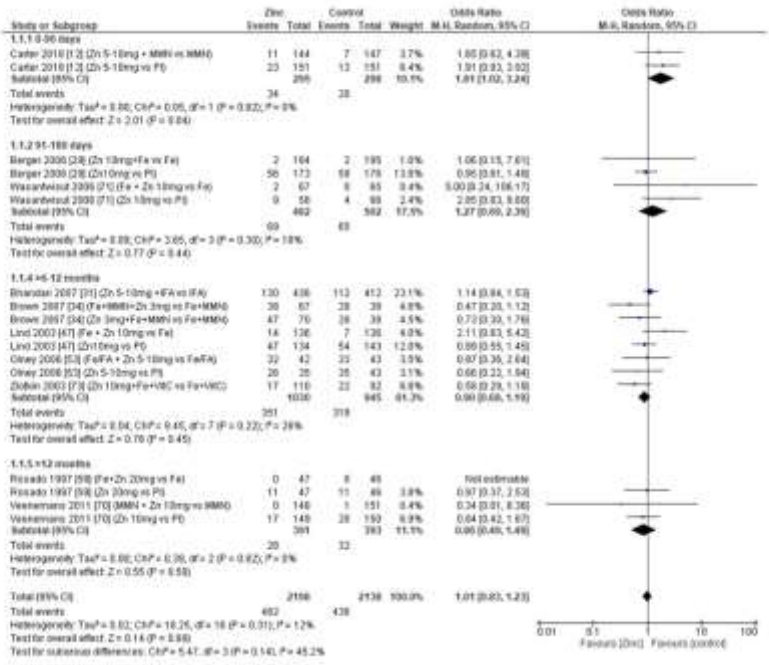

Figure 16. Effect of zinc supplementation in infants and children up to 3 years of age on iron deficiency (OR) by age group

Note: Zinc exposure corresponds to mg/d except for Rosado et al. (59) for which doses were given 6 days a week. CI: confidence interval, SD: standard deviation.

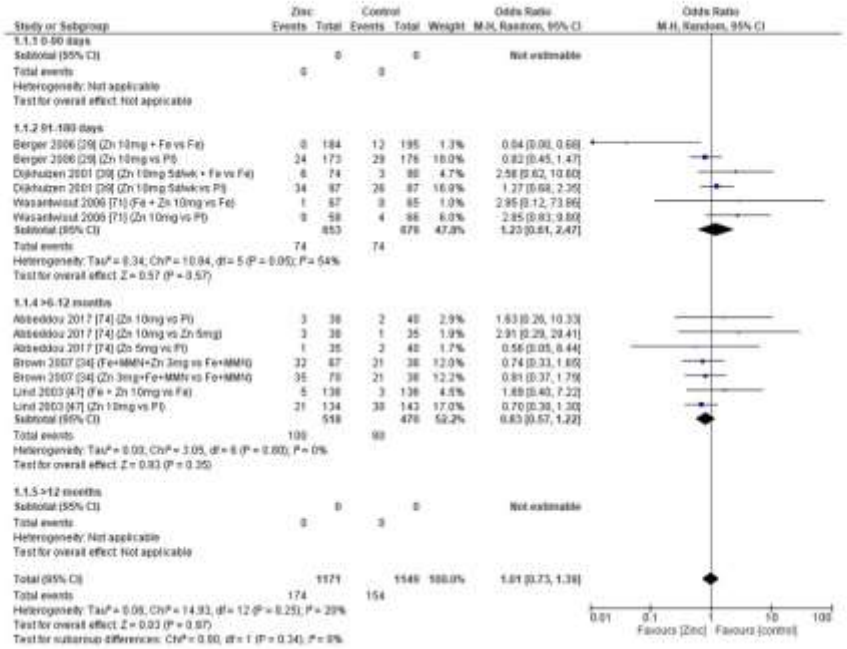

Figure 17. Effect of zinc supplementation in infants and children up to 3 years of age on iron deficiency anemia (OR) by age group

Note: Zinc exposure corresponds to mg/d. CI: confidence interval, SD: standard deviation.

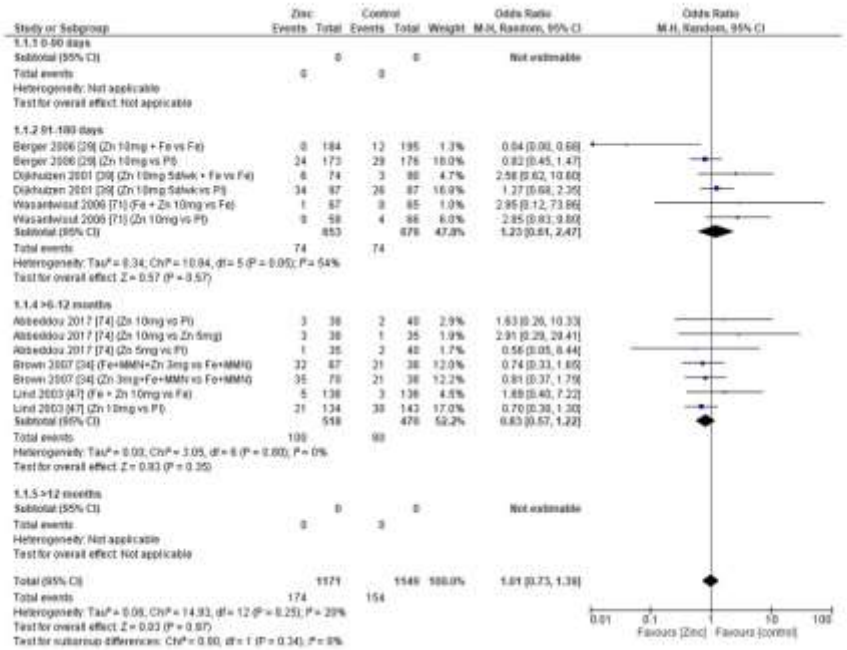

Figure 18. Effect of zinc supplementation in infants and children up to 3 years of age on serum/soluble transferrin receptor (mg/L) by age group

Note: Zinc exposure corresponds to mg/d except for Baqui et al. (28) for which dose was 20 mg/d once weekly, Chang et al. (36) for which dose was given in alternate days. CI: confidence interval, SD: standard deviation.

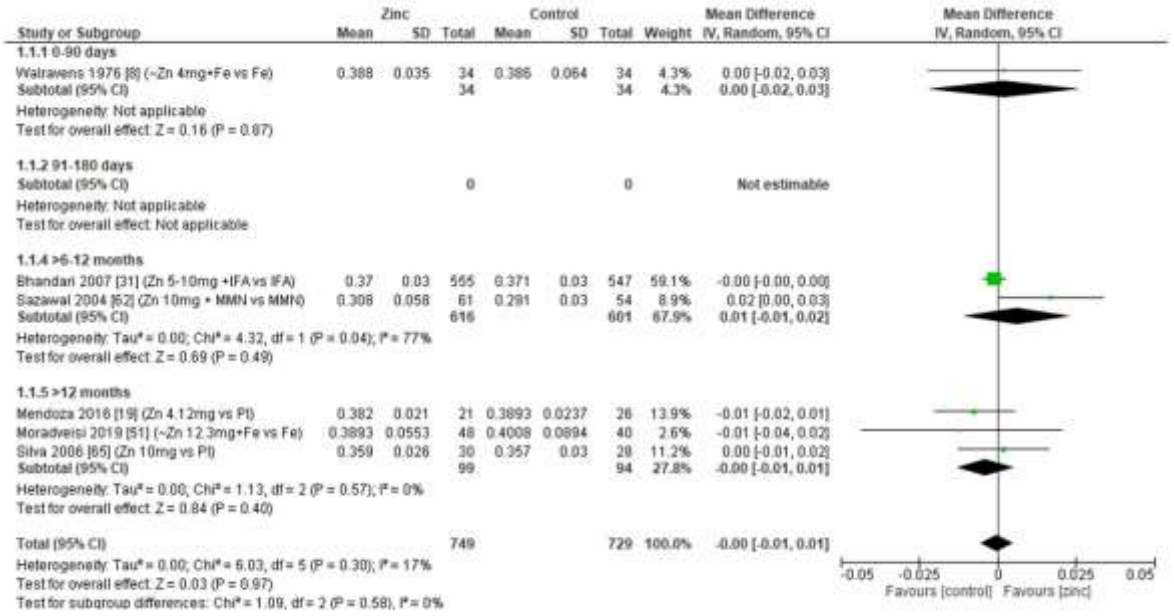

Figure 19. Effect of zinc supplementation in infants and children up to 3 years of age on hematocrit (proportion) by age group

Note: Zinc exposure corresponds to mg/d. CI: confidence interval, SD: standard deviation.

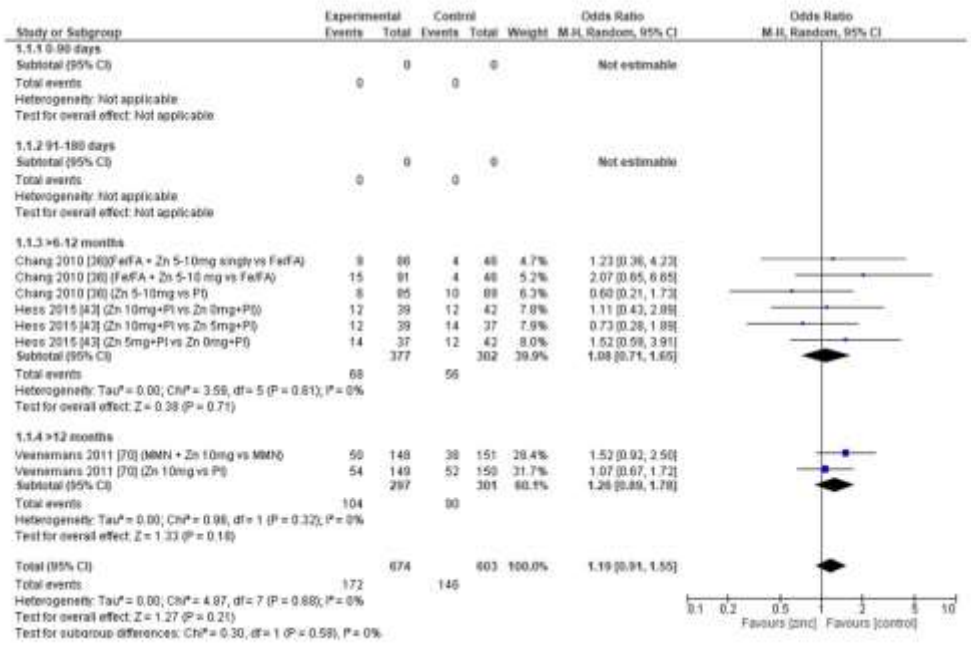

Figure 20. Effect of zinc supplementation in infants and children up to 3 years of age on raised CRP by age group

Note: Zinc exposure corresponds to mg/d except for Chang et al. (36) for which dose was given in alternate days. CI: confidence interval, SD: standard deviation.

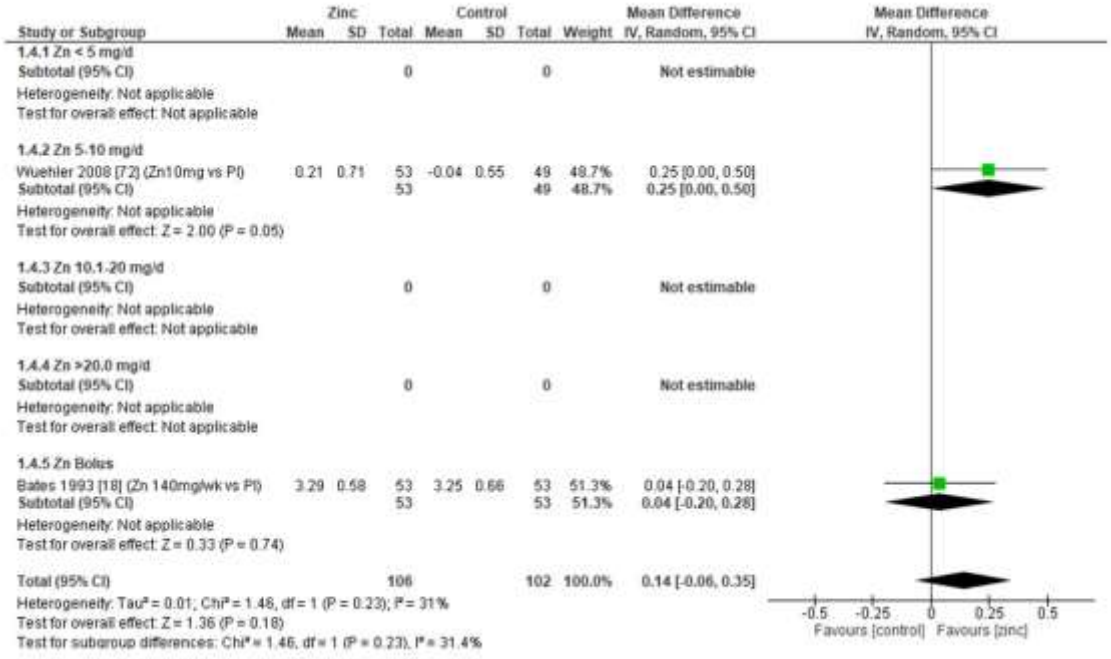

Figure 21. Effect of zinc supplementation on erythrocyte SOD (IU/mg Hb) in children aged over 12 months by zinc dose

Note: Zinc exposure corresponds to mg/d. CI: confidence interval, SD: standard deviation.

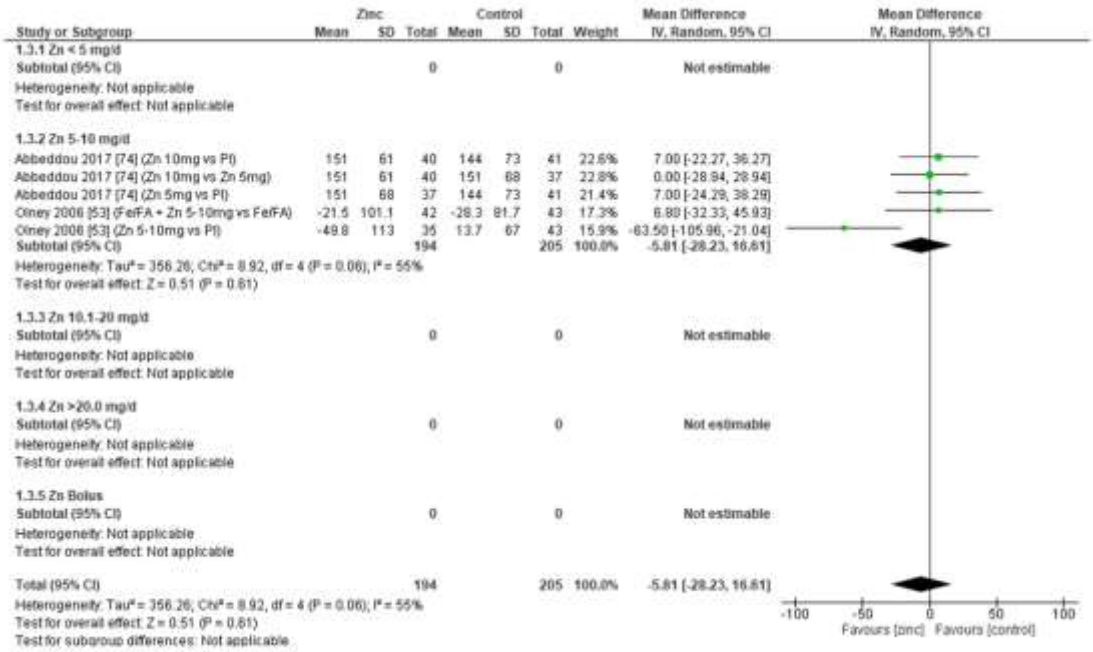

Figure 22. Effect of zinc supplementation on zinc Protoporphyrin (µmol/mol heme) in children over 6 months to 12 months by zinc dose

Note: Zinc exposure corresponds to mg/d. CI: confidence interval, SD: standard deviation.

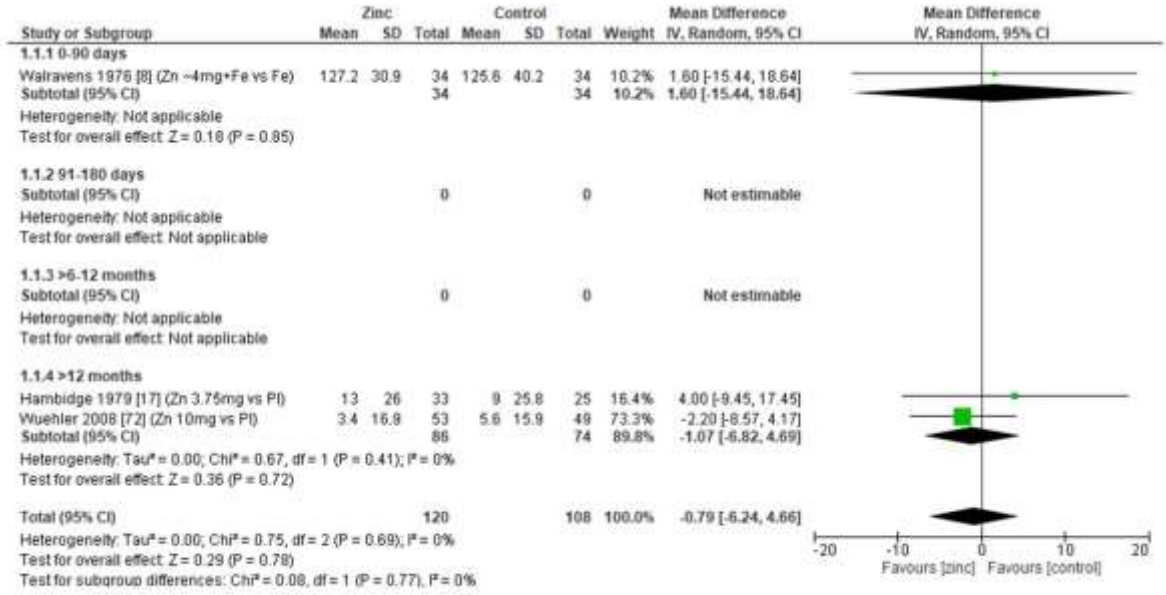

Figure 23. Effect of zinc supplementation in infants and children up to 3 years of age on serum total cholesterol (mg/dL) by duration of treatment

Note: Zinc exposure corresponds to mg/d except for Hambidge et al. (17) for which doses were given 6 days a week. CI: confidence interval, SD: standard deviation.

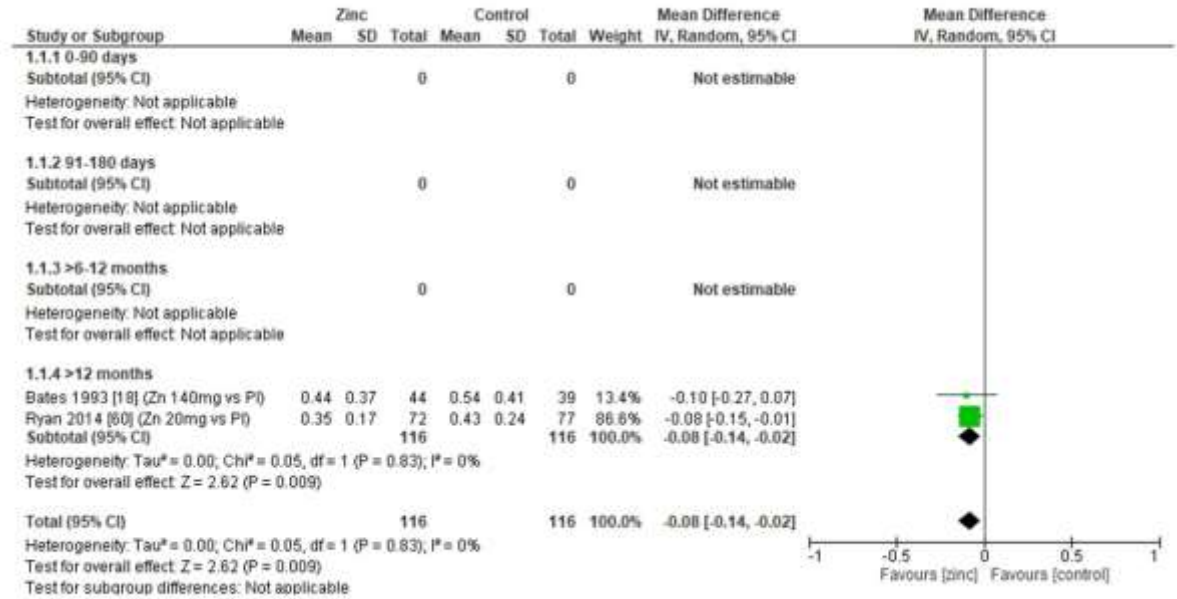

Figure 24. Effect of zinc supplementation in infants and children up to 3 years of age on lactulose mannitol by age group

Note: Zinc exposure corresponds to mg/d except for Bates et al. (18) for which doses of 70 mg/d were given twice weekly. CI: confidence interval, SD: standard deviation.

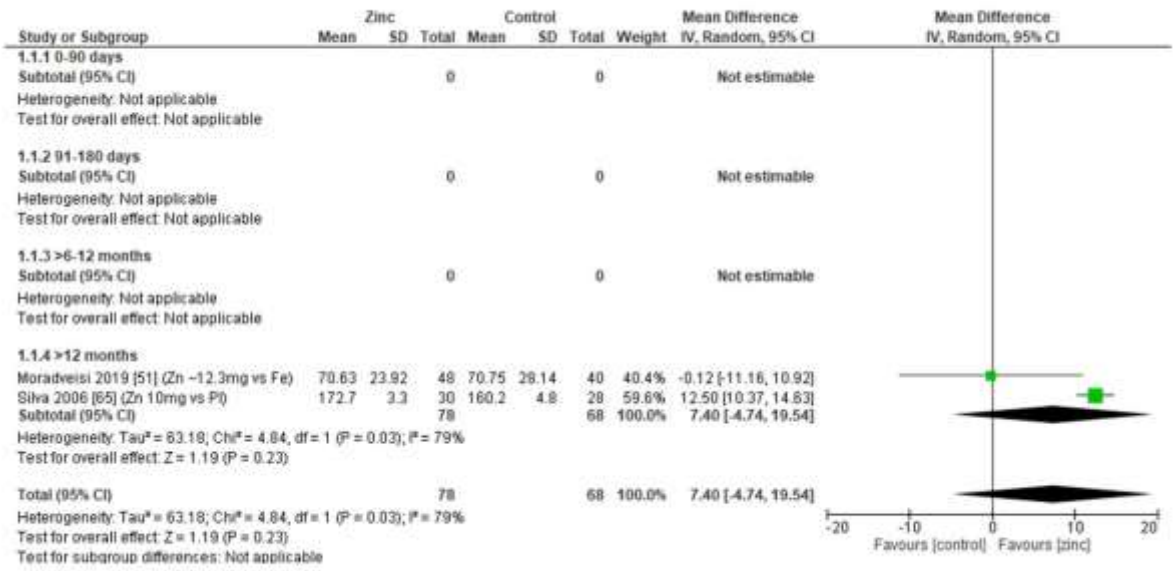

Figure 25. Effect of zinc supplementation in infants and children up to 3 years of age on serum Iron Concentration (µg/dL) by age group

Note: CI: confidence interval, SD: standard deviation.