

## Review Article

# The effect of zinc supplementation on body composition and hormone levels related to adiposity among children: a systematic review

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## Abstract

**Objective:** To provide a comprehensive synthesis of the effects of Zn supplementation on childhood body composition and adiposity-related hormone levels.

**Design:** Five electronic databases were searched for randomized controlled trials of Zn supplementation studies published before 28 February 2015. No statistical pooling of results was carried out due to diversity in study designs.

**Setting:** Community- or hospital-based, from fourteen developing and developed countries.

**Subjects:** Children and adolescents aged 0 to 10 years.

**Results:** Seven of the fourteen studies reported an overall or subgroup effect of Zn supplementation on at least one parameter of body composition, when determined by anthropometric measurements (increased mid upper-arm circumference, triceps skinfold, subscapular skinfold and mid upper-arm muscle area, and decreased BMI). Three out of the fourteen studies reported increased mean value of total body water estimated by bio-impedance analysis and increased fat-free mass estimated by dual energy X-ray absorptiometry and by total body water. Zn supplementation was associated with increased fat-free mass among stunted children. One study found supplementation decreased leptin and insulin concentrations.

**Conclusions:** Due to the use of anthropometry when determining body composition, a majority of the studies could not accurately address whether alterations in the fat and/or fat-free mass components of the body were responsible for the observed changes in body composition. The effect of Zn supplementation on body composition is not consistent but may modify fat-free mass among children with pre-existing growth failure.

**Keywords**  
Zinc supplementation  
Body composition  
Adiposity  
Adiposity-related hormone  
Children

The rapid increase in the prevalence of overweight and obesity in many parts of the world is now characterized as a global pandemic and so has become one of the more important contemporary public health issues<sup>(1,2)</sup>. Recent evidence suggests that the status of micronutrients such as vitamin A<sup>(3)</sup>, vitamin D<sup>(4)</sup>, vitamin B<sup>(5)</sup> and Zn<sup>(6)</sup> may affect energy balance and so play a role in obesity. Obese individuals have lower blood micronutrient concentrations

while micronutrient deficiencies are associated with increased fat deposition in both animal models and epidemiological studies<sup>(7)</sup>. Micronutrient supplementation of obese adults can lead to reductions in body weight, BMI and abdominal obesity, and improve serum lipid profiles, possibly through increased energy expenditure and fat oxidation<sup>(8)</sup>.

Obese adults have been found to have significantly lower serum Zn concentrations while Zn deficiency has been found to be a risk factor for central adiposity among Indian men<sup>(9,10)</sup>. Similarly, children with low hair Zn

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concentrations in Guatemala, New Zealand, Malawi and Ghana have been found to have higher weight-for-age Z-scores, higher BMI and lower mid upper-arm muscle area (MAMA) compared with children with greater hair Zn concentrations<sup>(11–13)</sup>. Other studies, in contrast, have found no differences in serum Zn concentrations between obese and normal-weight individuals<sup>(14,15)</sup>. However, Zn supplementation has been found to be associated with greater weight gain and increased lean tissue mass simultaneously with increased linear growth among malnourished and stunted children<sup>(16–18)</sup>.

Zn may determine body adiposity through its role in energy metabolism, appetite control and adipokine regulation. Marginal Zn deficiency solely or in conjunction with a low-protein diet is associated with reduced lean body mass, decreased appetite and excess adiposity, each of which can be altered or restored by Zn supplementation<sup>(19,20)</sup>. Zn is an important component of enzymes involved in energy metabolism and Zn metalloenzymes essential for nucleic acid and protein synthesis and new tissue synthesis<sup>(21)</sup>. It also regulates the hormones leptin, ghrelin, insulin and adiponectin, which, in turn, regulate adiposity and fat mass<sup>(22–24)</sup>. Any changes in tissue-specific adipokine concentrations associated with Zn status may subsequently determine changes in adipose tissue mass and in turn modify the risk of obesity.

Clinical and epidemiological evidence suggests that Zn status may be contributing to the increased burden of obesity reported in countries passing through the epidemiological and nutrition transitions due to its effect on body composition<sup>(10,18)</sup>. Meta-analyses have provided strong evidence for the growth-limiting effect of Zn deficiency<sup>(25)</sup>. There is increasing evidence that Zn status may play a role in determining both growth patterns and body adiposity<sup>(18,26)</sup>. Malnutrition in early childhood is associated with a greater risk of obesity and numerous chronic diseases later in life, possibly due to metabolic 'programming' and other physiological adaptations in response to nutrition constraints<sup>(27,28)</sup>. Such metabolic adaptations accompanying childhood stunting affect body fatness, growth rate, energy balance and fat oxidation, which can lead to obesity in the context of later availability of abundant energy (food)<sup>(29,30)</sup>. Pre-existing Zn deficiency may be contributing to the development of obesity via its relationship with fat deposition associated with childhood stunting and subsequent short stature.

There has been no systematic review to date of the effects of Zn supplementation on body adiposity and composition. Therefore, we carried out a review of the literature to clarify what is understood about the effects of Zn supplementation on children's body composition and address what factors may modify these associations. We also reviewed studies which reported the effect of Zn supplementation on adipokines and other hormones involved in adipogenesis that may be the mechanisms underlying associations between Zn status, body composition and risk of obesity.

## Methods and procedures

### Search strategy

A five-stage comprehensive search of the literature was conducted in the databases Cochrane Library, PubMed, Ovid Medline, CINAHL and EMBASE, for studies published before 28 February 2015. The search strategy for conducting the systematic review used the PICOS (Population, Intervention, Comparison, Outcome and Setting) method, in which search terms are separately developed for each component<sup>(31)</sup> to identify studies which assessed children as the study population (P) and supplementation with Zn alone or in combination with other micronutrients as the intervention/comparison (I/C). Zn could have been given with other micronutrients and the comparison group received the micronutrients (so that Zn was the only difference between the groups). Body composition, adiposity, leptin, ghrelin, insulin, adiponectin and adipokines are the outcomes (O) and studies conducted either in a community- or hospital-based setting from developing and developed countries (S). During the first stage the above databases were searched using the following keywords: 'Zinc' or 'Zn' or 'Zinc supplementation' or 'Zn supplementation' or 'Zinc therapy', AND 'child' or 'children' AND 'body composition' or ('adiposity', 'fat', 'fat free mass', 'weight', 'BMI', 'fat mass') or ('adipocytokine', 'adipokine', 'insulin', 'adiponectin', 'ghrelin', 'leptin').

In the second stage the total hits from the databases were pooled and duplicates were removed. This was followed by screening of the retrieved articles by reading the article 'title' in the third stage and the article 'abstract' in the fourth stage. In the fifth stage individual manuscripts were screened and those not satisfying inclusion criteria were excluded. To obtain additional data, a manual search of the reference lists of articles selected in the fifth stage was performed. This search process was conducted independently by two reviewers (I.R.G. and K.Z.L.) and the final group of articles to be included in the review was determined after an iterative consensus process. The present review was prepared in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines<sup>(32)</sup>.

The assessment of risk of bias within each study and the quality appraisal of each trial were evaluated using the quality checklists protocol of the CONSORT (Consolidated Standards of Reporting Trials) Statement 2001 for randomized controlled trials<sup>(33)</sup>. We appraised the methods section of the articles as the key quality parameters considered in the review (data not shown). However, since no quality evaluation of the studies was made, we did not exclude any studies due to concern about their quality.

We used the following inclusion and exclusion criteria: studies were included if they were controlled trials in human subjects, randomized trials of the effect of Zn supplementation efficacy (either as a single micronutrient

or in combination with other micronutrients) on body composition and adiposity-related hormone levels among children aged from infant to adolescent, from both developing and developed countries, in peer-reviewed, English-language journals. Papers were excluded if they were single clinical case studies or clinical discussion papers, or not primary sources. Book chapters and review papers were not considered primary sources and were excluded. The reference lists and citations of articles identified from the electronic search were assessed to ensure the inclusion of additional relevant studies. No communications with individual researchers were made.

The outcomes of interest for the present review were the different measures of body composition defined as the characteristic size and distribution of the component parts of total body weight<sup>(34)</sup>. Body composition in studies included in the review was assessed using two methods: 'direct' and 'indirect' methods. Studies employing direct methods used such methods as dual-energy X-ray absorptiometry (DXA) with the use of a densitometer and total body water (TBW) by bio-impedance analysis (BIA) and <sup>2</sup>H dilution. These direct methods provide greater accuracy and rapid, non-invasive estimates of fat-free mass (FFM), fat mass (FM) and percentage body fat (%BF)<sup>(35)</sup>. The indirect method of assessing body composition in the reviewed studies was anthropometric measurements<sup>(35)</sup>. The anthropometric methods used in the reviewed studies included BMI determined by the relationship of body weight to body height, and skinfold thickness. Skinfold measurements can provide estimates of the size of the subcutaneous fat depot and body composition after applying equations to these measurements<sup>(36)</sup>. We included studies which reported on FFM outcomes using anthropometric measures of mid upper-arm circumference (MUAC), mid upper-arm fat area (MAFA) and MAMA, which are derived from MUAC and triceps skinfold (TSF)<sup>(35)</sup>.

Zn plays a role in the regulation of adipokines and inflammatory responses, a mechanism which may underlie the effect of Zn supplementation on adiposity. Accordingly, studies that reviewed the effects of Zn on adipokines and other hormones involved in adipogenesis (leptin, ghrelin, adiponectin and insulin) were also screened as secondary outcomes for the present review.

### **Evaluation of studies**

Information on study outcomes, body composition measures, and the location, study design, sample size and characteristics was extracted from each included study. The reviewed studies were heterogeneous in design and outcomes; therefore statistical pooling was not possible. Instead, findings are presented in narrative form in which bivariate and/or multivariate associations between Zn supplementation and adiposity and adiposity-related hormones are summarized in tables and text to aid data

presentation where appropriate. As such, an effect of Zn supplementation was defined as a significant difference in body composition between Zn and control groups for at least one parameter of body composition or adiposity-related hormone.

## **Results**

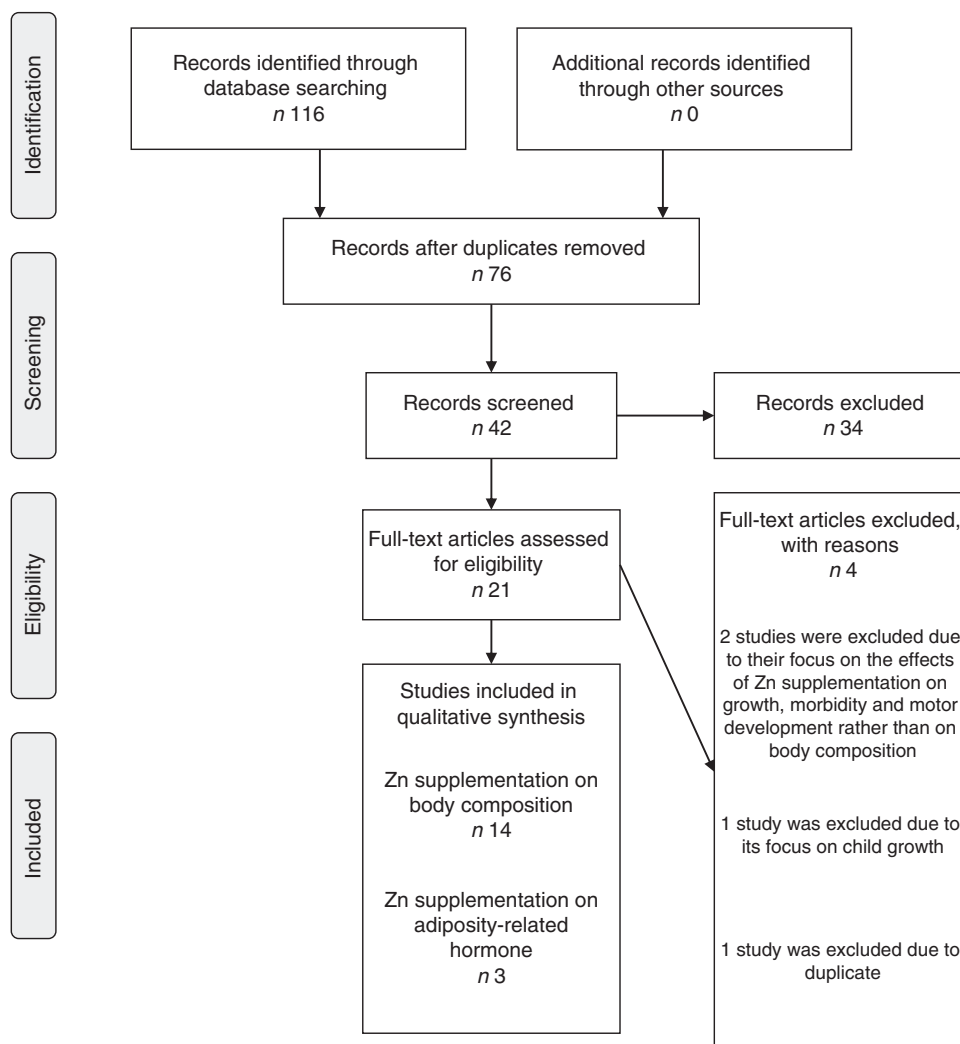
### **Search results**

The initial search identified 116 articles of which forty-two were identified as potentially eligible for inclusion after the screening of titles and abstracts. These articles were retrieved and reviewed in full, leading to the selection of sixteen articles reporting the effect of Zn supplementation on children's body composition in randomized controlled trials and five studies reporting Zn supplementation on adiposity-related hormones. Two of the studies on Zn supplementation on body composition<sup>(37,38)</sup> were considered as randomized controlled trials although the children were randomized into different treatment arms/multiple treatments instead of a placebo group. Two of the studies of Zn supplementation on body composition<sup>(17,39)</sup> and one study of Zn supplementation on adiposity-related hormones<sup>(16)</sup> were excluded due to their focus on childhood growth, morbidity and motor development, with no findings reported on the outcomes of interest. One study<sup>(24)</sup> was also excluded due to duplicate publication. This left fourteen studies on body composition and three studies<sup>(40–42)</sup> on adiposity-related hormone outcomes meeting the inclusion criteria and so were included in the present review (Fig. 1).

### **Overview of findings**

Table 1 presents the summary characteristics of reviewed studies and Table 2 presents the method used for assessing body composition and main findings. Out of the fourteen studies of Zn supplementation on body composition, ten studies<sup>(37,38,42–49)</sup> reported an overall or subgroup effect on at least one parameter of body composition, while the remaining four found no effects<sup>(50–53)</sup>.

Among the ten studies<sup>(37,38,42–49)</sup> which reported an effect of Zn on body composition, three studies used direct measurements of body composition as well as the indirect measures<sup>(37,38,46)</sup>. Diaz-Gomez *et al.*<sup>(38)</sup> reported higher mean values of TBW estimated by BIA among Zn-supplemented preterm infants in 6 months. No significant differences in subcutaneous fat accretion were found between children in the Zn and placebo groups, suggesting a positive effect of supplementation on FFM. Similarly, hospitalized children aged 4–10 years with sickle cell anaemia who were supplemented with Zn over 12 months were found to have greater MUAC and MAMA Z-scores and improved rates of linear growth compared with children receiving the placebo, while no significant



**Fig. 1** Systematic review flowchart (adapted from Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6, e1000097. doi:10.1371/journal.pmed1000097)

differences were found for body composition (whole-body FFM, FM and %BF) when using DXA<sup>(46)</sup>. However, girls who received the Zn supplement had a significant 0.87 kg increase in FFM. In the study by Arsenault *et al.*<sup>(37)</sup>, no effect of Zn supplementation was found on overall body composition among Peruvian children aged 6–8 months in 6 months. However, children with mild-to-moderate stunting (length-for-age Z-score <−1.1) who received a Zn supplement with Fe-fortified porridge had a greater increase in FFM and increase in mean TBW compared with children in the two other groups (Fe-fortified porridge plus Zn and control groups). These findings suggested that Zn supplementation may have a beneficial effect on FFM, especially among children with pre-existing growth failure.

Seven out of the ten studies<sup>(37,38,42–49)</sup> which reported an effect of Zn on body composition used only indirect measures of body composition. Friis *et al.*<sup>(44)</sup> reported that Zn-supplemented children (6–17 years old) in 12 months

had significantly greater increases in their MAMA-for-age Z-score (an indication of increased FFM) compared with children receiving the placebo. Their study also reported significant increases in weight gain while no effect was found on FM and linear growth. Rivera *et al.*<sup>(47)</sup> in their study among rural Guatemalan infants (6–9 months) found that Zn supplementation for 7 months was associated with an increase of 0.61 cm<sup>2</sup> in MAMA while at the same time it increased linear growth of children who were stunted at baseline. A 15-month Zn supplementation trial among Gambian children aged 6 months to 2 years reported a linear increase in body weight and a very small (2%) but significant increase in MUAC or MAMA among Zn-supplemented children<sup>(48)</sup>. Kikafunda *et al.*<sup>(45)</sup> found that Zn supplementation among Ugandan children (aged 33–89 months) in 6 months significantly increased MUAC but had no effect on height, weight and height-for-age Z-score. A study carried among obese Iranian children (aged 6–10 years) in 8 weeks reported a significant

**Table 1** Zinc supplementation studies on body composition

Study location; reference	Characteristics of study population	Study design	Data collected
1. Philadelphia, USA; Zemel <i>et al.</i> <sup>(46)</sup>	Hospital-based: prepubertal children with sickle cell disease, short stature (HAZ <2) Age: 4–10 years Sample size: 42	Stratified by sex and initial height, randomized to receive 10 mg ZnSO <sub>4</sub> /d in 5 ml syrup or placebo for 12 months	Randomization was stratified according to age, sex and initial height status 15% of the children had low Zn (<10.7 μmol/l)
2. Canaries; Diaz-Gomez <i>et al.</i> <sup>(36)</sup>	Hospital-based: preterm infants Age: premature infants Sample size: 36	Treatment group received 10 mg ZnSO <sub>4</sub> /l in standard term infant formula and 0.6 mg Cu/l, at 36 weeks post-conceptual age, at 3 and 6 months corrected postnatal age; whereas the placebo group received the same formula without supplementation (Zn content 5 mg/l and Cu content 0.4 mg/l); Fe supplement (1 mg/kg, as ZnSO <sub>4</sub> ) was given once daily during the study period	Dietary record for daily intake of Zn and Cu
3. Peru; Arsenault <i>et al.</i> <sup>(37)</sup>	Community-based: low-income, peri-urban children with initial LAZ <−0.5 Age: 6–8 months Sample size: 302	Children were randomly assigned to one of three groups, followed for 6 months: (i) Zn supplement (ZnSuppl) group received Fe-fortified, wheat-based porridge without added Zn liquid multivitamin supplement with 3 mg ZnSO <sub>4</sub> /d; (ii) Zn fortification (ZnFort) group received the same porridge with added ZnSO <sub>4</sub> (3 mg/d) and the liquid multivitamin supplement without Zn; (iii) control group received porridge and liquid multivitamin supplement without Zn	Dietary intake Morbidity assessment Body composition assessed in a subset of children at baseline and 6 months using <sup>2</sup> H dilution TBW was calculated using formula
4. Guatemala; Cavan <i>et al.</i> <sup>(43)</sup>	Community-based: low-income peri-urban area, mild Zn deficiency, plant-based diet, schoolchildren Age: 6–7 years Sample size: 162	Matched pairs by age and sex, randomized to receive 10 mg Zn tablet (amino acid chelate, chewable) daily or to control group for 25 weeks (6 months); both Zn and placebo provided multivitamin and multi-mineral	Plasma Zn and Cu Initial height/weight anthropometry Initial Zn status (hair Zn < 1.68 μmol/g and ≥1.68 μmol/g) No data on morbidity and diet
5. Zimbabwe; Friis <i>et al.</i> <sup>(44)</sup>	Community-based: rural area, generally healthy schoolchildren Age: 6–17 years Sample size: 313	Treatment group received either 30 mg or 50 mg ZnSO <sub>4</sub> tablets (based on body weight), daily, for 12 months (equivalent to 189 d)	Cut-off for Zn was 10.7 μmol/l Dietary phytic acid:Zn molar ratio = 15
6. Uganda; Kikafunda <i>et al.</i> <sup>(45)</sup>	Community-based: very low SES suburb, growth-retarded but generally healthy children Age: 33–89 months Sample size: 153	Stratified by sex, randomized to receive 10 mg ZnSO <sub>4</sub> tablet (dissolved in freshly prepared fruit juice) or placebo, 5 d/week for 6 months	Anthropometry measurements were taken at baseline and at 2, 3, 6, 7 and 8 months after the start of the trial
7. Guatemala; Rivera <i>et al.</i> <sup>(47)</sup>	Community-based: infants Age: 6–9 months Sample size: 89	Double-blind intervention trial, children were assigned randomly to receive 4 ml of a beverage containing 10 mg of Zn as ZnSO <sub>4</sub> ( <i>n</i> 45) or a placebo ( <i>n</i> 44) daily (7 d/week) for an average of 6.9 months. The children's weight, length, MUAC, head circumference and TSF were measured at baseline and at 1- to 2-month intervals until the end of supplementation. MAMA was derived from MUAC and TSF measurements	Maternal anthropometry Family SES Demographic characteristics Morbidity Dietary intake Appetite Physical activity pattern Compliance 95%
8. Iran; Kelishadi <i>et al.</i> <sup>(42)</sup>	Clinical-based: obese children, BMI ≥ 95th percentile (CDC) Age: 6–10 years Sample size: 60	Children were randomly assigned into two groups, received 20 mg of elemental Zn or placebo. The trial was triple-masked randomized, followed for 8 weeks and then, after a 4-week wash-out period, the group was crossed over, so that the children initially receiving Zn received the placebo and vice versa; the protocol was repeated again for 8 weeks of follow-up	Reported initial serum Zn Reported hormones related to adiposity: insulin, leptin

Table 1 Continued

Study location; reference	Characteristics of study population	Study design	Data collected
9. Gambia; Bates <i>et al.</i> <sup>(48)</sup>	Community-based: rural children Age: 0.57–2.30 years Sample size: 110	Children were divided into two matched groups, stratified by age and sex, one to receive 70 mg Zn (initially as Zn acetate, then as Zn gluconate for better compliance) twice weekly for 1.25 years (15 months) and the other a placebo. Growth and MUAC were measured at weekly intervals throughout the study	Illnesses were monitored Biochemical indices of Zn status
10. South India; Radhakrishna <i>et al.</i> <sup>(49)</sup>	Community-based: low-income, urban Full-term healthy infants, gestational age <37 weeks Sample size: 160 per group	Randomized, double-blind, placebo-controlled trial, community-based. Zn in syrup base, ZnSO <sub>4</sub> 10 mg elemental Zn/ml, 5 mg Zn/d and riboflavin 0.5 mg/d. Supplementation was initiated after the age of 4 months until 18 months of age for the period of 12 months. Follow-up at 12–18 months and 18–24 months	Nutritional status: weight, length, head circumference, chest circumference, MUAC, skinfold (triceps, biceps, subscapular) measured once in 3 months Morbidity Dietary intakes in a subset of 78 children Blood sample: Zn, Cu, Hb, vitamin A Zn deficiency (serum Zn <60 µg/dl): Zn group 26.5%, supplementation group 44.1%
11. Mexico; Rosado <i>et al.</i> <sup>(50)</sup>	Community-based: rural area, growth-stunted generally healthy pre-school children, inadequate dietary Zn Age: 18–36 months Sample size: 219	Stratified by age and sex, ranked by height, randomized to receive daily: (i) 20 mg Zn (Zn methionine), (ii) 20 mg Zn and 20 mg Fe (FeSO <sub>4</sub> ), (iii) 20 mg Fe or (iv) placebo in solution (contained sugar, citric acid, water, artificial flavour), for 12 months	Morbidity Hb, ferritin, CRP, plasma Zn (categorized into <10.7 µmol/l and ≥10.7 µmol/l) Initial plasma Zn was >13.8 µmol/l; 20% of children had baseline value <10.7 µmol/l
12. Ethiopia; Umata <i>et al.</i> <sup>(51)</sup>	Community-based: healthy breast-fed infants Age: 6–12 months Sample size: 200	A randomized, double-blind, placebo-controlled trial, 100 non-stunted (LAZ >−2) were matched for age, sex and recumbent length with 100 randomly selected stunted (LAZ <−2) infants. Zn supplement (10 mg Zn/d, as ZnSO <sub>4</sub> ) or placebo was given 6 d/week for 6 months	Data on illness and appetite were collected daily Samples of serum and hair for Zn analysis
13. California, USA; Heinig <i>et al.</i> <sup>(52)</sup>	Community-based: breast-fed infants Age: 4–10 months Sample size: 85	A randomized double-blind trial comparing Zn supplementation (5 mg/d as ZnSO <sub>4</sub> ) with placebo. Growth and indices of body composition and gross motor development were measured monthly from 3 to 10 months	Parental anthropometry Morbidity Dietary intake Plasma Zn, Fe, Cu, ferritin, IgG2 and IgG4
14. Santiago, Chile; Ruz <i>et al.</i> <sup>(53)</sup>	Day care-based: apparently healthy pre-school children, from low SES conditions Age: 27–50 months Sample size: 98	Double-blind Zn supplementation trial. Children were pair-matched according to sex and age, and randomly assigned to two experimental groups: the supplemented group, which received 10 mg Zn/d (as ZnSO <sub>4</sub> ) in glucose-based syrup, and the placebo group, for 14 months	Anthropometry Morbidity Dietary intake Biochemical: plasma Zn, Cu

HAZ, height-for-age Z-score; LAZ, length-for-age Z-score; SES, socio-economic status; CDC, US Centers for Disease Control and Prevention; MUAC, mid upper-arm circumference; TSF, triceps skinfold; MAMA, mid upper-arm muscle area; TWB, total body water; CRP, C-reactive protein.

**Table 2** Zinc supplementation studies and body composition outcome

	Study location; reference	Assessment of body composition	Main findings	Body composition outcome
1.	Philadelphia, USA; Zemel <i>et al.</i> <sup>(46)</sup>	Direct (DXA): FFM, FM, %BF Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>• The effect of Zn supplementation on FFM measured by DXA was not significant</li> <li>• From indirect methods: Zn supplementation had no significant effect on the fat-related measures TSF, MUAFA, FM and %BF. MUAC Z-scores were significantly greater in the Zn group. MAMA Z-scores were marginally significantly greater in the Zn group. FFM from skinfold was not significant in all samples. Only in girls, Zn supplementation was shown significantly increase FFM by 0.87 kg (<math>P=0.008</math>). Significant increased rates of growth in height, sitting height and knee height in Zn supplement group and in subgroup of children whose initial height status was low</li> <li>• No effect on BMI</li> </ul>	<p>Found effect on body composition</p> <p><i>Longitudinal mixed-effect models of the effect of Zn on:</i>            MUAC Z-score (coefficient, SE, <math>P</math> value)=0.27, 0.12, 0.03            MAMA Z-score (coefficient, SE, <math>P</math> value)=0.29, 0.15, 0.06            FFM in girls only (coefficient, SE, <math>P</math> value)=0.87, 0.32, 0.008</p>
2.	Canaries; Diaz-Gomez <i>et al.</i> <sup>(38)</sup>	Direct (BIA): TBW Indirect (anthropometric): MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>• Zn supplement had a positive effect on linear growth in premature infants, at least until 6 months. Improvement in TBW estimated by BIA found in the Zn group</li> <li>• Significant interaction between the independent variables (study period and group) in both length and TBW</li> <li>• Higher mean values of TBW in the supplementation group without significant differences in subcutaneous fat accretion could indicate a positive effect of supplementation on fat-free body mass</li> </ul>	<p>Found effect on body composition</p> <p><i>TBW:</i>            Significant interaction between the independent variables (study period and group) in TBW estimated by BIA (TBW <math>F(2, 62)=4.59, P=0.014</math>)            Significant difference between supplementation and placebo group at 3 and 6 months (TBW <math>&gt;0.247, P=0.01</math>)</p>
3.	Peru; Arsenault <i>et al.</i> <sup>(37)</sup>	Direct ( $^2\text{H}$ dilution): mean TBW, FFM, FM, %FM Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA, MUAC Z-score	<ul style="list-style-type: none"> <li>• Zn supplementation, either as a liquid supplement or in fortified food, did not affect overall energy intake or FFM accrual compared with those who did not receive additional Zn</li> <li>• Among the subset of children with mild-to-moderate stunting (LAZ <math>&lt;-1.1</math>), those who received liquid Zn supplement (ZnSuppl group) had a greater increase in FFM</li> <li>• It is possible that FFM accrual may be more sensitive to Zn deficiency and responds earlier to Zn supplementation than linear growth</li> <li>• TBW, FFM, FM and %FM did not differ among groups at baseline and there were no significant main effects of treatment group on the change in any of these variables from baseline to 6 months</li> </ul>	<p>Found effect on body composition</p> <p><i>Overall:</i>            Mean TBW increased from 3.97 to 4.74 kg            FFM increased from 4.95 to 6.00 kg during the 6-month study period (<math>P&lt;0.0001</math>)            FM increased from 2.66 to 2.93 kg (<math>P&lt;0.0001</math>)            %FM decreased from 34.7 to 32.65 (<math>P=0.0002</math>)  <i>Among children with LAZ <math>&lt;-1.1</math>:</i>            Children who received liquid Zn supplement (ZnSuppl group) had a greater increase in FFM (1.36 kg) than the ZnFort group (0.95 kg; <math>P=0.02</math>) or the control group (0.95 kg; <math>P=0.04</math>)</p>
4.	Guatemala; Cavan <i>et al.</i> <sup>(43)</sup>	Indirect (anthropometric): MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>• Children responded to the Zn supplement with changes in indices of body composition rather than growth</li> <li>• The Zn group had significantly higher median Z-scores for MUAC and TSF at the end of the study</li> <li>• Supplemented children showed a smaller deficit in median MUAC Z-score</li> <li>• Supplemented children showed increased in fat status, as indicated by the significant rise in median TSF Z-score</li> </ul>	<p>Found effect on body composition</p> <p><i>MUAC Z-score (median (1st, 3rd quartile)):</i>            Zn group            Initial = -0.60 (-0.92, -0.30)            Final = -0.72 (-1.05, -0.26)            Change = -0.03 (-0.30, 0.16), <math>P&lt;0.05</math>            Placebo            Initial = -0.44 (-0.86, -0.19)            Final = -0.66 (-1.08, -0.35)            Change = -0.20(-0.42, 0.02), <math>P&lt;0.05</math>  <i>TSF Z-score (median (1st, 3rd quartile)):</i>            Zn group            Initial = -0.20 (-0.50, 0.10)            Final = 0.33 (-0.07, 0.95)            Change = 0.50 (0.25, 0.99), <math>P&lt;0.05</math></p>

Table 2 Continued

Study location; reference	Assessment of body composition	Main findings	Body composition outcome
5. Zimbabwe; Friis <i>et al.</i> <sup>(44)</sup>	Indirect (anthropometric): MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>Significance effects on weight, WAZ and MAMA-for-age Z-score over 3 months but no effects over 12 months</li> <li>Zn supplementation had favourable effects on lean body mass and weight gain, but no effects on fat and linear growth</li> <li>The mean weight gain was 0.51 kg in the Zn-supplemented group v. 0.14 kg in the placebo group (<math>P=0.01</math>)</li> <li>The change in MAMA-for age Z-score was significantly larger among Zn-supplemented children compared with children receiving placebo (0.10 v. 0.001, <math>P=0.03</math>)</li> </ul>	<p>Placebo Initial = -0.27 (-0.57, 0.03) Final = 0.14 (-0.16, 0.43) Change = 0.38 (0.18, 0.66), <math>P &lt; 0.05</math></p> <p>Found effect on body composition MAMA-for-age Z-score (<math>P=0.03</math>): Zn group Baseline (mean (sd)) = -0.92 (0.59) At 3 months (mean (95% CI)) = 0.10 (0.04, 0.15)</p> <p>Placebo Baseline (mean (sd)) = -0.85 (0.58) At 3 months (mean (95% CI)) = 0.01 (-0.05, 0.07)</p>
6. Uganda; Kikafunda <i>et al.</i> <sup>(45)</sup>	Indirect (anthropometric): MUAC, MAFA, MAMA	<ul style="list-style-type: none"> <li>No effect on height, weight and HAZ</li> <li>Zn supplementation had a significant effect on MUAC (<math>P=0.029</math>)</li> <li>Greater weight gain only in children from medium SES</li> <li>Significantly increased MUAC (<math>P=0.03</math>)</li> </ul>	<p>Found effect on body composition MUAC (mean (sd), in mm) (<math>P=0.03</math>): Zn group Baseline = 163.9 (11.6) LSM changes At 3 months = -0.88 (0.42) At 8 months = 0.86 (0.57)</p> <p>Placebo Baseline = 165.9 (11.1) LSM changes At 3 months = -1.25 (0.42) At 8 months = -0.82 (0.60)</p>
7. Guatemala; Rivera <i>et al.</i> <sup>(47)</sup>	Indirect (anthropometric): MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>Zn supplementation was associated with an overall increase of 0.61 cm<sup>2</sup> in MAMA (<math>P &lt; 0.001</math>)</li> <li>Children who received Zn supplements had a mean length increment that was 0.75 cm greater than those who did not (<math>P=0.12</math>)</li> <li>There was a significant interaction between treatment group and initial LAZ (<math>P=0.04</math>), such that supplemented children who were stunted at baseline (LAZ &lt; -2) gained 1.40 cm more than stunted children who received the placebo</li> <li>Zn supplementation increased accretion of FFM and enhanced the linear growth of those who were stunted at baseline</li> </ul>	<p>Found effect on body composition MAMA (mean (sd), in cm<sup>2</sup>): Zn group Baseline = 9.9 (1.8) Final = 11.1 (1.8) Difference = 1.2 (1.7)</p> <p>Placebo Baseline = 10.0 (2.1) Final = 10.6 (1.6) Difference = 0.6 (1.9)</p> <p>Main effect of multiple regression models of treatment for MAMA: Coefficient of regression = 0.17, <math>P=0.0002</math></p>
8. Iran; Kelishadi <i>et al.</i> <sup>(42)</sup>	Indirect (anthropometric): BMI	<ul style="list-style-type: none"> <li>In both groups, mean BMI Z-scores remained high after receiving Zn supplementation. Mean weight decreased significantly (<math>P=0.01</math>). Mean BMI (<math>P=0.01</math>) and BMI Z-score (<math>P=0.02</math>) decreased significantly and these values increased after receiving placebo</li> </ul>	<p>Found effect on body composition (i) Children receiving Zn supplementation followed by placebo BMI (mean (sd), in kg/m<sup>2</sup>) (<math>P=0.01</math>): Zn group Before = 25.05 (3.70), <math>P &lt; 0.05</math> (after v. before receiving Zn) After = 23.29 (3.64), <math>P &lt; 0.05</math> (after v. before receiving Zn)</p> <p>Placebo group Before = 23.21 (3.28), <math>P &lt; 0.05</math> (after v. before receiving placebo) After = 24.87 (3.40), <math>P &lt; 0.05</math> (after v. before receiving placebo)</p>



Table 2 Continued

Study location; reference	Assessment of body composition	Main findings	Body composition outcome
9. Gambia; Bates <i>et al.</i> <sup>(48)</sup>	Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<ul style="list-style-type: none"> <li>• Body weight and arm circumference showed a linear increase</li> <li>• For body weight there was no significant overall effect of the supplement</li> <li>• For arm circumference, a very small (2%) but significant (<math>P &lt; 0.01</math>) difference favoured the supplemented group</li> </ul>	<p>BMI Z-score (mean (sd)) (<math>P = 0.02</math>):</p> <p>Zn group            Before = 3.00 (0.41), <math>P &lt; 0.05</math> (after v. before receiving Zn)            After = 2.50 (0.42), <math>P &lt; 0.05</math> (after v. before receiving Zn)</p> <p>Placebo group            Before = 2.50 (0.37), <math>P &lt; 0.05</math> (after v. before receiving placebo)            After = 3.00 (0.45), <math>P &lt; 0.05</math> (after v. before receiving placebo)</p> <p>(ii) Children receiving placebo followed by Zn supplement</p> <p>BMI (mean (sd), in <math>\text{kg/m}^2</math>) (<math>P = 0.03</math>):</p> <p>Zn group            Before = 24.71 (3.70), <math>P &lt; 0.05</math> (after v. before receiving placebo)            After = 25.19 (3.64), <math>P &lt; 0.05</math> (after v. before receiving placebo)</p> <p>Placebo group            Before = 25.28 (3.27), <math>P &lt; 0.05</math> (after v. before receiving Zn)            After = 23.75 (3.40), <math>P &lt; 0.05</math> (after v. before receiving Zn)</p> <p>BMI Z-score (mean (sd)) (<math>P = 0.01</math>):</p> <p>Zn group            Before = 3.00 (0.38)            After = 3.00 (0.46)</p> <p>Placebo group            Before = 3.00 (0.42)            After = 2.50 (0.37)</p>
10. South India; Radhakrishna <i>et al.</i> <sup>(49)</sup>	Indirect (anthropometric): skinfold thickness at triceps, biceps, subscapular	<ul style="list-style-type: none"> <li>• Skinfold thicknesses taken at three sites showed a significant increase in SSF and TSF at 18, 21 and 24 months when compared with baseline</li> </ul>	<p>Found effect on body composition</p> <p>MUAC (mean (SE), in mm):</p> <p>Unsupplemented group            1st assessment = 137 (1.4)            2nd assessment = 145 (1.3)            Change = 7.7 (1.4)</p> <p>Zn group            1st assessment = 133 (1.6)            2nd assessment = 143 (1.5)            Change = 8.7 (1.0)</p> <p>Found effect on body composition</p> <p>SSF:</p> <p>At 18 and 21 months age, SSF was significantly (<math>P = 0.022</math> and <math>P = 0.033</math>) higher in the Zn group compared with the placebo group by mean of 0.331 (CI 0.049, 0.613) cm and 0.318 (CI 0.025, 0.611) cm</p> <p>TSF:</p> <p>At 21 and 24 months, TSF was significantly (<math>P = 0.011</math> and <math>P = 0.024</math>) higher in the Zn group compared with the placebo group by mean of 0.425 (CI 0.095, 0.755) cm and 0.389 (CI 0.047, 0.731) cm</p>

DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FM, fat mass; %BF, percentage body fat; MUAC, mid upper-arm circumference; TSF, triceps skinfold; MAFA, mid upper-arm fat area; MAMA, mid upper-arm muscle area; BIA, bio-impedance analysis; TBW, total body water; %FM, percentage fat mass; LAZ, length-for-age Z-score; WAZ, weight-for-age Z-score; HAZ, height-for-age Z-score; SES, socio-economic status; SSF, subscapular skinfold; SE, standard estimate; LSM, least-square mean.

decrease in mean weight and mean BMI and BMI Z-score, respectively, after Zn supplementation<sup>(42)</sup>. In a study carried out in peri-urban communities of Guatemala, Cavan *et al.*<sup>(43)</sup> reported that primary-school children aged 6–7 years supplemented with Zn for 6 months had significantly increased median triceps skinfold Z-score (a measure of fat status) but a small decrease in median MUAC Z-score. Radhakrishna *et al.*<sup>(49)</sup> reported that Zn supplementation of full-term normal infants (<37 weeks) for a mean period of 190 d had significant effect on skinfold thicknesses, but not on linear growth.

The four studies that found no significant effect of supplementation on measures of body composition were carried out in very different settings; all of them used indirect measures of body composition (Table 3). Rosado *et al.*<sup>(50)</sup> reported the lack of effect of a 12-month Zn supplementation among Mexican children (18–36 months) on anthropometry measurements. A study in Ethiopia carried out by Umeta *et al.*<sup>(51)</sup> found that Zn supplementation among children aged 6–12 months for 6 months did not have any measurable effects on body composition although it increased the length of stunted infants. Heinig *et al.*<sup>(52)</sup> found no effect of Zn on anthropometric indices among breast-fed infants (4–10 months) followed for 10 months. Similarly, Ruz *et al.*<sup>(53)</sup> reported no effect of Zn supplementations on body composition among pre-school children (aged 27–50 months) followed for 14 months, although there was a significant trend for increased MAMA among supplemented boys.

Three studies concerned with the effect of Zn supplementation on adiposity-related hormone levels were included in the present review (Table 4). Kelishadi *et al.*<sup>(42)</sup> found that supplementing children (aged 6–10 years) with 20 mg Zn/d in 8 weeks was associated with significantly reduced serum leptin and insulin. In contrast, Arsenault *et al.*<sup>(40)</sup> found no effect on plasma leptin, ghrelin and insulin concentrations among 6-month-old children supplemented with 3 mg ZnSO<sub>4</sub>/d in 7 months. The third study by Bueno *et al.*<sup>(41)</sup> found no effect of Zn supplementation for 6 months on serum leptin concentrations among newborns with intra-uterine growth retardation and asymmetric growth retardation. Interestingly, serum leptin concentrations correlated significantly with changes in skinfold measurements and weight-for-age Z-score among children in the placebo group. There was no clear association between Zn formulation and its dosage on hormonal levels from those three studies.

## Discussion

The present systematic review of the effects of Zn supplementation on childhood body composition can only be inconclusive at this point due to the small number of trials and their diverse study designs. There is evidence

that Zn supplementation overall has an effect on body composition when determined by anthropometric measurements, i.e. increased MUAC<sup>(43,45,46,48)</sup>, TSF<sup>(43,49)</sup>, subscapular skinfold<sup>(49)</sup> and MAMA<sup>(44,46,47)</sup>, and decreased BMI<sup>(42)</sup>. Zn supplementation also showed an effect on body composition when measured by a direct method: i.e. increased mean values of TBW estimated by BIA<sup>(37,38)</sup>, especially among children with mild-to-moderate stunting<sup>(37)</sup>; increased FFM estimated by TBW<sup>(37)</sup>; and increase in FFM assessed by DXA in girls who received the Zn supplement<sup>(46)</sup>. Only one study<sup>(42)</sup> found that Zn supplementation was associated with reduced serum leptin and insulin. Thus, Zn supplementation may have a beneficial effect on body composition, especially on FFM among children with pre-existing growth failure.

These findings suggest that the effect of Zn supplementation on body composition may be less consistent than its effect on growth. Three previously published meta-analyses of Zn supplementation trials on growth have reported strong evidence of the growth-limiting effect of Zn deficiency<sup>(25,54,55)</sup>. Zn directly influences the growth hormone and insulin-like growth factor-I systems<sup>(56)</sup>, affects bone metabolism<sup>(57)</sup> and is involved in DNA synthesis, all of which may affect linear growth<sup>(58)</sup>. However, the effect of Zn supplementation on body composition may depend on the pre-existing nutritional status of children. The study by Arsenault *et al.*<sup>(37)</sup> suggests that pre-existing Zn deficiency may be contributing to the development of obesity via its relationship with fat deposition associated with childhood stunting.

It is not clear whether the observed changes of body composition resulted from changes in FM or FFM and whether stunted children are at greater risk of obesity as a result of such changes, since only a few studies reported the findings for FFM and FM<sup>(37,46)</sup> from direct measures and most of the studies used the anthropometric method in determining body composition. However, Zn is essential for lean body mass synthesis and its deficiency was reported to increase the energy cost of tissue deposition<sup>(18)</sup>. Zn deficiency may also cause altered fatty acid metabolism leading to an increase in FM<sup>(18,26)</sup>. It is important, therefore, to concurrently study how the effect of Zn supplementation on body fat or FFM relates to its effect on linear growth.

Reduced Zn absorption and bioavailability, the combination of Zn with other micronutrients and the form by which Zn is delivered may be determining the efficacy of Zn on body composition<sup>(59)</sup>. Zn and Fe, for example, compete for mucosal binding sites as well as in the absorption process<sup>(60)</sup>. Dietary Fe:Zn greater than 2:1 will inhibit Zn absorption, as the Fe carrier, transferrin, which also carries Zn, becomes saturated<sup>(61)</sup>. Zn is absorbed most efficiently from aqueous solutions when Zn is in solution form, but not when it is part of a complex meal<sup>(62)</sup>. Zn-fortified foods generally produce a small reduction in fractional absorption, although a positive impact on net absorption<sup>(63)</sup>.

**Table 3** Studies that did not find any effect of zinc supplementation on body composition

Study location; reference	Methods of body composition assessment	Main finding
1. Mexico; Rosado <i>et al.</i> <sup>(50)</sup>	Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<p>Did not find effect on body composition</p> <ul style="list-style-type: none"> <li>• Zn and Zn + Fe supplements reduced morbidity but had no effect on growth and/or body composition</li> <li>• No effect of Zn perhaps due to the children not being severely stunted or Zn deficient and the small sample size in each treatment group</li> </ul>
2. Ethiopia; Umeta <i>et al.</i> <sup>(51)</sup>	Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<p>Did not find effect on body composition</p> <ul style="list-style-type: none"> <li>• The length (mean (SE)) of stunted infants increased significantly more (<math>P &lt; 0.001</math>) when supplemented with Zn (7.0 (1.1) cm) than with placebo (2.8 (0.9) cm); and the effect was greater (<math>P &lt; 0.01</math>) than in non-stunted infants (6.6 (0.9) v. 5.0 (0.8) cm for the Zn and placebo groups respectively, <math>P &lt; 0.01</math>)</li> <li>• Zn supplementation also increased the weight (mean (SE)) of stunted children (1.73 (0.39) v. 0.95 (0.39) kg for the corresponding placebo group, <math>P &lt; 0.001</math>) and of non-stunted children (1.19 (0.39) v. 1.02 (0.32) kg for the corresponding placebo group, <math>P &lt; 0.05</math>)</li> <li>• TSF and MUAC did not change, suggesting that the supplementation period was too short to find any measurable effects on body composition</li> </ul>
3. California, USA; Heinig <i>et al.</i> <sup>(52)</sup>	Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<p>Did not find effect on body composition</p> <ul style="list-style-type: none"> <li>• The baseline characteristics, attained weight or length at 10 months, growth velocity, gross motor development and morbidity did not differ significantly between groups, even after controlling for potentially confounding variables</li> </ul>
4. Santiago, Chile; Ruz <i>et al.</i> <sup>(53)</sup>	Indirect (anthropometric): BMI, MUAC, TSF, MAFA, MAMA	<p>Did not find effect on body composition</p> <ul style="list-style-type: none"> <li>• Height gain after 14 months was on average 0.5 cm higher in the supplemented group (<math>P = 0.10</math>)</li> <li>• Boys from the supplemented group gained 0.9 cm more than those in the placebo group (<math>P = 0.045</math>). No effect was seen in girls</li> <li>• Although no significant differences were observed in the rest of the variables studied, trends (<math>0.05 &lt; P &lt; 0.10</math>) in the supplemented group compared with the placebo group for increased MAMA in boys (<math>P = 0.08</math>), improved response to tuberculin and reduced rates of parasite re-infestation were noted</li> </ul>

MUAC, mid upper-arm circumference; TSF, triceps skinfold; MAFA, mid upper-arm fat area; MAMA, mid upper-arm muscle area; SE, standard estimate.

The study by Arsenault *et al.*<sup>(37)</sup> included Zn in a liquid multivitamin supplement and in a wheat-based, Fe-fortified porridge, both of which could reduce Zn absorption.

Zn status can have an effect on adipokines and other hormones involved in adipogenesis which can determine body composition and risk of obesity. The adipokine leptin that is produced primarily in adipose tissue regulates food intake and energy expenditure<sup>(64)</sup> and is positively associated with body weight<sup>(65)</sup>, BMI, %BF and FM among children<sup>(66,67)</sup>. Zn deficiency leads to reduced serum leptin concentration in rats<sup>(68)</sup> and man<sup>(69)</sup>, and reduced leptin secretion by rat adipocytes while repletion reversed this effect<sup>(70)</sup>. However, two of the three studies that examined the effect of Zn on adipokines and other hormones involved in adipogenesis found no clear effect.

A child's overall adiposity may contribute to this lack of an effect of Zn on leptin and subsequently body composition, since leptin levels reflect the amount of energy stored in somatic adipose tissue in man and other mammals<sup>(71,72)</sup> and are highly correlated with body fat in both adults and children<sup>(73,74)</sup>. Sex may also modify the effect of Zn on leptin since studies have reported that women have markedly higher leptin concentrations than men for any given degree of FM<sup>(75)</sup> and this may be present at birth<sup>(76)</sup>. In addition, infection and/or

inflammation may alter the effect of Zn on leptin since the induction of leptin is part of the acute-phase response to inflammatory stimuli such as lipopolysaccharide and pro-inflammatory cytokines<sup>(77)</sup>.

### Sources of study outcome heterogeneity

There were important differences between studies in factors that may have modified associations between Zn supplementation and body composition and so contributed to the inconsistent findings. Most importantly, methods used in measuring body composition varied between the different studies. All of the Zn supplementation studies included in the present review used anthropometry as indirect measures to determine body composition while only three studies used more direct methods such as DXA, BIA and <sup>2</sup>H dilution in assessing body composition<sup>(37,38,46)</sup>. Anthropometric methods have poor accuracy and precision when used alone and so have reduced utility for predicting body adiposity outcomes compared with direct methods<sup>(36)</sup>. The direct method of body composition assessment is more ideal where the aim is to quantify FM or FFM with greater accuracy<sup>(36)</sup>.

The initial nutritional status of children varied between the different studies and so may have modified the effects of Zn supplementation. An effect on body composition

**Table 4** Zinc supplementation studies on adiposity-related hormonal levels

	Study location; reference	Characteristics of study population	Study design	Data collected	Finding
1.	Peru; Arsenault <i>et al.</i> <sup>(40)</sup>	Community-based: low-income, peri-urban communities, LAZ < -0.5 Age: 5–7 months Sample size: 142	Children were randomly assigned to one of three groups, followed for 7 months: (i) Zn supplement group received Fe-fortified, wheat-based porridge without added Zn liquid multivitamin supplement with 3 mg ZnSO <sub>4</sub> /d; (ii) Zn fortification group received the same porridge with added ZnSO <sub>4</sub> (3 mg/d) and the liquid multivitamin supplement without Zn; (iii) control group received porridge and liquid multivitamin supplement without Zn	Anthropometric measurements Body composition (TBW) Plasma hormone (ghrelin, insulin, leptin) and glucose concentrations Dietary intake before supplementation	<u>Did not find effect on hormone</u> <ul style="list-style-type: none"> <li>Supplemental Zn did not affect the children's growth anthropometric indices or plasma hormone concentrations</li> </ul>
2.	Spain; Bueno <i>et al.</i> <sup>(41)</sup>	Hospital-based: gestational age 38–41 weeks, birth weight <10th percentile for gestational age and ponderal index <2.4 Gestational age: 38–41 weeks Sample size: 31	Treatment group received 3 mg ZnSO <sub>4</sub> /d in 3 ml syrup/d, followed for 6 months	Anthropometric data: weight, length, ponderal index, head and arm circumferences, skinfold Hb, IGF-I, IGFBP-3 Hair Zn	<u>Did not find effect on hormone</u> <ul style="list-style-type: none"> <li>Changes in serum leptin concentrations showed significant correlation with changes in sum of four skinfolds and weight-for-age Z-score, only in the placebo group</li> </ul>
3.	Iran; Kelishadi <i>et al.</i> <sup>(42)</sup>	Clinical-based: obese children, BMI ≥ 95th percentile (CDC) Age: 6–10 years Sample size: 60	Children were randomly assigned into two groups, received 20 mg of elemental Zn or placebo. The trial was triple-masked randomized, followed for 8 weeks and then, after a 4-week wash-out period, the groups were crossed over, so that the children initially receiving Zn received the placebo and vice versa, the protocol was repeated again for 8 weeks of follow-up	Anthropometry: weight, BMI, BMI Z-score, WC Reported initial Zn status Reported hormone-related to adiposity: Insulin, leptin No report about diet and morbidity	<u>Found effect on hormone (insulin and leptin)</u> <ul style="list-style-type: none"> <li>In both groups, significant decrease was reported for leptin after receiving Zn, without significant change after receiving placebo</li> <li>In both groups: hs-CRP and markers of IR decreased significantly after receiving Zn, but increased after receiving placebo</li> </ul>

LAZ, length-for-age Z-score; CDC, US Centers for Disease Control and Prevention; TBW, total body water; IGF-I, insulin-like growth factor-I, IGFBP-3, insulin-like growth factor binding protein-3; WC, waist circumference; hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance.

was found in three<sup>(45–47)</sup> of the six studies where study subjects were growth stunted<sup>(37,45,47,50,51)</sup> and in children with sickle cell disease whose stature was below 2 sd<sup>(46)</sup>. The study by Arsenault *et al.*<sup>(37)</sup> did not find an overall effect of Zn supplementation on FFM but did find an increase in FFM among mild-to-moderately stunted children (length-for-age Z-score < -1.1). Interestingly, this finding suggests that a positive response to Zn supplementation of body composition is more likely to be apparent among children with pre-existing growth failure. Diaz-Gomez *et al.*<sup>(38)</sup> found an improvement in TBW among premature infants in the Zn group (birth weight between 1000 and 2500 g). Two of four studies conducted among generally healthy normal children also found a positive effect of Zn supplementation on lean body mass<sup>(44)</sup> and a very small but significant increase in MUAC or MAMA<sup>(48)</sup>.

It is not clear whether children's initial Zn status and serum adipokine concentrations may also have modified the effect of Zn supplementation on body composition. One<sup>(43)</sup> out of two studies<sup>(43,50)</sup> where children had a high initial Zn status (>13.5 µmol/l) reported an effect of Zn supplementation on body composition; while an effect was also found in three studies among children with low initial Zn status<sup>(42,44,46)</sup>. The studies concerned with the effect of Zn on adiposity-related hormones did report the initial Zn status of children but did not consistently report on the initial hormone levels.

The inclusion of other micronutrients in the control group may also contribute to the inconsistent findings. Five studies compared the effect of Zn supplementation on body composition with the effects produced with multi-micronutrients<sup>(37,38,43,49,50)</sup> while nine studies compared Zn with a placebo group. The supplementation regimen for the treatment groups in two studies<sup>(37,40)</sup> was a wheat-based, Fe-fortified porridge and a liquid multivitamin. Zn supplementation was found to have an effect on body composition (increase in FFM) among the subset of children with mild-to-moderate stunting. In the study by Cavan *et al.*<sup>(43)</sup>, significantly higher median Z-scores for MUAC and TSF were found in the Zn-supplemented group when children in both the Zn and placebo groups were provided with multi-micronutrient supplements. In contrast, Zn and Zn plus Fe supplements had no effect on growth and/or body composition in the study by Rosado *et al.*<sup>(50)</sup>. Diaz-Gomez *et al.*<sup>(38)</sup> reported an improvement in TBW among children in the Zn group fed with a standard term formula supplemented with Zn (final content: 10 mg/l) and a small quantity of Cu (final content: 0.6 mg/l); the placebo group received the same formula without supplementation (final content of Zn: 5 mg/l; final content of Cu: 0.4 mg/l), and an Fe supplement was given to all children once daily during the study period. These findings suggest that combining micronutrients may dilute the effects of Zn<sup>(59)</sup>. A trial that supplemented Chinese obese women with Zn combined with other micronutrients did report reductions in body

weight, BMI and abdominal obesity and improvement in serum lipid profiles<sup>(8)</sup>.

Children's age and sex may be a source of heterogeneity in findings. For example, two studies<sup>(43,44)</sup> conducted among school-aged children found an effect of Zn supplementation while one study<sup>(45)</sup> reported an effect among pre-school children. Studies that included infants<sup>(38)</sup>, 5–7-month-old children<sup>(37)</sup> and 4–10-year-old children<sup>(46)</sup> found no effect. The modification of Zn supplementation effect on hormone levels by age is difficult to determine since there were only limited studies of Zn supplementation on adiposity-related hormone levels<sup>(40–42)</sup>. There are significant sex differences in body composition before the onset of puberty. Prepubertal girls generally have higher total body fat and %BF but lower FFM<sup>(78)</sup> than do boys matched for age, weight and height. Fat distribution also differs between sexes, with prepubertal girls generally having greater trunk fat than do boys<sup>(79)</sup>. Thus, the pattern of the effect of Zn supplementation on body composition might not be consistent if studies have children of different age groups. In certain age ranges children might have different impacts by Zn supplementation on body composition.

The formulation, dosage and duration of Zn supplementation may also have contributed to the inconsistencies in the effect of Zn, since these varied considerably between the different studies. An effect of Zn on body composition was found in seven studies when ZnSO<sub>4</sub> was used as a supplement. Studies that used Zn as an amino acid chelate<sup>(43)</sup>, elemental Zn<sup>(42)</sup>, and Zn acetate and Zn gluconate<sup>(48)</sup> also showed an effect. The dosages of Zn supplementation varied considerably (from 3 to 70 mg/d) between the different studies. Dosages of 10 mg/d given to school-aged children<sup>(43)</sup>, 10 mg/d given to pre-school children<sup>(45)</sup> and 30 and 50 mg/d given to schoolchildren whose weight was below 29.5 kg and ≥29.5 kg, respectively<sup>(44)</sup>, all had effects on body composition. Studies that gave 20 mg Zn/d to pre-school children, 10 mg Zn/d to school-aged children<sup>(46)</sup> and 10 mg Zn/d to infants<sup>(38)</sup> found no effect on body composition. Another study by Arsenault *et al.*<sup>(37)</sup>, which supplemented children with 3 mg Zn/d (either in liquid supplement or fortified porridge), showed no effect. Four studies<sup>(38,43,45,47)</sup> which supplemented children with Zn for 6–7 months and three studies<sup>(44,46,48)</sup> which supplemented children with Zn for 10–15 months reported an effect on body composition. The findings suggest that at least 6 months of Zn supplementation can have an effect on body composition but differing dosages and the formulation of Zn have no clear association with body composition.

Additional sources of heterogeneity that may modify study outcomes are dietary Zn intake, dietary factors influencing Zn absorption and morbidity/illness. Although the influence of diet and morbidity were not adequately tested in the reviewed analyses, ten studies<sup>(37,38,44–48,51–53)</sup>

carried out dietary data collection to determine mean daily intakes of energy, protein, Zn<sup>(44–46)</sup>, phytic acid<sup>(45)</sup> and Cu<sup>(38)</sup>. In addition, the children were generally selected from low socio-economic areas<sup>(44,45,47–49)</sup> in which Zn deficiency prevalence is usually high due to poor dietary intake. Only one study<sup>(40)</sup> concerned with the effect of Zn supplementation on adipokines measured the dietary intake of Zn and the relative content of phytic acid to Zn in ingested food, although it was reported in another paper<sup>(37)</sup> that reported the body composition results.

Another source of outcome heterogeneity was compliance. For example, the study by Cavan *et al.*<sup>(43)</sup> had a low average number of treatment days because of children's frequent absences from school. Zn supplementation was administered only on school days in the studies by Friis *et al.*<sup>(44)</sup> and Kikafunda *et al.*<sup>(45)</sup>, resulting in sporadic intake. Similarly, the mild effect of Zn supplementation on MUAC reported by Kikafunda *et al.*<sup>(45)</sup> may partly result from high study attrition and low compliance.

There are limitations of the current review, which include publication bias from selective inclusion of studies published in English and the lack of statistical pooling of the studies.

## Conclusions

Overall, the present review has found that the effect of Zn supplementation on body composition may not be consistent. The review has suggested that Zn supplementation may have a beneficial effect on body composition, especially on FFM among children with pre-existing growth failure. However, variable findings resulting from technical difficulties in measuring body composition in community settings still need to be addressed. A majority of the studies could not accurately address whether alterations in the FM and/or FFM components of the body were responsible for the observed weight or body composition changes due to the use of anthropometry when determining body composition.

Further well-designed studies which measure body composition directly and address the limitations from previous studies are required. The determination of how adiposity-related hormone concentrations relate to body composition among supplemented children that differ in nutritional status may further clarify these relationships. Confirmation that Zn has an effect on body composition would allow the development of new public health interventions that may contribute to efforts to reduce the long-term risk of stunting, obesity and related diseases in the context of the global obesity pandemic.

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