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Effect of zinc intake on serum/plasma zinc status in infants: A meta-analysis.

Nissensohn M[1'2]*, Sánchez Villegas A[1'2], Fuentes Lugo D[3], Henríquez Sánchez P[1'2], Doreste Alonso J[1], Lowe NL[4], Hall Moran V[5], Skinner AL[4], Warthon Medina M[4], Serra-Majem L[1'2]

[1] *Department of Clinical Sciences, University of Las Palmas de Gran Canaria, P.O. Box 550, 35080 Las Palmas de Gran Canaria, Spain* (MN mnissensohn@acciones.ulpgc.es; ASV asanchez@dcc.ulpgc.es; PHS phenriquez@dcc.ulpgc.es; JDA jdoreste@dcc.ulpgc.es; LSM lserra@dcc.ulpgc.es)

[2] *Ciber Fisiopatología Obesidad y Nutrición (CIBEROBN, CB06/03), Instituto de Salud Carlos III, Spain* (MN mnissensohn@acciones.ulpgc.es; ASV asanchez@dcc.ulpgc.es; PHS phenriquez@dcc.ulpgc.es; LSM lserra@dcc.ulpgc.es)

[3] *Faculty of Health Sciences, Unacar, Ciudad del Carmen, Mexico* (DFL daniel.fuentes@mac.com)

[4] *International Institute of Nutritional Sciences and Food Safety Studies, University of Central Lancashire, Preston PR1 2HE, UK* (NL NMLowe@uclan.ac.uk; ALS ASkinner@uclan.ac.uk; MWM MWathonmedina1@uclan.ac.uk)

[5] *Maternal & Infant Nutrition & Nurture Unit, University of Central Lancashire, Preston PR1 2HE, UK.* (VHM vlmoran@uclan.ac.uk)

*Corresponding Author:
Nissensohn Mariela, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
phone: 0034 928 459816
fax: 0034 928 451416

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Abstract

A systematic review and meta-analysis of available RCTs was conducted to evaluate the effect of zinc (Zn) intake on serum/plasma Zn status in infants. Out of 5500 studies identified through electronic searches and reference lists, 13 RCTs were selected after applying the exclusion/inclusion criteria. The influence of Zn intake on serum/plasma Zn concentration 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. RESULTS: The pooled β of status was 0.09 (CI 0.05 to 0.12). However, a substantial heterogeneity was present in the analyses (I^2= 98%; p=0.00001). When we performed a meta-regression, the effect of Zn intake on serum/plasma Zn status changed depending on the duration of the intervention, the dose of supplementation and the nutritional situation (p ANCOVA= 0.054; <0.001 and <0.007 respectively). After stratifying the sample according to the effect modifiers the results by duration of intervention showed a positive effect when Zn intake was provided during medium and long periods of time (4-20 weeks and >20 weeks). A positive effect was also seen when doses ranged from 8.1 to 12 mg/day. In all cases, the pooled β showed high evidence of heterogeneity. CONCLUSION: Zn supplementation increases serum/plasma Zn status in infants, although high evidence of heterogeneity was found. Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of Zn supplementation upon infant serum/plasma Zn status, particularly in Europe.

Keywords: EURRECA, zinc intake, serum/plasma Zn status, 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 the 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 and cellular proliferation, differentiation, and growth (Chesters 1978).

Plasma Zn accounts for only about 0.1 per cent of the total body content. Zn has a rapid turnover, and its level appears to be under close homeostatic control. There is no 'store' for Zn in the conventional sense (Milne et al. 1983) and it is present in the body almost exclusively as Zn2+ bound to cellular proteins (Makonnen et al. 2003).

Assessment of the Zn nutriture of individuals is complicated by the fact that no generally accepted, sensitive and specific biomarker of serum/plasma Zn status exists (King 1990). Although it is true that serum/plasma Zn concentrations decrease within several weeks of the introduction of a diet containing a severely restricted amount of Zn (Baer et al. 1985), serum/plasma Zn concentrations are generally maintained within the normal range with small or moderate reductions in Zn intake. Moreover, factors unrelated to the level of Zn nutriture, such as recent meals, time of day, infection, tissue catabolism, and pregnancy, can also affect serum/plasma Zn concentrations (King 1990; Hambidge & Krebs 1995). Thus, the serum/plasma Zn concentration may not always be a reliable indicator of an individual's true Zn status (Brown et al. 2002). Nevertheless a recent systematic review concluded that serum/plasma Zn concentration was responsive to both Zn supplementation and depletion and it remains the most widely used biomarker for Zn (Lowe et al. 2009).

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). 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 biomarkers

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of Zn status in infants, and combines these studies in meta-analyses to model Zn concentrations in serum or plasma as a function of Zn intake.

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 (www.eurreca.org). This review was part of a wider review process to identify studies assessing the effect of Zn intake on different outcomes (biomarkers of Zn status and health outcomes). The wider searches were performed of literature published up to and including February 2010, and an updated search was carried out in January 2013. The databases MEDLINE, EMBASE and Cochrane using search terms for "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 procedure for the identification, selection of articles and data extraction is illustrated in Figure 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. complementary foods), fortified foods (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 & narrative literature reviews); duplicate publications; studies where the Zn intake – status relationship was not reported or biomarkers of Zn other than serum / plasma Zn were used.

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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 two reviewers in even parts. Following the initial screening process, full-text articles were obtained. Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-analysis if they were: randomised controlled trials; had an intervention duration of at least 2 weeks; and reported baseline data for all outcome measures. Non-randomised controlled trials, uncontrolled trials or trials reporting insufficient or unclear data were excluded. Data were extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA).

Data synthesis

When Zn status in serum/plasma was measured at different time points within the same population, we used the measures as different estimations (Bates et al. 1993**;** Makonnen et al. 2003 I/II). One study reported data from the total of infants included, between males and females separately, and according to age (<11 months and > 11 months) (Sazawal et al. 1996; 2004) and it was treated as five estimations within the meta-analysis. One study reported data from two groups of infants (stunted and non stunted) and these were treated as two different estimations (Umeta et al. 2000). One study reported data from two groups according to the form of Zn supplementation (tablets or liquid) and these were treated as two estimations within the meta analysis (Wessells et al. 2012). Of the selected studies, two RCTs were companion papers (Makonnen et al. 2003 I; Sazawal et al. 2004). 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 serum/plasma Zn concentrations were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline serum/plasma Zn concentrations (n= 12). Only one study, Bates et al. 1993, failed to report anything regarding baseline serum /plasma Zn concentrations.

Exposure and outcome and other covariates assessment:

The influence of Zn intake on serum/plasma Zn concentrations was considered in the overall meta-analysis. 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

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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

Nutritionally at risk was defined as infants who lived in low income families with a low socioeconomic situation and poor nutritional status was defined as infants with protein energy malnutrition (PEM) but without congenital abnormalities or cerebral palsy or heart disease or infants with low birth weight during their first year. PEM occurs characteristically in children under 5 years of age in circumstances where the diet is poor in protein, calories and micronutrients, and insufficient to satisfy the body's nutritional needs. It remains one of the most common causes of morbidity and mortality among children worldwide (WHO, 1999).

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 and 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 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 & Green 2009) (Table 2).

Statistical analyses

Mean and standard deviation (SD) or standard errors (SE) of the outcome (serum/plasma Zn) 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 serum/plasma Zn (y- variable) is based on the assumption that this intake-serum/plasma Zn status curve is a logarithmic function and that both intake and serum/plasma Zn status follow a log-normal distribution (the natural logarithm of intake and serum/plasma Zn status have a normal distribution). Thus, the expected value of the serum/plasma Zn status score is expressed as:

$\mu y = \beta * \mu x + \text{intercept}$, where μy represents the mean of the natural logarithm of the y–variable (= serum/plasma Zn status score), β represents the regression coefficient, and μx represents the mean of the natural logarithm of the x-variable (= Zn intake). The method used to systematically review

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differences was a formal meta–analysis (Greenland 1998). 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 status parameter (serum / plasma), 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 status 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% confidence interval 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 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 all studies combined in each outcome, 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 of the different status parameters 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 ANCOVA. Additionally we carried out additional meta-analyses by subgroups considering only those groups which provided significant values in the meta-regression. Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review Manager 5.1, were used to conduct the statistical analyses.

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

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applying the additional eligibility criteria and grouping the studies by outcome, 9 randomized controlled trials (17 estimations) were selected (Walravens et al. 1989; Bates et al. 1993; Sazawal et al. 1996, 2004; Umeta et al. 2000; Osendarp et al. 2002; Lind et al. 2003; Makonnen et al. 2003; Wasantwisut et al. 2006; Chang et al 2010). The 2013 update of the original search identified 4 additional articles (Berger et al. 2006; Mazariegos et al. 2010; Ba Lo et al. 2011; Wessells et al. 2012), providing a total of 13 articles (22 estimates) for meta-analysis (Figure 1).

Descriptive characteristics of the studies included in the meta-analysis are presented in Table 2. Of the 13 studies included, only six comply strictly with the age infants (0 to 12 months) (Umeta et al. 2000; Osendarp et al. 2002; Lind et al. 2003; Berger et al. 2006; Wasantwisut et al. 2006; Mazariegos et al. 2010). The other seven studies included this age among their sample, but did not clarify how many are actually aged 0 to 12 months (Walravens et al. 1989; Bates et al. 1993; Sazawal et al. 1996, 2004; Makonnen et al. 2003; Chang et al 2010; Ba Lo et al. 2011; Wessells et al. 2012). None of the ages extended beyond 27 months, except Makonnen et al. 2003 which included children up to 5 years. Thus the age range of the studies included was from 3 weeks to 60 months.

Six studies were conducted in Asia, one in North America, one in Latin America and the Caribbean and five in Africa. The duration of the interventions ranged from 2 to 24 weeks. Doses of Zn intake ranged from 2.5 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; Umeta et al. 2000; Osendarp et al. 2002; Lind et al. 2003; Wasantwisut et al. 2006; Wessells et al. 2012), six studies were conducted on infants who were nutritionally at risk (Walravens et al. 1989; Sazawal et al. 1996, 2004; Berger et al. 2006; Chang et al 2010; Mazariegos et al. 2010; Ba Lo et al. 2011;), and one study was conducted on infants with poor nutritional status (Makonnen et al. 2003).
Table 3 summaries the internal validity of the included studies, assessed as described in the data synthesis section. The risk of bias was high in two studies (Bates et al. 1993; Umeta et al. 2000), five had a moderate risk (Sazawal et al. 1996; 2004; Osendarp et al. 2002; Makonnen et al. 2003; Berger et al. 2006; Wessells et al. 2012) and six had a low risk of bias (Walravens et al. 1989; Lind et al 2003; Wasantwisut et al. 2006; Chang et al 2010; Mazariegos et al. 2010; Ba Lo et al. 2011).

In general, most of the studies found a significant and direct association between Zn intake and serum/plasma Zn status, with β values ranged from 0.031 and 0.233. Only four studies reported no statistically significant association between Zn intake and serum/plasma Zn status (Walravens et al.

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1989; Bates et al. 1993; Makonnen et al. 2003; Wessells et al. 2012 (a) Tablets group). In order to summarize the results we performed a formal meta-analysis (Figure 2).

Differences between serum/plasma Zn status measured according to the intervention group in each particular study and in the pooled analysis are shown in Figure 2. The pooled β was 0.09 (95%CI 0.05, 0.12). However, a substantial heterogeneity was present in the analyses (I^2 for status = 98%). In order to investigate which variables may be potential effect modifiers, we performed a meta-regression (Table 4). The effect of Zn intake on serum/plasma Zn status changed depending on the duration of the intervention, the dose of supplementation and the nutritional situation (p ANCOVA= 0.054; <0.001 and <0.007) respectively. After stratifying the sample according to the effect modifiers identified in the meta-regression (Table 5) the results by duration of intervention showed no significant effect when the duration was short (1 to 3 weeks) (β = 0.02; CI 95% -0.03 to 0.07). Nevertheless, a positive effect was shown when Zn intake was provided over medium (4 to 20 weeks)(β = 0.09; CI 95% 0.06 to 0.13) and long periods of time (>20 weeks) (β = 0.12; CI 95% 0.07 to 0.16). However these pooled β still revealed high evidence of statistically significant heterogeneity (I^2= 91 and 96 %) respectively. When doses of Zn ranged from 4.1 to 8 mg/day, there was no significant effect of Zn intake on the serum/plasma Zn; whereas a positive effect was seen when doses ranged from 8.1 to 12 mg/day (β = 0.12; CI 95% 0.09 to 0.16). For doses higher than 12 mg/day we found no effect. However high evidence of heterogeneity was observed (I^2= from 77 to 96 %). When studies were categorised by nutritional situation, those studies based on healthy infants and on infants at nutritional risk reported a positive association between Zn intake and serum/plasma Zn status (β= 0.19; CI 95% 0.04 to 0.13 and β= 0.10; CI 95% 0.05 to 0.15) respectively. However, no association was found when the nutritional situation was poor (β= 0.05; CI 95% -0.02 to 0.12). Once again, the pooled β still showed high evidence of heterogeneity (I^2= from 95 to 99 %). Due to the high heterogeneity found in all the analyses, we decided to avoid calculating the dose-response relationship between Zn intake and serum/plasma Zn status.

Discussion

Our results indicate that Zn supplementation increases serum/plasma Zn status in infants, as suggested by most of the individual studies. However the results obtained in the meta-analyses were highly heterogeneous. Moreover, after carrying out several subgroup analyses, the pooled β for each sub analysis still showed high evidence of heterogeneity. We argue that conducting a meta-analysis with such data is important in order to highlight the differences between the results of the studies

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available, rather than to present a unifying synthesis (Delgado-Rodríguez & Sillero Arenas in press).

The interpretation of these results should be carefully considered for a number of reasons. First, the number of studies that were eligible for inclusion in this meta-analysis was small, which limited the statistical power of the analyses to examine the relation between status responses to Zn supplementation. Thus, the small effect size we found may be explained by the limited amount of available information. Also, it is well acknowledged that when many statistical comparisons are carried out, one or more might reach significance due to chance alone (Bland & Altman 1995). It is also important to consider the scientific quality of included studies. Although meta-analyses 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 depend on the methodological quality of the original studies, the appropriateness of the study inclusion criteria, and the thoroughness of the review and synthesis of information (Brown et al. 2002). While strict systematic review protocols were followed adhering to EURRECA's quality standards (Matthys et al 2011), an assessment of the risk of bias of included studies revealed that the majority (n=7) had a high to moderate risk of bias.

Positive effects of Zn supplementation on mean serum Zn concentrations have also been reported in previous meta-analyses conducted in children, pregnant women and adults (Brown et al. 2002; Hess et al. 2007; Hall Moran et al 2012a, Hall Moran et al 2012b; Lowe et al 2012). In these meta-analyses, there was a significantly positive effect of Zn supplementation over the mean serum Zn concentrations of the studied population. However, to our knowledge, meta-analytical methods have not yet been used to model serum/plasma Zn status as a function of Zn intake levels in infants. Understanding the relationship between dietary intake and micronutrient status is essential for deriving dietary recommendations.

Population mean concentration of serum Zn is a useful indicator of the successful delivery and absorption of Zn supplements in infants. Both serum and plasma Zn concentrations are the most widely used biochemical indicators of serum/plasma Zn status but their levels are not necessarily identical. For instance, several biochemical studies designed to compare plasma and serum Zn concentrations observed higher levels of Zn in serum than in plasma (Kasperek et al. 1981; English & Hambidge 1988). These differences may have occurred because serum samples were separated from blood cells after a longer period of time than plasma samples, so more Zn went out from the cells into serum than into plasma. By controlling both, the amount of blood collected and the time

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of cell separation, no differences were found in the Zn concentrations of serum and plasma (English & Hambidge 1988). For the sake of simplicity, this paper referred to "serum/plasma Zn" without making any distinction between them.

Some confounders should be considered in evaluating the effect of Zn intake on infant serum/plasma Zn status. Those confounders include low birth weight, breastfeeding, protein energy malnutrition, poverty and social deprivation. The pre-existing serum/plasma Zn status of the study subjects, the content and bioavailability of Zn in the local diets, and the incidence of common infections that can affect individual's serum/plasma Zn status are others important confounders to take into account. 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 their results (Brown et al. 2002). However, with the exception of Bates et al (1993), all the RCTs included in the meta-analysis assumed no baseline differences in serum/plasma Zn. As all the studies included in our meta-analysis are RCTs we may assume that the randomization has been correct and these factors should not bias the results.

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 reason, 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 (Walravens et al. 1989; Bates et al. 1993; Makonnen et al. 2003; Sazawal et al. 2004, 1996; Chang et al. 2010; Ba Lo et al. 2011; Wessells et al. 2012) and assumed the consequences of this possible bias. Another confounding factor that might explain the inconsistency in our findings is that serum Zn concentrations vary according to the time of day, proximity of previously consumed meals, and occurrence of recent physical activity or other forms of stress, fluctuating by as much as 20% during a 24-hour period (Hambidge et al. 1989). The diurnal variation in circulating Zn concentration is largely a result of metabolic changes after meal consumption, although some variation may occur as a result of normal circadian variation in metabolism (Guillard et al. 1979; Wallock et al. 1993). Meal consumption results in a decrease in serum/plasma Zn concentrations, which add up following repeated meals (Goode 1991; Wallock et al. 1993), whereas overnight and daytime fasting result in increased circulating Zn concentrations (Wallock et al. 1993). Of the studies included in our meta-analyses, those conducted by Walravens et al. 1989, Umeta et al. 2000, Osendarp et al. 2002, Berger et al. 2006, Wasantwisut et al. 2006, Ba lo et al. 2011 and Wessells et al. 2012 reported the time of the day when the blood samples were collected (during the morning).

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Due to small numbers it was not possible to conduct a subgroup analysis on the time of the day that the samples were collected.

Infection and inflammation can decrease serum/plasma Zn values, with the magnitude of change depending on the severity and stage of infection (Brown 1998). In community- based surveys, the reductions in serum/plasma Zn concentration due to infection average ~10% to 12% compared with healthy reference groups (Thurnham et al. 2005). Several other factors, such as low serum albumin, elevated white blood cell counts, use of hormones, can also affect serum/plasma Zn levels and must be considered in the interpretation of laboratory results (IZiNCG 2004). In our meta-analysis, all studies accounted for the presence of disease over the duration of the intervention and whether or not Zn levels were affected by that.

Infants suffering from protein-energy malnutrition have low concentrations of Zn in serum/plasma, muscle and liver (Hansen & Lehman 1969; Cheek et al. 1970). Because Zn is needed for tissue synthesis during nutritional rehabilitation, the amount required may exceed dietary supply (Castillo-Duran et al. 1987; Gibson et al. 1998). Makonnen et al 2003 were the only authors in our meta-analysis which included infants with PEM. In this study, improvement in serum/plasma Zn status became evident only after 60 days. In children with PEM it takes over one month for serum levels to increase significantly, so this could explain the limited effect Zn supplementation had on serum/plasma Zn levels at 30 days. Inclusion of a study conducted in malnourished children might have contributed to the lack of significance in the present meta-analysis. Finally, most of the studies were carried out among low-income populations of Asia and Africa and some of them were based on nutritionally at risk subjects so the generalization of the reported estimations to European populations could be compromised.

In conclusion, a positive significant association was found between Zn intake and serum/plasma Zn status in infants. The magnitude of effect we found was in all cases rather small. Based on this limited group of studies and their heterogeneity, we found insufficient current information to suggest that supplementation of Zn has a positive effect on infants' serum/plasma Zn status or to recommend mean serum/plasma Zn concentration of a given population as a useful predictor of response to Zn supplementation. Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of Zn supplementation upon infant serum/plasma Zn status, particularly in Europe and other affluent societies.

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The authors´ responsibilities were as follows: MN: analysis of the data and writing the manuscript, ALS & MN: review the papers, MWM: contribution to selection of papers and data extraction, ASV: support in data-analysis, DFL, PHS, JDA, NL, VMH and LSM provision of significant advice. All authors directly participated in the planning, execution or analysis of the study and reviewed the manuscript.

CONFLICT OF INTEREST: Authors declare no conflicts of interest.

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Figure 1: Flow diagram for the systematic review.

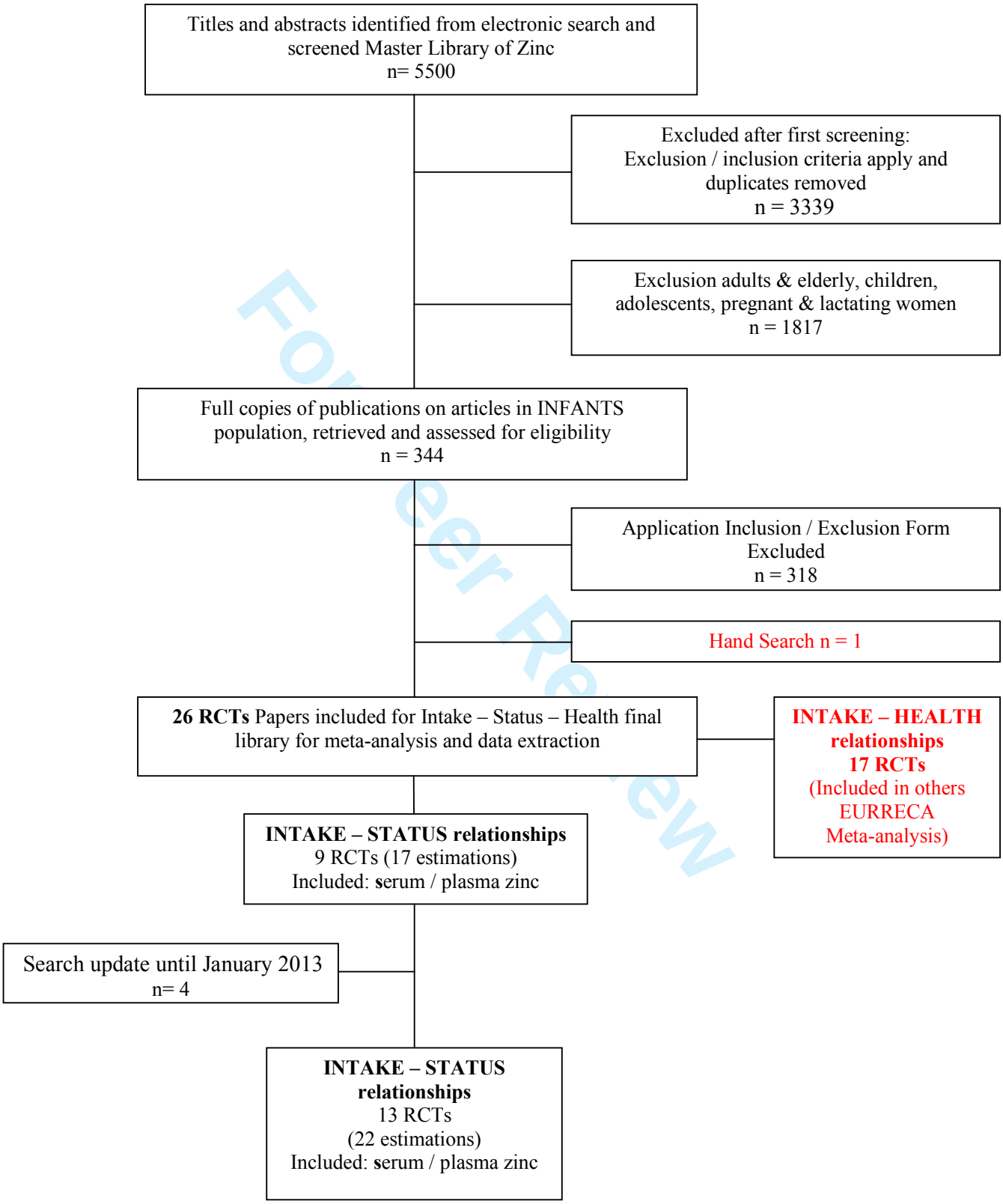

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Figure 2: Forest Plot of RCTs evaluating the effect of zinc intake on serum/plasma zinc status in infants

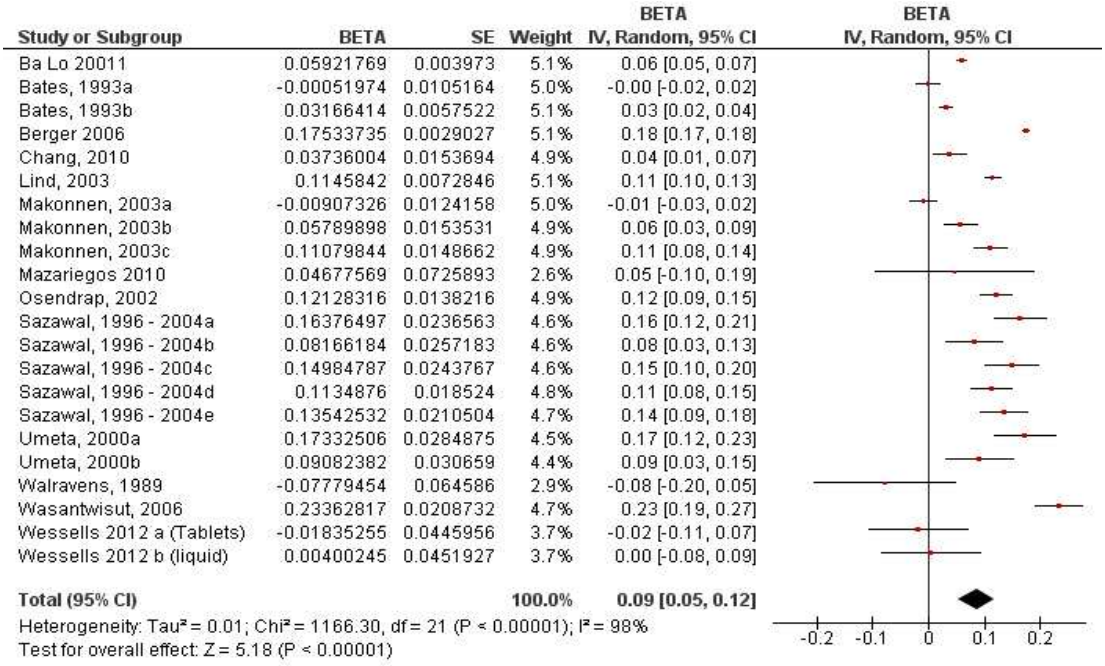

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Table 1: Search strategy: MEDLINE February 2010

(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 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.	16,681
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 13 (22 estimations) Status studies included in the meta-analysis

Author	Study year	Country	Sample Age range or Mean (SD)	Number of Infants (n)		Doses of Zinc/ day	Time of the intervention	Outcome (measure)	Nutritional situation	Risk of bias [2]
				Zn [1]	C [1]					
Ba Lo	2011	Senegal	9 to 17 months	33	32	6 mg	15 days	Status (plasma)	Nutritionally at risk	Low risk
Bates (a)	1993	Gambia	5.7 to 27 months	30	28	20 mg	2 weeks	Status (plasma)	Healthy	High risk
(b)				46	44		8 weeks			
Berger	2006	Vietnam	4 to 7 month	161	155	10 mg	24 weeks	Status (serum)	Nutritionally at risk	Moderate risk
Chang	2010	Bangladesh	6 to 18 months	85	89	2,5 mg	24 weeks	Status (serum)	Nutritionally at risk	Low risk
Lind	2003	Indonesia	6.1 (0.5) months	134	143	10 mg	24 weeks	Status (serum)	Healthy	Low risk
(a)	2003	Lesotho	6 to 60 months	142	121	10 mg	4 weeks	Status (serum)	Poor nutritional status	Moderate risk
Makonnen (b)				141	119		8 weeks			
(c)				138	116		12 weeks			
Mazariegos	2010	Guatemala	6 to 12 months	24	29	5 mg	24 weeks	Status (plasma)	Nutritionally at risk	Low risk
Osendarp	2002	Bangladesh	3 to 5 weeks	138	133	5 mg	20 weeks	Status (serum)	Healthy	Moderate risk
Sazawal (a)	1996-	India	6 to 35 months	223	224	10 mg	16 weeks	Status (plasma)	Nutritionally at risk	Moderate risk
(b)	2004[3]		6 to 11 months	78	78					
(c)			> 11 months	69	73					
(d)			Females	115	106					
(e)			Males	108	118					

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(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	25 25	25 25	8,57 mg	24 weeks	Status (serum)	Healthy	High risk
Walravens	1989	USA	8 to 27 months	16	25	5,7 mg	24 weeks	Status (plasma)	Nutritionally at risk	Low risk
Wasantwisut	2006	Thailand	4 to 6 months	58	66	10 mg	24 weeks	Status (serum)	Healthy	Low risk
Wessells (a)Tablets (b)Liquid	2012	Burkina Faso	6 to 23 month	 149 146	150	5 mg	3 weeks	Status (plasma)	Healthy	Moderate risk

(a - e): Estimations

[1]Zn: Zinc group / [1]C: Control group

[2] Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All others studies were considered as moderate when they meet any of the above criteria or high risk of bias when they meet any of the criteria. (Higgins 2009, Cochrane Handbook)

[3]Companion paper

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Table 3: Assessment of internal validity in RCTs of serum/plasma Zn status.

Author, Year	Method of sequence generation	Adequate allocation	Blinding adequate	Number at start, dropouts & dropouts reasons Outcome data complete	Funder adequate	Others potential funding bias	Overall risk of bias
Ba Lo 2011	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Bates 1993	Yes	No	Unclear	Yes	No	No	High risk
Berger 2006	Unclear	Unclear	Yes	Yes	Yes	Yes	Moderate risk
Chang 2010	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Lind 2003	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Makonnen 2003	Yes	Unclear	Yes	Yes	Unclear	Yes	Moderate risk
Mazariegos 2010	Yes	Yes	Yes	Unclear	Yes	Yes	Low risk
Osendarp 2002	Unclear	Unclear	Yes	Yes	Yes	Yes	Moderate risk
Sazawal 1996-2004	Unclear	Unclear	Yes	Unclear	Yes	Yes	Moderate risk
Umeta 2000	Unclear	Unclear	Yes	Unclear	Unclear	Yes	High risk
Walravens 1989	Yes	Unclear	Yes	Yes	Yes	Yes	Low risk
Wasantwisut 2006	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Wessells 2012	Yes	Yes	No	Yes	Yes	Yes	Moderate risk

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Table 4: Meta-regression. Multivariate adjusted mean beta for Status (95% confidence interval) by different characteristics of the studies included in the meta-analysis

	n	Mean Beta's	CI (95%)	P Ancova*
Status				
By duration of the intervention				
1 to 3 weeks	4	0.0221	-0.0752 to 0.1194	
4 to 20 weeks	10	0.0543	0.0142 to 0.0943	
> 20 weeks	8	0.1331	0.0805 to 0.1858	
				0.054
By Dose				
1 to 4 mg	1	-0.1025	-0.2081 to 0.0031	
4,1 to 8 mg	6	0.1893	0.1021 to 0.2764	
8,1 to 12 mg	13	0.1070	0.0650 to 0.1491	
> 12 mg	2	0.0855	0.0215 to 0.1495	
				<0.001
By Nutritional situation				
Healthy	9	0.0456	0.0048 to 0.0863	
Nutritionally at risk	10	0.1184	0.0686 to 0.1681	
Poor nutritional situation	3	0.0456	0.0048 to 0.0863	
				<0.007
By Risk of Bias				
Low	6	0.0978	0.0351 to 0.1606	
Moderate	12	0.0558	0.0140 to 0.0976	
High	4	0.0558	0.0140 to 0.0976	
				0.255

* Adjusted for the rest of variables in the table

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Table 5: Pooled beta (95% confidence intervals) in Status according to the intervention group. Subgroup analyses.

	Pooled estimates (β)	Chi² (df, P)	I²
Status			
All Studies (n=22)	0.09 (0.05 to 0.12)	1166.30 (21, < 0.00001)	98%
By duration of the intervention			
1 to 3 weeks (n=4)	0.02 (-0.03 to 0.07)	31.78 (3, < 0.00001)	91%
4 to 20 weeks (n=10)	0.09 (0.06 to 0.13)	141.21 (9, < 0.00001)	94%
> 20 weeks (n=8)	0.12 (0.07 to 0.16)	162.64 (7, < 0.00001)	96%
By dose			
1 to 4 mg (n=1)	0.04 (0.01 to 0.07)		
4,1 to 8 mg (n=6)	0.04 (-0.01 to 0.09)	22.08 (5, 0.0005)	77%
8,1 to 12 mg (n=13)	0.12 (0.09 to 0.16)	341.12 (12, < 0.00001)	96%
> 12 mg (n=2)	0.02 (-0.01 to 0.05)	7.21 (1, 0.007)	86%
By Nutritional Situation			
Healthy (n=9)	0.09 (0.04 to 0.13)	220.90 (8, < 0.00001)	96%
Nutritionally at risk (n=10)	0.10 (0.05 to 0.15)	615.54 (9, < 0.00001)	99%
Poor nutritional status (n=3)	0.05 (-0.02 to 0.12)	39.26 (2, < 0.00001)	95%

*I² Index measures the extent of the heterogeneity

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