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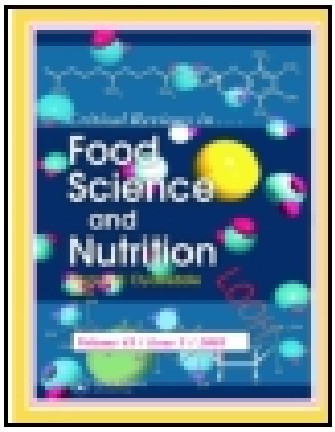

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Effect of zinc intake on growth in infants: A meta-analysis

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Effect of zinc intake on growth in infants: A meta-analysis

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Abstract

A systematic review and meta-analysis of available randomised controlled trials (RCTs) was conducted to evaluate the effect of zinc (Zn) intake on growth in infants. Out of 5500 studies identified through electronic searches and reference lists, 19 RCTs were selected after applying the exclusion/inclusion criteria. The influence of Zn intake on growth was considered in the overall meta-analysis. Other variables were also taken into account as possible effect modifiers: doses of Zn intake, intervention duration, nutritional status and risk of bias. From each select growth study, final measures of Weight, Length, Mid upper arm circumference (MUAC), Head circumference, Weight for age z-score (WAZ), Length for age z-score (LAZ) and Weight for Length z-score (WLZ) were assessed. Pooled β and 95% confidence interval (CI) were calculated. Additionally we carried out a sensitivity analysis. Zn intake was not associated to Weight, Length, MUAC, Head Circumference and LAZ in the pooled analyses. However, Zn intake had a positive and statistically effect on WAZ (β = 0.06; 95%CI 0.02 to 0.10) and WLZ (β = 0.05; 95%CI 0.01 to 0.08). The dose response relationship between Zn intake and these parameters indicated that a doubling of Zn intake increased WAZ and WLZ by approximately 4%. Substantial heterogeneity was present only in Length analyses. (I^2 = 45 %; p = 0.03). Zn intake was positively associated with length values at short time (4 to 20 weeks) (β = 0.01; CI 95% 0 to 0.02) and at medium doses of Zn (4.1 to 8 mg/day) (β = 0.003; CI 95% 0 to 0.01). Nevertheless, the effect magnitude was small. Our results indicate that Zn intake increases growth parameters of infants. Nonetheless, interpretation of these results should be carefully considered.

Keywords: EURRECA, zinc intake, growth, infants

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Introduction

Zinc (Zn) is an essential nutrient, present in all body tissues and fluids. The biologic role of Zn is now recognized in structure and function of proteins, including more than 300 enzymes, transcription factors, hormonal receptor sites, and biologic membranes. Zn has numerous central roles in DNA and RNA metabolism (MacDonald 2000), and it is involved in signal transduction, gene expression, and apoptosis. Zn enzymes are involved in nucleic acid metabolism, cellular proliferation, differentiation and growth (Chesters 1978). Zn is a critical micronutrient for normal growth, haematopoiesis, immune function and neurologic development during infancy. Infants have a relatively high requirement of Zn per unit body weight during a sensitive period of rapid growth and development (Hermoso et al. 2010).

Physiological functional consequences (e.g. growth retardation) of mild Zn deficiency are often apparent before the Zn concentrations in plasma and/or tissues are significantly reduced (Gibson et al. 1989; Ruz et al. 1991). Inadequate Zn intake is likely to be an important contributing factor of growth failure in children that are malnourished, because diets lows in protein tend to be low in Zn (Golden & Golden 1981). Growth faltering starts at 6 mo of age in less-developed countries with rapid progression (The World Bank 2006) and coincides with a critical time in the dietary supply of Zn, labelled as a ‘‘problem’’ nutrient in complementary feeding by WHO. (Dewey & Brown 2003)

Human Zn deficiency was described since the early 1960s. But it was not until 1990, when the Zn became to be a micronutrient of major interest until the current date, due the important function for immune system integrity (Shankar & Prasad 1998), the know losses of Zn in

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diarrheal fluids (Ruz & Solomons 1990), and pilot data on the association between Zn deficiency and diarrhea (Hambidge 1992). Zn supplementation RCT´s focused on growth velocity in young children. A comprehensive meta-analysis of results of 33 studies provided convincing evidence of a significant increase in linear growth (Brown et al. 1998; IZNCG 2004).

A considerable number of intervention trials have been completed in multiple countries to assess the effect of supplemental Zn on children's growth. However, these studies have yielded inconsistent results, possibly because of differences in 1) the pre-existing Zn status of the study subjects, 2) the content and bioavailability of Zn in the local diets, and 3) the incidence of common infections that can affect growth independently of an individual's Zn status. Moreover, methodology aspects of these studies, such as variations in the dose, the sample sizes, the method of administration or the duration of supplementation, may have influenced their results (Brown et al. 2002).

Recommendations for Zn intake during infancy vary widely across Europe, ranging from 1 mg/day up to 5 mg/day (Hermoso et al. 2010). The EURRECA project attempts to consolidate the basis for the definition of micronutrient requirements across Europe, taking into account relationships among intake, status and health outcomes, in order to harmonise these recommendations (Ashwell et al. 2008). This paper presents a systematic review of the data from all available randomized controlled trials (RCTs) meeting EURRECA's quality standard (Matthys et al. 2011), which investigated Zn intake and growth parameters in infants, and combines these studies in meta-analyses to model of growth as a function of Zn intake.

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Materials and Methods

Search strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (http://www.eurreca.org).

This review was part of a wider review process to identify studies assessing the effect of Zn intake

on different outcomes (health outcomes). The wider searches were performed for literature

published up to and including February 2010. The databases MEDLINE, EMBASE and Cochrane

were accessed using search terms ‘study designs in humans’ and ‘zinc’ and ‘intake’. Both indexing

and text terms were used and languages included were restricted to those spoken in the EURRECA

Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek

and Serbian.). The Ovid MEDLINE search strategy can be found in Table 1. Reference lists of

retrieved articles and published literature reviews were also checked for relevant studies. The

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procedure for the identification, selection of articles and data extraction is illustrated in Fig. 1.

Selection of articles

Titles of articles identified from the searches were entered into an EndNote library. Papers were considered eligible for inclusion if they were RCTs, conducted in human infants (aged 0-12 months), and studied the effect of supplements, fortified foods or micronutrient intake from natural food sources, and assessed Zn concentrations in serum/plasma. Zn intake was assessed from breast milk, infant formula and food sources (e.g. fortified formula or cereal) and supplements.

Exclusion criteria applied were: studies conducted in animals; combined interventions e.g. >1 micronutrient or micronutrient + lifestyle intervention which did not study the effect of the micronutrient separately; non primary studies (e.g. letters and narrative literature reviews); duplicate publications; studies where the Zn intake - growth relationship was not reported or health outcomes other than growth assessed.

Briefly, titles and abstracts of the 10% of the library were screened in duplicate for eligibility by two reviewers and any discrepancies were discussed and resolved before screening the remaining references. Only when both reviewers agreed that titles and abstracts met the inclusion criteria were the articles included. When a title and abstract could not be included with certainty, the full text of the article was obtained and then further evaluated. The remaining 90% was distributed among the reviewers in even parts. Following the initial screening process, full-text articles were

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obtained. Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-analysis if they were: RCTs; had an intervention duration of at least 2 weeks and reported baseline data for all outcome measures. Non-RCTs, uncontrolled trials or trials reporting insufficient or unclear data were excluded. Data was extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA, USA).

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Data synthesis

When growth was measured at different time points within the same population, we used the measures as different estimations (Hamadani et al. 2001; Heinig et al. 2006; Sur et al 2003). One study reported data separately for boys and girls (Walravens et al. 1989). One study report data from two groups of infants (stunted and non stunted) and also these were treated as two different estimations within the meta-analysis (Umeta et al. 2000). Different estimations were also considered for the following studies: the study of Osendrap et al. 2002 that analyzed three groups of infants (all, infants with low serum Zn < 9.18 μmol/L, and infants with normal serum Zn > 9.18 μmol/L) and the study of Arsenault et al. 2008 that assessed two groups (Zn intake in a liquid supplement and in a fortified porridge).

If dietary intake of Zn (in addition to the intervention) was not reported in the RCTs we imputed a value of 1.3 mg/day, the mean dietary intake level of the RCTs that did report dietary Zn intake. As mean baseline growth parameters were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline measures (n= 16). Arsenault and Rivera et al. (2008; 1998) performed an adjustment for initial differences. Only the study of Bates et al. (Bates et al. 1993) failed to report anything regarding this matter.

Exposure and outcome and other covariates assessment:

The influence of Zn intake on growth parameters was considered in the overall meta-analysis. From each select growth study, final measures were assessed: Weight, Length, MUAC, Head circumference, WAZ, LAZ and WLZ in all the included studies.

Other variables were also taken into account as possible effect modifiers. We considered doses of Zn intake (1 to 4 mg, 4.1 to 8 mg, 8.1 to 12 mg and >12.1 mg), intervention duration (1 to 3 weeks, 4 to 20 weeks and > 20 weeks), nutritional situation (healthy, nutritionally at risk and poor nutritional status) and risk of bias (low, moderate or high).

Assessment of nutritional situation in included studies

Poor nutritional status was defined as infants with low birth weight during their first year, undernourished or current growth retardation evidenced by a WAZ and LAZ scores below -2; nutritionally at risk was defined as infants who lived in low-income families with a low socioeconomic situation.

Assessment of risk of bias in included studies

Risk of bias was assessed in order to evaluate the quality of the studies included. The following indicators of internal validity specific to the RCT methodology were collected during data extraction: 1) method of sequence generation 2) adequate allocation, 3) blinding, 4) number of participants at start, dropouts and dropout reasons, 5) outcome data complete, 6) funder adequate, 7) other potential funding bias. Based on these indicators, two reviewers assessed the

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overall risk of bias. Disagreements were resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook for Systematic Reviews (Higgins JPT & Green S 2009).

Statistical analysis

Mean and standard deviation (SD) or standard errors (SE) of the outcome (Weight, Length, MUAC, Head circumference, WAZ, LAZ and WLZ) were assessed. From the mean and SD of each study, beta values (β) and their SE were calculated because the statistical model that we used to estimate the relation between Zn intake (x-variable) and growth (y- variable) is based on the assumption that this intake-growth linear relationship is a logarithmic function and that both intake and growth follow a log-normal distribution (the natural logarithm of intake and growth have a normal distribution). Thus, the expected value of the growth score is expressed as:

$\mu y = \beta * \mu x + \text{intercept}$

where μy represents the mean of the natural logarithm of the y–variable (= growth score), β represents the regression coefficient, and μx represents the mean of the natural logarithm of the x-variable (= Zn intake).

This shape of this linear relationship on the $\log_e$–$\log_e$-scale corresponds to a monotonic concave function on the original scale for $\beta < 1$. This shape is assumed to be realistic for the biological relationship between Zn intake and growth parameters. As the true dose-response curve is unknown, this approximation provides a practical methodology to estimate the dose-response relationship.

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The method used to systematically review differences was a formal meta–analysis (Greenland 1998).

Procedures of formal meta-analysis have been applied to combine the results from previously reported studies (Dickersin 2002).

A random-effects model was considered to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's (DerSimonian & Laird 1986) to pool the estimates of betas across studies. Under this model, the pooled effect was the beta in the growth parameters (Weight, Length, MUAC, Head circumference, WAZ, LAZ and WLZ), for an increment of 1 unit in Zn intake. A pooled beta estimate was calculated as a weighted average of the beta reported in each study.

The formula we used to estimate the weighted effect size was (Hedges 1982):

$\beta\ pooled = \sum \beta i\ wi\ / \sum wi$

where $\beta\ pooled$ is the pooled estimate of the beta in growth parameters; the weight *(wi)* of each study was computed as*:*

$wi = 1 / Vi + \varsigma^2$

where *V* is the variance of each study and ς^2 is the inter-study variance.

Besides this, we calculated a 95% CI for the pooled estimated of effect size:

95% CI= $\beta\ pooled \pm$ (1.96 x SE pooled)

where SE is the standard error of the pooled estimate (Greenland 1998).

A test of heterogeneity was calculated, estimating Q statistics, which follows a chi-square distribution with degrees of freedom *n*-1, *n* being the number of studies included in the analysis. The I^2 Index measures the extent of the heterogeneity. A low *P* value for this statistic (lower than

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0.05) indicates the presence of heterogeneity, which somewhat compromises the validity of the pooled estimates (Takkouche et al. 1999). Because significant heterogeneity was clearly evident in the pooled beta estimates for Length studies, we evaluated potential sources of heterogeneity by linear meta-regressions (Greenland 1998). We fitted a meta-regression using the duration of the intervention, the doses of Zn intake, the risk of bias and the nutritional situation as independent variables. The betas obtained in each study for the Length parameter according to Zn intake were used as the dependent variable.

Statistical differences in multivariate adjusted mean beta values between each possible heterogeneity sources were determined by analysis of covariance ANCOVA. Additionally we carried out additional meta-analyses by subgroups considering only those groups which provided significant values in the meta-regression.

Sensitivity analyses were also conducted. We excluded the studies considered outliers and recalculated the pool estimate of the beta in each growth parameter.

Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review Manager 5.1, were used to conduct the statistical analyses.

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Results

Five thousand five hundred articles were identified in the initial search strategy. After applying the exclusion/inclusion criteria, 344 articles from the search appeared to be potentially relevant. After applying the additional eligibility criteria and grouping the studies by outcome, nineteen RCTs (38 estimations) were selected for the growth meta-analysis (Arsenault et al. 2008; Bates

et al. 1993; Berger et al. 2006; Dijkhuizen et al. 2001; Fischer Walker et al. 2009; Gardner et al. 2005; Hamadani et al. 2001; Heinig et al. 2006; Lind et al. 2004; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Olney et al. 2006; Osendarp et al. 2002; Rivera et al. 1998; Sur et al. 2003; Umeta et al. 2000; Walravens et al. 1989; Wasantwisut et al. 2006). (Fig. 1)

Descriptive characteristics of the studies included in the meta-analysis are presented in Table 2.

Of the nineteen studies included, only twelve comply strictly with the age infants (0 to 12 months) (Arsenault et al. 2008; Berger et al. 2006; Dijkhuizen et al. 2001; Fischer Walker et al. 2009; Heinig et al. 2006; Lind et al. 2004; Olney et al. 2006; Osendarp et al. 2002; Rivera et al. 1998; Sur et al. 2003; Umeta et al. 2000; Wasantwisut et al. 2006). The other seven studies included this age among their sample, but did not clarify how many are actually aged 0 to 12 months (Bates et al. 1993; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Walravens et al. 1989). None of the ages extended beyond 27 month, except Gardner et al. and Ninh et al. (2005; 1996), which included children up to 30 and 36 months respectively.

Four studies were conducted in Latin America and the Caribbean, two in North America, nine in Asia and four in Africa. The duration of the interventions ranged from 2 to 60 weeks. Some studies assessed growth parameters at several time points within the study (Hamadani et al. 2001; Heinig et al. 2006; Sur et al. 2003). Doses of Zn intake ranged from 1.78 to 20 mg per day. The nutritional situation of infants also varied between studies: six studies were conducted in healthy infants (Bates et al. 1993; Heinig et al. 2006; Lind et al. 2004; Osendarp et al. 2002;

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Umeta et al. 2000; Wasantwisut et al. 2006), six studies were conducted on infants who were nutritionally at risk (Arsenault et al. 2008; Berger et al. 2006; Fischer Walker et al. 2009; Müller et al. 2003; Rivera et al. 1998; Walravens et al. 1989), and seven studies were conducted on infants with poor nutritional status (Dijkhuizen et al. 2001; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Ninh et al. 1996; Olney et al. 2006; Sur et al. 2003). The risk of bias varied also between studies: six studies had a high risk of bias, seven had a moderate risk and six showed a low risk of bias.

Differences in Growth outcomes (Weight, Length, MUAC, Head Circumference, WAZ, LAZ and WLZ) according to Zn intake in each particular study and in the pooled analyses are showed in Figures 2 to 8. Zn intake was not associated to Weight, Length, MUAC, Head Circumference and LAZ in the pooled analyses. However, Zn intake had a positive and statistically effect on WAZ ($\beta = 0.06$; 95%CI 0.02 to 0.10) and WLZ ($\beta = 0.05$; 95%CI 0.01 to 0.08).

Since we applied a base-*e* logarithmic transformation on the Zn intake and growth parameters before calculation of the study-specific β´s, the overall β represents the difference in the $\log_e$transformed predicted value of WAZ and WLZ for each one-unit difference in the $\log_e$transformed value in Zn intake. Therefore, an overall β of 0.06 means that for every doubling in Zn intake, the difference in WAZ is $2^{\hat{\beta}}$ ($2^{0.06} = 1.04$). For an overall β of 0.05, the difference in WLZ is 1.035. That means that a person with a double intake of Zn has aproximately 4% higuer WAZ and WLZ than a person with the half intake. (Fig. 9, 10)

Excepting for Length ($I^2 = 45$ %, $p= 0.03$), heterogeneity was not present in any analysis. In order to investigate which variables may be potential effect modifiers on Length, we performed a

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meta-regression (Table 3). The effect of Zn intake on Length changed depending on the duration of the intervention and the dose (p ANCOVA= 0.008 and 0.023) respectively.

Table 4 shows the results of Length analyses after stratifying the studies according to the effect modifiers identified in the meta-regression. After stratifying by duration of the intervention and by dose, the heterogeneity disappeared. At short time (4 to 20 weeks), Zn intake was positively associated with length values (β=0.01; CI 95% 0 to 0.02). However, no effect was found when supplementation lasted for more than 20 weeks (β = -0.001; CI 95% -0.003 to 0.002). At medium doses of Zn (4.1 to 8 mg/day), Zn intake was positively associated with length values (β= 0.003; CI 95% 0 to 0.01). Nevertheless, the effect magnitude was small. However, no effect was found at low or high doses of Zn (1 to 4 or >12 mg/day) (β= 0; CI 95% -0.01 to 0.004 and β= 0.01; CI 95% -0.02 to 0) respectively.

The results of the sensitivity analyses are shown in Table 5. The study of Osendrap et al. (b) (2002), Walravens et al. (a) and Walravens et al. (b) (1989) were considered as outliers in the analysis of weight because the limits of beta were very wide (from CI 95% 0.01 to 0.23; CI 95% -0.04 to 0.10 and CI 95% -0.04 to 0.10) respectively. When we excluded these studies, the null association previously seen remained. In WAZ studies the study of Sur et al. (d, e, f, g, h, I, j, k, l) (2003) were considered as outliers for the same reasons. When we excluded these studies, we observed an attenuation of the positive effect of Zn supplementation on WAZ (β = 0.03; CI 95% 0 to 0.07). The study of Osendrap et al. (b) (2002) was considered as an outlier in the analysis of Length. When we excluded this study, the null association previously seen persisted and also the heterogeneity (I^2= 47%; p= 0.03).

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Discussion

Our results indicate that Zn supplementation increases some growth parameters in infants. Zn intake had a positive and statistically effect on WAZ (β = 0.06; 95%CI 0.02 to 0.10) and WLZ (β = 0.05; 95%CI 0.01 to 0.08). We only found significant heterogeneity while analysing length (I^2 = 45 %, p = 0.03). After stratifying by several factors, heterogeneity disappeared.

To our knowledge, this meta-analysis is the only in providing an estimate of the dose-response relationship of Zn intake and growth parameters in infants aged 1–12 months. An infant with a Zn intake of 10 mg/day has a WAZ and WLZ that is 4 % higher than an infant who has a Zn intake of 5 mg/day. However, interpretation of these results should be carefully considered for a number of reasons. It is a well acknowledged that when many statistical comparisons are carried out, one or more might reach significance due to chance alone (Bland & Altman 1995). Although meta-analysis are increasingly used to consolidate results from multiple studies of the same topic and to develop evidence-based policies for clinical practice and public health programmes, the reliability of reached conclusions depends on the methodological quality of the original studies, the appropriateness of the study inclusion criteria, the thoroughness of the review and the synthesis of information (Brown et al. 2002). It is unlikely that confounding factors might have affected our results since all the studies included in our meta-analyses are RCTs. However, if some baseline differences were observed because any failure in the randomize process, several authors performed an adjustment of initial differences (Arsenault et al. 2008 and Rivera et al. 1998). Other authors assumed no initial differences. The only exception was Bates et al (1993) that did not mention anything regarding this matter.

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A limitation of this study was the small amount of studies that were eligible for inclusion in this meta-analysis. Although nineteen RCTs of the effect of Zn supplementation on infant's growth were included, for the association between Zn intake and head circumference the meta-analysis only included 4 studies which led to a reduction of the statistical power to detect significant differences.

Age of the study populations considered in this meta-analysis was another important point. We believe that there was no reason to exclude any study that did not adhere exclusively to the group of 0 to 12 months of age. For this reasons, we took into account all the studies which included this age group in the study, even if they were not analysed according to their age group (Bates et al. 1993; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Walravens et al. 1989) and assumed the consequences of this possible bias.

The small magnitude of effect that we observed might be due to some effect modifiers that should be considered whenever the effect of Zn deficiency on growth is being evaluated. Those include factors in close relation to infancy such as prematurity, low birth-weight (LBW), breastfeeding, protein energy malnutrition, infectious morbidity, poverty, and social deprivation, the pre-existing Zn status of the study subjects; and others directly related to Zn such as content and bioavailability of Zn in local diets. Moreover, methodological aspects of these studies, such as variations in the dose, chemical form, method of administration of Zn and duration of supplementation, may have influenced our results (Brown et al. 2002). Some of these aspects were analysed in the sub-groups analyses of the meta-analysis. Duration of the intervention in some studies was other possible explication to the small magnitude of the effect founded. Time

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of the intervention appears to be too short to obtain a positive impact on growth (Heinig et al. 2006; Lind et al. 2004; Meeks Gardner et al. 1998; Sur et al. 2003). However, this becomes more relevant when studies are conducted in LBW infants because the low weight together with the immaturity associated with premature infants requires adjustment of gestational age with chronological age for proper assessment of catch-up growth (Rugolo 2005). This is the case of the Meeks Gardner and Sur's studies (Meeks Gardner 1998; Sur et al. 2003) which were conducted in infants with a poor nutritional status. Nonetheless, on healthy infants Lind et al. (2004) reported improvements in growth in a 3 month period. Opposite results were obtained by Bates et al. and Heinig et al. (1993; 2006) which failed to observe a positive effect in longer periods of time. Also Rivera et al. and Umeta et al. (1998; 2000) found that MUAC did not change due to the extension of the supplementation period which was apparently too short to find any measurable effect. Thus, clinical trials are required to analyze the long-term effects on growth of Zn supplementation before reaching significant conclusions.

Other consideration to take into account is that the data from our meta-analysis was obtained generally from countries in a developing stage and included data from LBW and malnourished infants which might have resulted in the poor effect we found. Several studies have been carried out worldwide and many of these showed a positive effect of Zn supplementation on growth among groups of children who were nutritionally disadvantaged in some way, including stunted children (Walravens et al. 1989; Wasantwisut et al. 2006), and in particular among malnourished children (Arsenault et al. 2008; Ninh et al. 1996; Rivera et al. 1998). On the other hand, there was no growth response to supplementation in healthy Gambian nor healthy USA infants (Bates et al. 1993; Heinig et al. 2006). A meta-analysis of 25 studies (Brown et al. 1998) of Zn

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supplements on growth of children in developing countries found smaller but significant effects on growth (an effect size of +0.22 for height and +0.26 for weight increments). However, an updated version of that meta-analysis (Brown et al. 2002) based on 33 RCT, showed a highly significant aggregate effect size of 0.350 (95% CI: 0.189, 0.511) for height, 0.309 (95% CI: 0.178, 0.439) for weight, and ≈0 for WLZ increments. Thus Zn supplementation on child growth has been studied extensively in developing countries, but relatively little information is available from industrialized ones (Brown et al. 2002). Therefore, it is unclear whether children in industrialized countries would benefit from increased Zn intakes.

In conclusion, our meta-analyses provided us an estimate of the dose-response relation between Zn intake and some growth's parameters (WAZ and WLZ) in infant population. These data can be used as complementary evidence for underpinning Zn reference values, although restrictions on extrapolation of our results to other populations should be acknowledged mainly to developed populations.

For the others growth's parameters included in the meta-analyses, no effect was found. Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of Zn supplementation upon infant growth mainly in Western populations.

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(FOOD). The original concept of the systematic review was undertaken by the EURRECA Network and coordinated by partners based at Wageningen University (WU), the Netherlands and the University of East Anglia (UEA), United Kingdom. Susan Fairweather-Tait (UEA), Lisette de Groot (WU), Pieter van' t Veer (WU), Kate Ashton (UEA), Amélie Casgrain (UEA), Adriënne Cavelaars (WU), Rachel Collings (UEA), Rosalie Dhonukshe-Rutten (WU), Esmée Doets (WU), Linda Harvey (UEA) and Lee Hooper (UEA) designed and developed the review protocol and search strategy.

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The authors´ responsibilities were as follows: NM: analysed the data and wrote the manuscript, NM, DAJ, LSA and WMM: reviewed the papers and contributed to the selection of the papers and the data extraction, SVA: provided support in data-analysis, FLD, HSP, PQL, RC, LN, MHV and SML provided significant advice. All authors directly participated in the planning, execution or analysis of the study and reviewed the manuscript.

CONFLICT OF INTEREST: Authors declare that they have no conflicts of interest.

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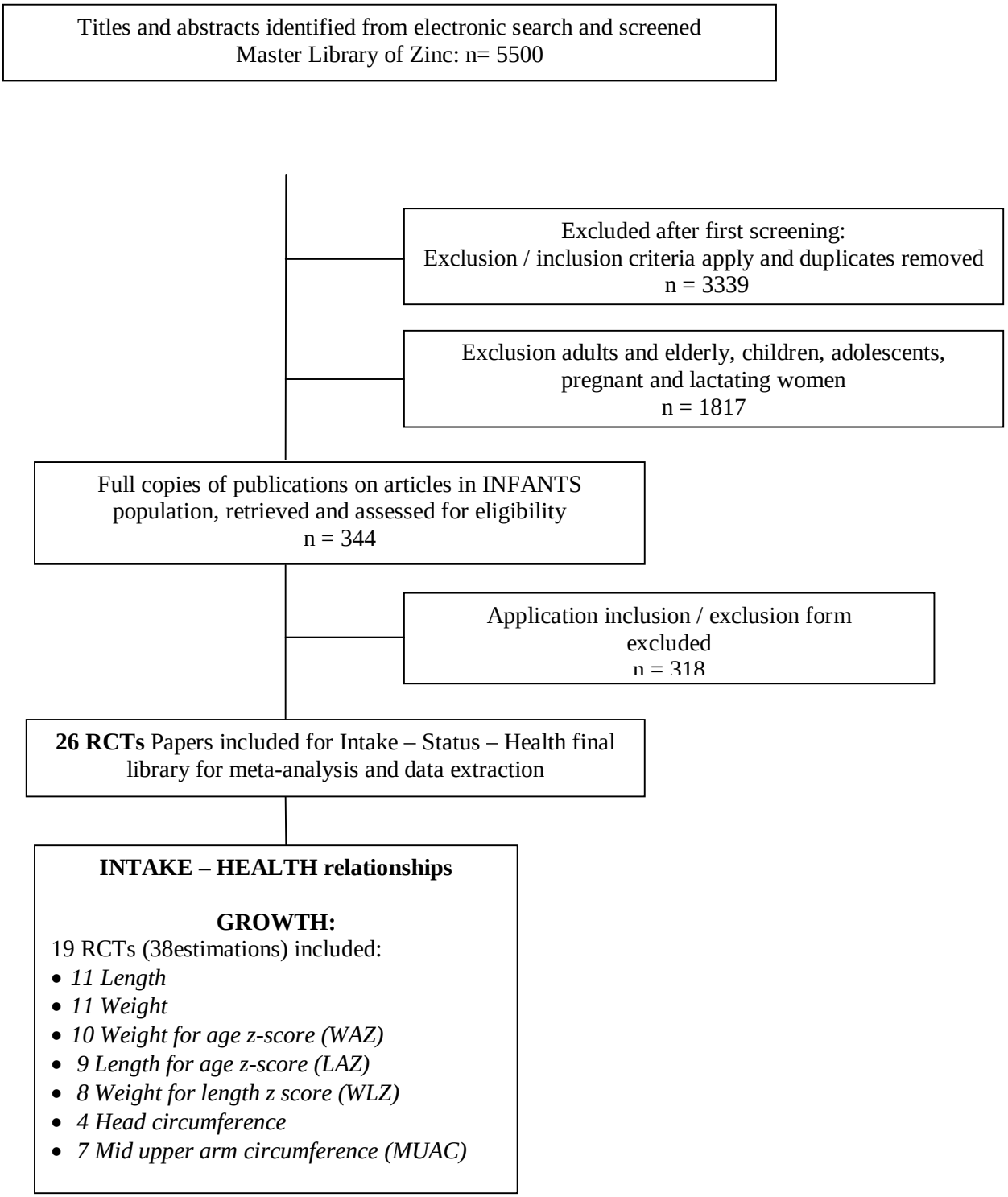

Figure 1: Flow diagram for the systematic review.

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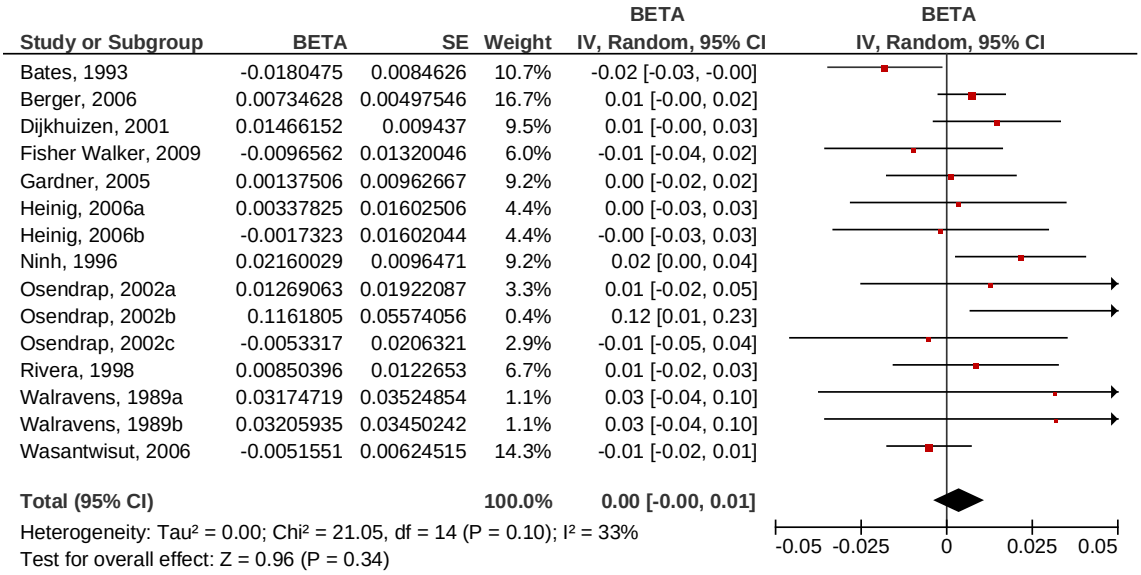

Figure 2: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (Weight) in infants.

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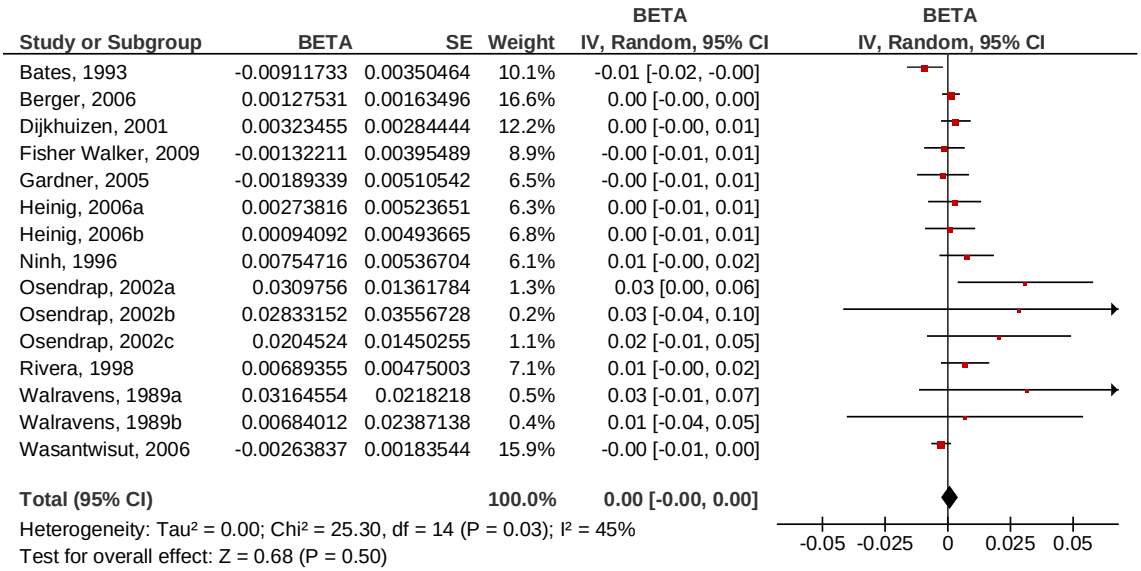

Figure 3: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (Length) in infants.

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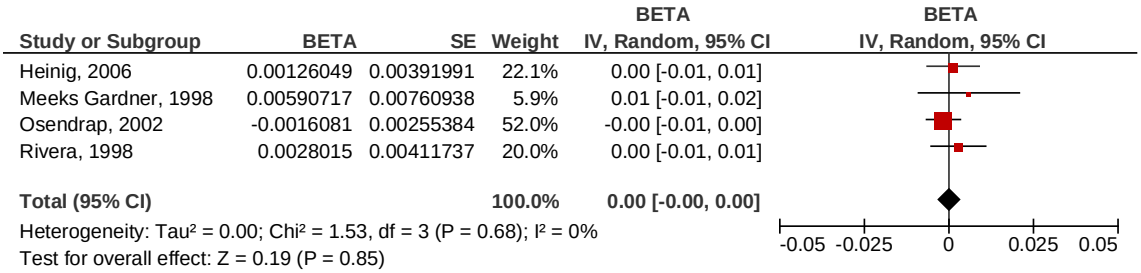

Figure 4: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (Head Circumference) in infants.

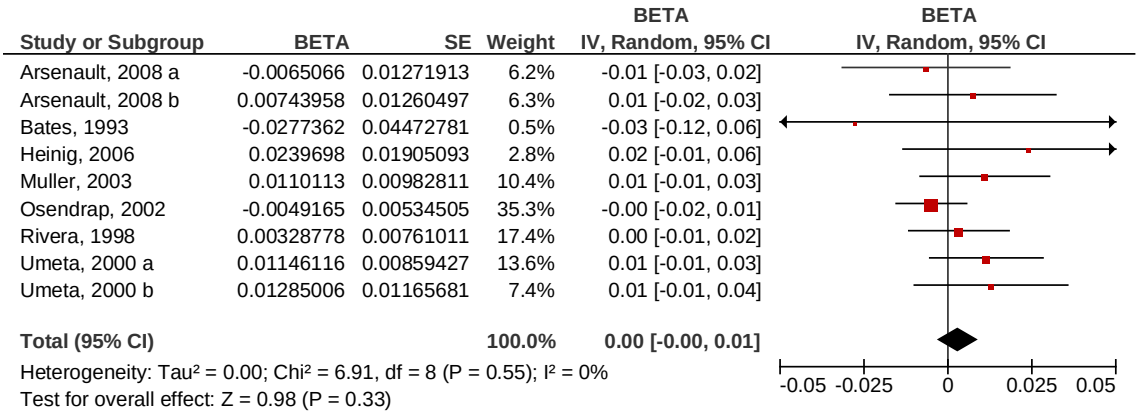

Figure 5: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (MUAC: Mid upper arm circumference) in infants.

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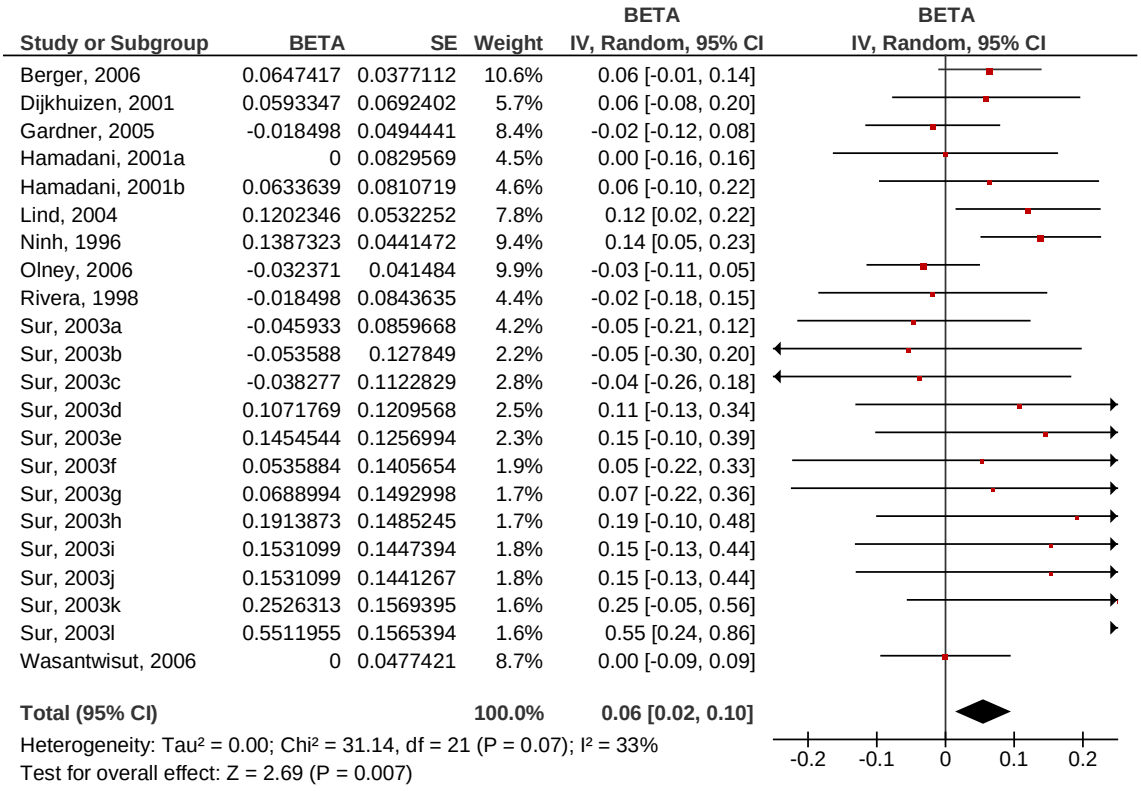

Figure 6: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (WAZ: Weight for age Z – score) in infants.

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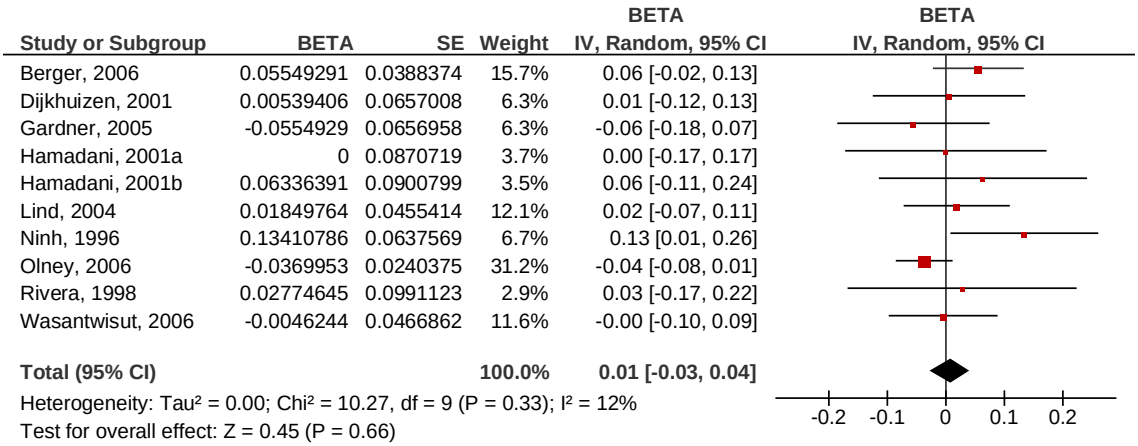

Figure 7: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (LAZ: Length for age Z- score) in infants.

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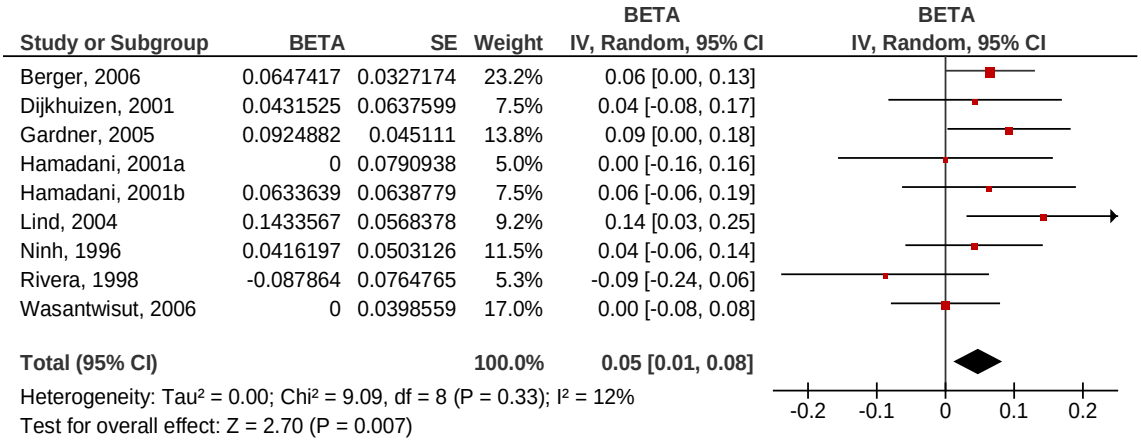

Figure 8: Forest plot of randomized controlled trials evaluating the effect of zinc intake on Growth (WLZ: Weight for length Z- score) in infants.

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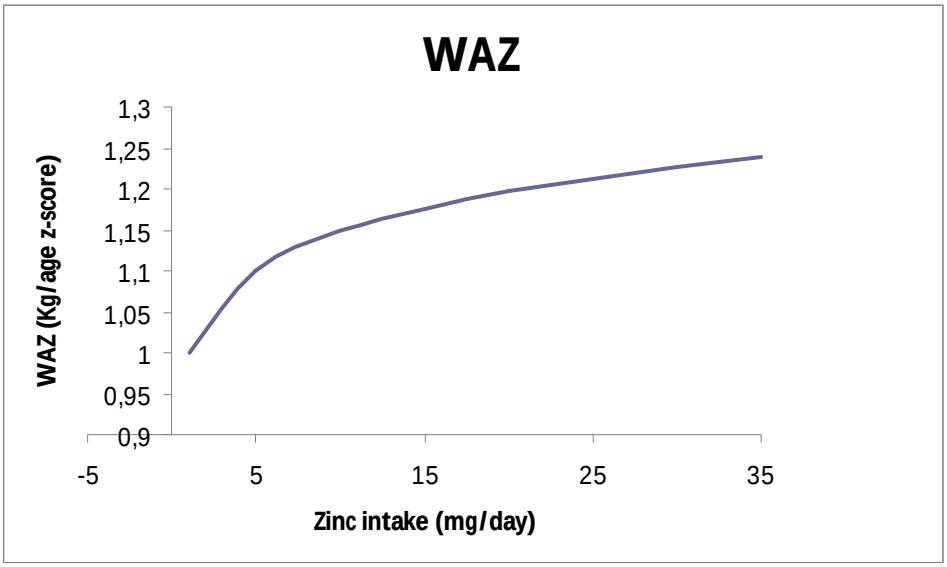

Figure 9: WAZ (Kg/age z-score) as a function of dietary zinc intake (mg/day), estimated by random effects meta-analyses of RCTs of infants.

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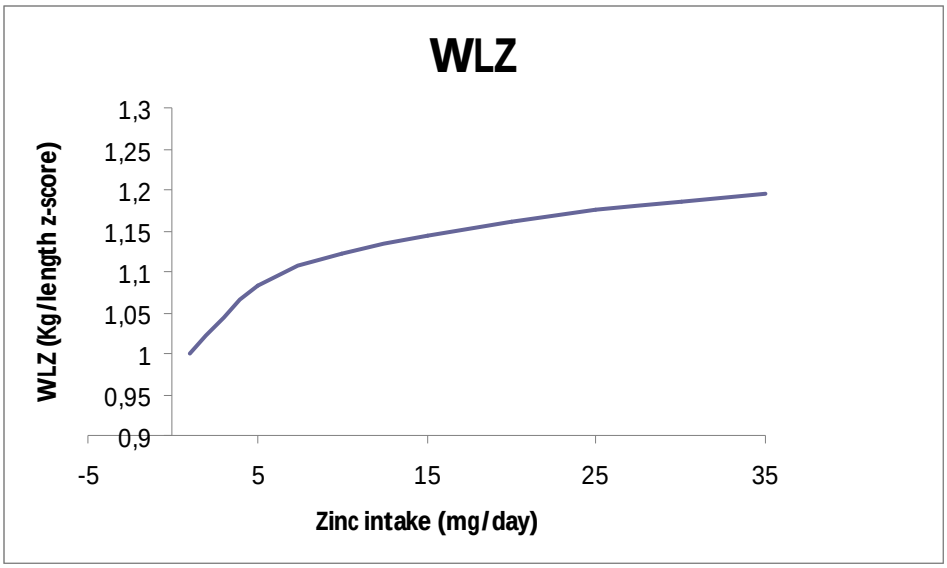

Figure 10: WLZ (Kg/Length z-score) as a function of dietary zinc intake (mg/day), estimated by random effects meta-analyses of RCTs of infants.

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Table 1: Search strategy: MEDLINE February 2010

(MEDLINE home page. Available online: http://www.ncbi.nlm.nih.gov/pubmed/)

No.	*Search term*	*Results*
1	randomized controlled trial.pt.	280,821
2	controlled clinical trial.pt.	79,998
3	randomised.ab.	196,604
4	placebo.ab.	117,891
5	clinical trials as topic.sh.	146,242
6	randomly.ab.	145,491
7	trial.ab.	203,467
8	randomised.ab.	38,423
9	6 or 3 or 7 or 2 or 8 or 1 or 4 or 5	734,511
10	(animals not (human and animals)).sh.	4,482,479
11	9 not 10	642,665
12	(cohort* or "case control*" or cross-sectional* or "cross sectional" or case-control* or prospective or "systematic review*").mp.	768,885
13	exp meta-analysis/ or expmulticenter study/ or follow-up studies/ or prospective studies/ or intervention studies/ or epidemiologic studies/ or case-control studies/ or exp cohort studies/ or longitudinal studies/ or cross-sectional studies/	1,013,635
14	13 or 12	1,203,767
15	14 not 10	1,154,385
16	11 or 15	1,599,094
17	((zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or	16,681

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	zinc isotope*) adj3 (intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair)).ti,ab.	
18	Nutritional Support/ or Dietary Supplements/ or nutritional requirements/ or Breast feeding/ or exp infant food/ or bottle feeding/ or infant formula/	63,098
19	exp Nutritional Status/ or exp Deficiency Diseases/ or supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or Diet/ or Food, Fortified/ or nutrition assessment/ or Nutritive Value/	176,014
20	(intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair).ti,ab.	3,166,092
21	18 or 19 or 20	3,263,114
22	zinc/	41,027
23	22 and 21	20,745
24	23 or 17	26,943
25	24 and 16	2410

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Table 2: Characteristics of the 19 (38 estimations) Growth studies included in the meta-analysis

Author	Study year	Country	Sample Age range or Mean (SD)	Number of infants (n)		Doses of Zn/day	Time of the intervention	Outcome (measure)	Nutritional situation	Risk of bias[1]
				C	Zn					
Arsenault (a) (b)	2008	Peru	6 to 8 months	44 44	36 38	3 mg in a liquid supplement 3 mg in a fortified porridge	24 w	Growth: MUAC	Nutritionally at risk	High risk
Bates	1993	Gambia	5.7 to 27 months	50	53	20 mg	60 w	Growth: Weight - Length MUAC	Healthy	High risk
Berger	2006	Vietnam	4 to 7 months	195	191	10 mg	24 w	Growth: Weight - Length WAZ – LAZ - WLZ	Nutritionally at risk	Moderate risk
Dijkhuizen	2001	Indonesia	Mean 4.2 months	90	98	7 mg	24 w	Growth: Weight- Length WAZ – LAZ – WLZ	Poor nutritional status	Moderate risk
Fischer Walker	2009	Bangladesh	6.3 ± 0.3 months	140	141	2,8 mg	24 w	Growth: Weight -	Nutritionally	Moderate

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								Length	at risk	risk
Gardner	2005	Jamaica	9 to 30 months	59	55	10 mg	24 w	Growth: Weight - Length WAZ – LAZ - WLZ	Poor nutritional status	Moderate risk
Hamadani (a) (b)	2001	Bangladesh	1 to 13 months	109 101	103 97	5 mg	28 w 52 w	Growth: WAZ – LAZ WLZ	Poor nutritional status	High risk
Heinig (a) (b) For Head Circunf.	2006	USA	3 to 10 months	37 37 37	33 33 33	5 mg	16 w 40 w 24 w	Growth: Weight - Length MUAC Head Circumference	Healthy	Low risk
Lind	2004	Indonesia	6 to 12 months	164	163	10 mg	8 w	Growth: WAZ - LAZ-WLZ	Healthy	Low risk
Meeks Gardner	1998	Jamaica	6 to 24 months	24	31	5 mg	12 w	Growth: Head Circumference	Poor nutritional status	Moderate risk
Muller	2003	Burkina Faso	Zn group: 18.7 ± 7.0 moPlacebo group: 17.6 ± 6.5 mo	329	332	1,78 mg	24 w	Growth: MUAC	Nutritionally at risk	Low risk
Ninh	1996	Vietnam	4 to 36 months	73	73	10 mg	20 w	Growth: Weight - Length WAZ –	Poor nutritional status	Low risk

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Olney	2006	Zanzibar	5 to 12 months	58	44	10 mg	24 w	LAZ - WLZ Growth: WAZ – LAZ	Poor nutritional status	High risk
Osendarp(a) (b) low serum zn < 9.18 µmol/L(c) normal serum zn > 9.18 µmol/L	2002	Bangladesh	3 to 5 weeks	13316115	1382 1117	5 mg	20 w	Growth: Weight - Length MUAC Head Circumference	Healthy	Moderate risk
Rivera	1998	Guatemala	6 to 9 months	44	45	10 mg	28 w	Growth: Weight - Length WAZ – LAZ - WLZ- MUAC Head Circumference	Nutritionally at risk	High risk
(a) (b) (c) (d) (e)Sur (f) (g) (h) (i) (j) (k) (l)	2003	India	0 to 12 months	50 50 50 50 50 50 50 50 50 50 50 50	50 50 50 50 50 50 50 50 50 50 50 50	3,57 mg3,57 mg3,57 mg3,57 mg3,57 mg3,57 mg3,57 mg3,57	1 w 2 w 3 w 4 w 5 w 6 w 7 w 8 w 9 w 10 w 11 w 12 w	Growth : WAZ	Poor nutritional status	Moderate risk

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					50 50 50	mg3,57 mg3,57 mg3,57 mg				
(a) Umeta (b)	2000	Ethiopia	Zinc stunted: 9.5 ± 2.0 mo Placebo stunted: 9.7 ± 2.0 mo Zinc non stunted: 9.3 ± 2.1 mo Placebo non stunted: 9.2 ± 2.0 mo	47 45	47 45	8,57 mg	24 w	Growth: MUAC	Healthy (stunted – non stunted)	High risk
Walravens Boys Girls	1989	USA	8 to 27 months	13 12	13 12	5,7 mg 5,7 mg	24 w	Growth: Weight - Length	Nutritionally at risk	Low risk
Wasantwisut	2006	Thailand	4 to 6 months	153	151	10 mg	24w	Growth: Weight - Length WAZ – LAZ - WLZ	Healthy	Low risk

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Table 3: Meta-regression. Multivariate adjusted mean beta for Growth (Length) (95% confidence interval) by different characteristics of the studies included in the meta-analysis

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	n	Mean beta's	Confidence interval (95%)	*P* ANCOVA*
Growth: Length				
By time				
4 to 20 weeks	5	0.0113	0.008 to 0.0219	
> 20 weeks	10	-0.0026	-0.0089 to 0.0037	
				0.008
By Dose				
1 to 4 mg	1	-0.0058	-0.0245 to 0.0130	
4,1 to 8 mg	8	0.0162	0.0078 to 0.0245	
8,1 to 12 mg	5	0.0057	-0.0016 to 0.0130	
> 12 mg	1	0.0014	-0.0200 to 0.0229	
				0.023
By Nutritional situation				
Healthy	7	0.0010	-0.0066 to 0.0086	
Nutritionally at risk	5	0.0128	0.0025 to 0.0230	
Poor nutritional situation	3	-0.0006	-0.0122 to 0.0110	
				0.083
By Risk of Bias				
Low	6	0.0016	-0.0084 to 0.0116	
Moderate	7	0.0074	-0.0013 to 0.0161	
High	2	0.0042	-0.0105 to 0.0189	
				0.409

* Adjusted for the rest of variables in the table

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Table 4: Pooled beta (95% confidence intervals) for Growth according to the intervention group.

Subgroup analyses.

	Pooled estimates (β)	Chi² (d.f., P)	I² *
Growth: Length			
All Studies (n=15)	0.001 (-0.002 to 0.004)	25.30 (14, 0.03)	45%
By time			
4 to 20 weeks (n=5)	0.01 (0 to 0.02)	4.93 (4, 0.29)	19%
> 20 weeks	-0.001 (-0.003 to 0.002)	15.18 (9, 0.09)	41%
By dose			
1 to 4 mg (n=1)	0 (-0.01 to 0.01)		
4,1 to 8 mg (n=8)	0.003 (0 to 0.01)	7.81 (7, 0.35)	10%
8,1 to 12 mg (n=5)	0 (-0.002 to 0.004)	6.85 (4, 0.14)	42%
> 12 mg (n=1)	0.01 (-0.02 to 0)		

*I² Index measures the extent of the heterogeneity

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Table 5: Pooled beta (95% confidence intervals) for Growth according to the intervention group.

Sensitivity Analyses

	Pooled estimates (β)	Chi² (dif, P)	I²
Growth: Weight			
All studies (n=15)	0.004 (-0.004 to 0.01)	21.05 (14, 0.10)	33%
All Studies excluding (n=3)	0 (-0.005 to 0.01)	15.53 (11, 0.16)	29%
Osendrap et al. 2002 b	0.12 (0.01 to 0.23)		
Walravens et al. 1989 a	0.03 (-0.04 to 0.10)		
Walravens et al. 1989 b	0.03 (-0.04 to 0.10)		
Growth: MUAC			
All studies (n=9)	0.003 (-0.003 to 0.01)	6.91 (8, 0.55)	0%
All Studies excluding (n=1)	0 (-0.003 to 0.01)	6.43 (7, 0.49)	0%
Bates et al.1993	-0.03 (-0.12 to 0.06)		
Growth: WAZ			
All studies (n=22)	0.06 (0.02 to 0.10)	31.14 (21, 0.07)	33%
All Studies excluding (n=9)	0.03 (0 to 0.07)	15.67 (12, 0.21)	23%
Sur et al. 2003 d	0.11 (-0.13 to 0.34)		
Sur et al. 2003 e	0.15 (-0.10 to 0.39)		
Sur et al. 2003 f	0.05 (-0.22 to 0.33)		
Sur et al. 2003 g	0.07 (-0.22 to 0.36)		
Sur et al. 2003 h	0.19 (-0.10 to 0.48)		
Sur et al. 2003 i	0.15 (-0.13 to 0.44)		
Sur et al. 2003 j	0.15 (-0.13 to 0.44)		
Sur et al. 2003 k	0.25 (-0.05 to 0.56)		

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Sur et al. 2003 l	0.55 (0.24 to 0.86)		
Growth: Length			
All studies (n=15)	0.001 (-0.002 to 0.004)	25.30 (14, 0.03)	45%
All Studies excluding (n=1)	0 (-0.002 to 0.004)	24.67 (13, 0.03)	47%
Osendrap et al. 2002 b	0.03 (-0.04 to 0.10)		

I^2 Index measures the extent of the heterogeneity

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