Original Communication

Effect of Zinc Intake on Mental and Motor Development in Infants: A Meta-Analysis

Mariela Nissensohn[1,2], Almudena Sánchez-Villegas[1,2], Daniel Fuentes Lugo[3], Patricia Henríquez Sánchez[1,2], Jorge Doreste Alonso[1,2], Anna Louise Skinner[4], Marisol Warthon Medina[4], Nicola M. Lowe[4], Victoria Hall Moran[5], and Lluis Serra-Majem[1,2]

[1]Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Spain
[2]Ciber Fisiopatología Obesidad y Nutrición (CIBEROBN, CB06/03), Instituto de Salud Carlos III, Madrid, Spain
[3]Faculty of Health Sciences, Unacar, Ciudad del Carmen, Mexico
[4]International Institute of Nutritional Sciences and Food Safety Studies, University of Central Lancashire, Preston, UK
[5]Maternal & Infant Nutrition & Nurture Unit, University of Central Lancashire, Preston, UK

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Abstract: A systematic review and meta-analysis of available randomized controlled trials (RCTs) was conducted to evaluate the effect of zinc (Zn) intake on mental and motor development in infants. Out of 5500 studies identified through electronic searches and reference lists, 5 RCTs were selected after applying the exclusion/inclusion criteria. The influence of Zn intake on mental and motor development 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 situation, and risk of bias. Indices of mental and motor development assessed were the Mental Development Index (MDI) and Psychomotor Development Index (PDI). Additionally we carried out a sensitivity analysis. The pooled β was –0.01 (95 %CI –0.02, 0) for MDI and 0 (95 %CI –0.03, 0.02) for PDI, with a substantial heterogeneity in both analyses. When we performed a meta-regression, the effect of Zn supplementation on MDI changed depending on the dose of supplementation. Regarding PDI, there was a differential effect of Zn intake depending on intervention duration, dose of supplementation, nutritional situation, and risk of bias. Zn supplementation showed a negative, weak and significant effect on PDI score in those studies with a length of 4 to 20 weeks (β= –0.05; CI 95 % –0.06 to –0.04). In conclusion, no association was found between Zn intake and mental and motor development in infants. Further standardized research is urgently needed to clarify the role of Zn supplementation upon infant mental and motor development, particularly in Europe.

Key words: EURRECA, zinc intake, mental and motor development, infants

Introduction

Our perception of zinc (Zn) has progressed from that of a rather obscure essential trace mineral of doubtful significance for human health to that of a micronutrient of exceptional biologic and public health importance, in a remarkably short time. This is most evident in relation to both prenatal and postnatal development [1].

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 [2], 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 [1].

Zn is also present in the brain and contributes to its structure and function. Limited evidence from animal and human studies suggests that its deficiency may lead to delays in cognitive development. Severe Zn deficiency in animals has been associated with structural malformations of the brain, such as anencephaly, microcephaly, and hydrocephaly; and with behavioral problems, such as reduced activity and deficits in short-term memory and spatial learning. In humans, severe Zn deficiency can cause abnormal cerebellar function and impaired behavioral and emotional responses [3].

Although the mechanisms linking Zn deficiency with cognitive development remain unclear, it appears that Zn deficiency may lead to deficits infant's neuropsychological functioning, activity, or motor development, and thus interferes with cognitive performance [3].

A considerable number of intervention trials have been completed in multiple countries to assess the effect of supplemental Zn on the infant's mental and motor development. However, these studies have yielded inconsistent results, possibly because of differences in the pre-existing Zn status of the study subjects, in the content and bioavailability of Zn in the local diets, and in the incidence of common infections that can affect growth independently of an individual's Zn status. Moreover, methodological aspects of these studies, such as variations in the dose, in the method of administration, or in the duration of supplementation, may have influenced their results [4]. Finally, in some cases, the sample sizes may have been inadequate to detect potentially important differences in growth with statistical confidence.

To our knowledge meta-analytic methods have not yet been used to evaluate the influence of Zn intake on infant mental and motor development. This paper presents a systematic review of the data from all available randomized controlled trials (RCTs), meeting European Micronutrient Recommendations Aligned (EURRECA)'s quality standard [5], that assessed the effect of Zn supplementation on infant mental and motor development.

Materials and Methods

Search strategy

This research was conducted within the framework of the 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 (biomarkers of Zn status and 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 was been published elsewhere [6] and can be found in Table I. 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 on mental and motor development.

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

Table I: Search strategy.

Search no:	*Search term*	Results	Search type
1	randomized controlled trial.pt.	280821	Advanced
2	controlled clinical trial.pt.	79998	Advanced
3	randomized.ab.	196604	Advanced
4	placebo.ab.	117891	Advanced
5	clinical trials as topic.sh.	146242	Advanced
6	randomly.ab.	145491	Advanced
7	trial.ab.	203467	Advanced
8	randomised.ab.	38423	Advanced
9	6 or 3 or 7 or 2 or 8 or 1 or 4 or 5	734511	Advanced
10	(animals not (human and animals)).sh.	4482479	Advanced
11	9 not 10	642665	Advanced
12	(cohort* or ,‘case control*‘‘ or cross-sectional* or ,‘cross sectional‘‘ or case-control* or prospective or ,‘systematic review*‘‘).mp.	768885	Advanced
13	exp meta-analysis/ or exp multicenter 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/	1013635	Advanced
14	13 or 12	1203767	Advanced
15	14 not 10	1154385	Advanced
16	11 or 15	1599094	Advanced
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.	16681	Advanced
18	Nutritional Support/ or Dietary Supplements/ or nutritional requirements/ or Breast feeding/ or exp infant food/ or bottle feeding/ or infant formula/	63098	Advanced
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/	176014	Advanced
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.	3166092	Advanced
21	18 or 19 or 20	3263114	Advanced
22	zinc/	41027	Advanced
23	22 and 21	20745	Advanced
24	23 or 17	26943	Advanced
25	24 and 16	2410	Advanced

Search strategy: MEDLINE February 2010
(MEDLINE home page. Available online: http://www.ncbi.nlm.nih.gov/pubmed/)
Pt = publication type; ab = abstract; sh = subject heading; adj3 = adjacency (terms in any order with three words or less between them; ti = title.

did not study the effect of the micronutrient separately; non-primary studies (e. g., letters and narrative literature reviews); duplicate publications; studies where Zn intake-cognitive development/functioning association was not reported or health outcomes other than mental and motor development assessed.

Briefly, titles and abstracts of 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 % of the titles and abstracts were distributed among the two reviewers in even parts.

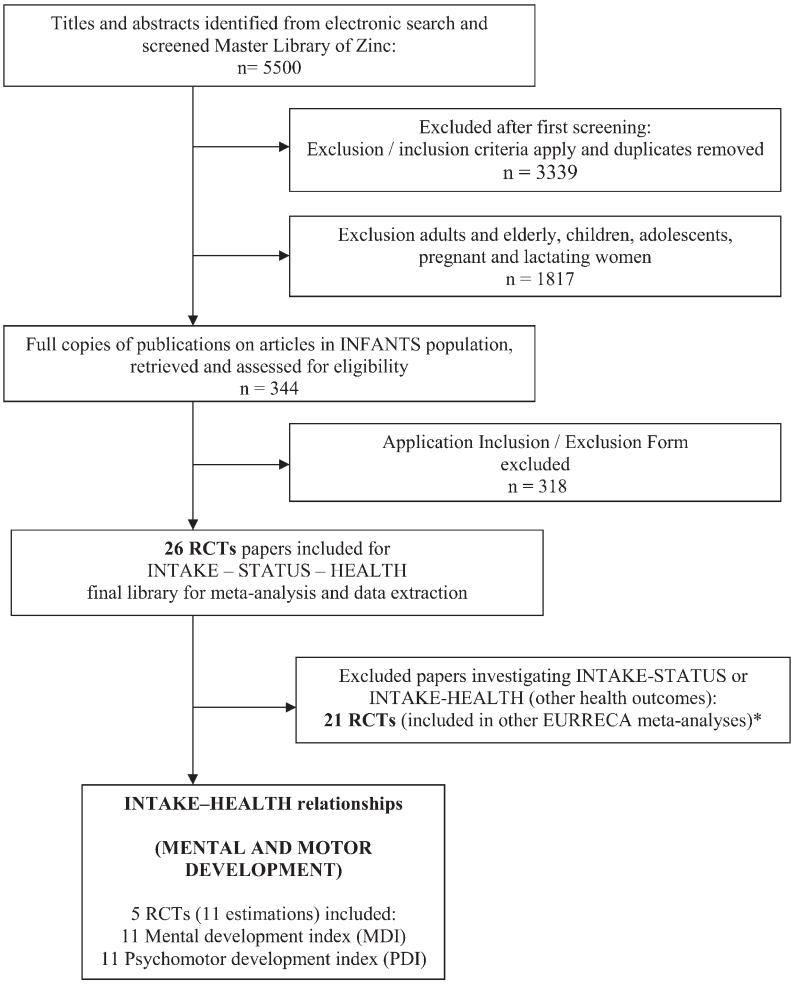

Figure 1: Flow diagram for the systematic review.
* European Micronutrient Recommendations Aligned (EURRECA) (http://www.eurreca.org) assessed the effect of Zn intake on different outcomes (biomarkers of Zn status and health outcomes: growth). The 21 RCTs are excluded from this paper, and are published elsewhere.

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: 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 were extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA, USA).

Data synthesis

When mental and motor development was measured at different time points within the same population, we used the measures as different estimations [7–9].

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 Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline measures [8, 10, 11]. Ashworth *et al.* [7] performed an adjustment for initial differences. Only the study of Jimenez *et al.* [9] failed to mention anything regarding this matter.

Exposure and outcome and other covariates assessment

The influence of Zn intake on infant mental and motor development measures 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 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; poor nutritional status was defined as infants with protein energy malnutrition (PEM) but without congenital abnormalities, cerebral palsy, heart disease, or low birth weight during their first year.

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; and 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 [12].

Indices of mental and motor development assessed in the included studies were MDI (Mental Development Index) and PDI (Psychomotor Development Index). Both indices were measured by Bayley Scales of Infant Development II in all studies.

Statistical analysis

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

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

where μy represents the mean of the natural logarithm of the y-variable (mental and motor development 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 differences was a formal meta-analysis [13]. Procedures of formal meta-analysis have been applied to combine the results from previously reported studies [14].

A random-effects model was considered to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's model [15] to pool the estimates of betas across studies. Under this model, the pooled effect was the beta in the mental and motor development parameters (MDI – PDI), 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 [16]:

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

where $\beta\ pooled$ is the pooled estimate of the beta in mental and motor development parameters; the weight *(wi)* of each study was computed as: $wi = 1/Vi + R^2$

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

Besides this, we calculated a 95 % confidence interval (CI) for the pooled estimate of effect size:

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

where SE is the standard error of the pooled estimate [13].

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 [17].

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 [13].

We fitted a meta-regression using the duration of the intervention, the doses of Zn intake, the nutritional situation, and the risk of bias as independent variables. The betas of the different mental and motor development parameters according to Zn intake were used as the dependent variable.

Statistical differences in multivariate adjusted mean beta values between each possible heterogene-

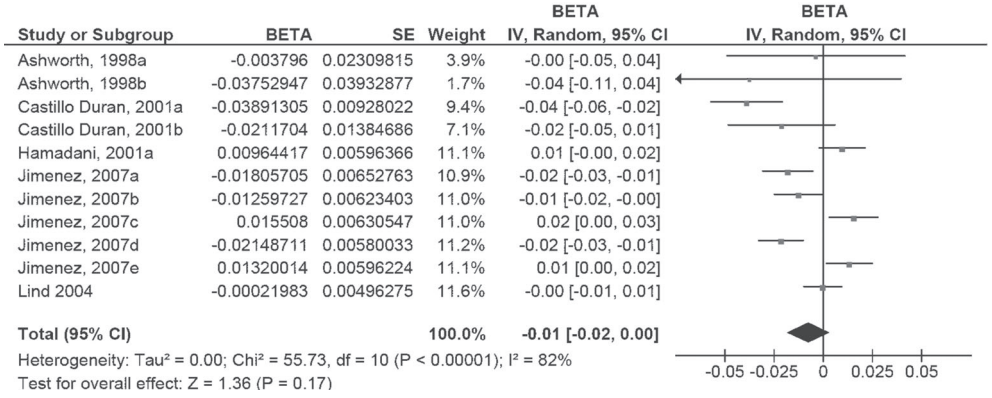

Figure 2: Forest plot of randomized controlled trials evaluating the effect of zinc intake on MENTAL DEVELOPMENT INDEX (MDI) in infants.

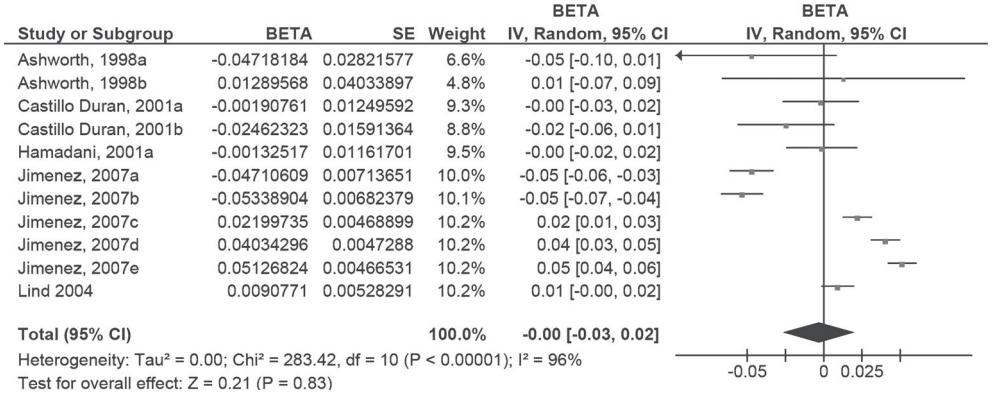

Figure 3: Forest plot of randomized controlled trials evaluating the effect of zinc intake on PSYCHOMOTOR DEVELOPMENT INDEX (PDI) in infants.

ity source were determined by analysis of covariance (ANCOVA).

Moreover, 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 pooled estimate of the beta in each mental and motor development parameter.

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 appeared to be of potential relevance. After applying the additional eligibility criteria and grouping the studies by outcome (mental and motor development), five RCTs (11 estimations) were selected for the meta-analysis [7–11]. (Figure 1)

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

Three studies were conducted in Latin America and the Caribbean and two in Asia. The duration of the interventions ranged from 4 to 52 weeks. Doses of Zn intake ranged from 1 to 10 mg per day. Age of infants was between 1 to 12 months. The nutritional situation of infants also varied between studies: two studies were conducted in healthy infants [8,11] and three studies were conducted on infants with poor nutritional status [7, 9, 10]. There were no studies including infants nutritionally at risk. Risk of bias varied also between studies: two studies had a high risk of bias [7, 10], one had a moderate risk [8], and two had low risk of bias [9, 11].

Differences between mental and motor development outcomes (MDI and PDI) according to Zn intake in each particular study and in the pooled analysis are shown in Figures 2 and 3. The pooled β was –0.01 (95 % CI –0.02, 0) for MDI and 0 (95 % CI –0.03, 0.02) for PDI. However, a substantial heterogeneity was present in both analyses (I^2 for MDI = 82 % and I^2 for PDI = 96 %).

In order to investigate which variables may be potential effect modifiers, we performed a meta-regression (Table III). The effect of Zn supplementation on

Table II: Characteristics of the 5 (11 estimations) mental and motor development 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*
					Zn	C					
Ashworth	(a) (b)	1998	Brazil	6 to 12 months	56 48	53 44	1 mg	24 weeks 48 weeks	MDI-PDI	Poor nutritional status	High risk
Castillo Duran (8)	(a) (b)	2001	Chile	Mean 6 months	57 57	55 55	5 mg	24 weeks 48 weeks	MDI-PDI	Healthy	Moderate risk
Hamadani (10)	(a)	2001	Bangladesh	1 to 13 months	103	109	5 mg	28 weeks	MDI-PDI	Poor nutritional status	High risk
Jiménez (9)	(a) (b) (c) (d) (e)	2007	Cuba	1 to 12 months	87 87 87 87 87	76 76 76 76 76	10 mg	4 weeks 13 weeks 26 weeks 39 weeks 52 weeks	MDI-PDI	Poor nutritional status	Low risk
Lind (11)		2004	Indonesia	6 to 12 months	161	162	10 mg	8 weeks	MDI-PDI	Healthy	Low risk

(a – e): Estimations
Zn: Intervention group / C: Control group
*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 & Green 2009, Cochrane Handbook)
MDI: Mental Development Index
PDI: Psychomotor Development Index

Table III: Meta-regression. Multivariate adjusted mean beta for mental and motor development (MDI; PDI) (95 % confidence interval) by different characteristics of the studies included in the meta-analysis.

	n	Mean beta's	Confidence interval (95 %)	P Ancova *
MDI				
By duration of the intervention				
4 to 20 weeks	3	–0.0005	–0.0144 to 0.0133	
>20 weeks	8	–0.0148	–0.0224 to –0.0072	
				0.078
By Dose				
1 to 4 mg	2	–0.0273	–0.0432 to –0.0114	
4,1 to 8 mg	3	0.0107	–0.0035 to 0.0249	
8,1 to 12 mg	6	–0.0064	–0.0164 to 0.0037	
				0.001
By Nutritional situation				
Healthy	3	–0.0014	–0.0146 to 0.0119	
Poor nutricional situation	8	–0.0140	–0.0272 to –0.0008	
				0.245
By Risk of Bias				
Low	6	–0.0051	–0.0165 to 0.0062	
Moderate	2	–0.0128	–0.0359 to 0.0103	
High	3	–0.0051	–0.0165 to 0.0062	
				0.616
PDI				
By duration of the intervention				
4 to 20 weeks	3	–0.0757	–0.0994 to –0.0521	
> 20 weeks	8	0.0124	0.0006 to 0.0253	
				<0.001
By Dose				
1 to 4 mg	2	–0.0553	–0.0824 to –0.0282	
4,1 to 8 mg	3	–0.0395	–0.0637 to –0.0153	
8,1 to 12 mg	6	0.0003	–0.0174 to 0.0168	
				<0.001
By Nutritional situation				
Healthy	3	–0.0020	–0.0246 to 0.0205	
Poor nutricional situation	8	–0.0613	–0.0839 to –0.0388	
				0.001
By Risk of Bias				
Low	6	–0.0079	–0.0273 to 0.0114	
Moderate	2	–0.0792	–0.1185 to –0.0398	
High	3	–0.0079	–0.0273 to 0.0114	
				0.001

* Adjusted for the rest of variables in the table

MDI changed depending on the dose of supplementation (p ANCOVA=0.001). Regarding PDI, there was a differential effect of Zn intake depending on duration of the intervention, dose of supplementation, nutritional situation, and risk of bias (p ANCOVA =<0.001, <0.001, 0.001, 0.001), respectively.

Table IV: Pooled beta (95 % confidence intervals) in mental and motor development according to the intervention group. Subgroup analyses.

	Pooled estimates (β)	Chi² (d.f., P)	I²
MDI			
All Studies (n = 11)	–0.01 (–0.02 to 0)	55.73 (10, < 0,00001)	82 %
By dose			
1 to 4 mg (n = 2)	–0.04 (–0.08 to 0)	0 (1, 0.98)	0 %
4,1 to 8 mg (n = 3)	0 (–0.01 to 0.01)	1.07 (2, 0.59)	0 %
8,1 to 12 mg (n = 6)	–0.01 (–0.02 to 0.01)	38.19 (5, < 0.00001)	87 %
PDI			
All Studies (n = 11)	0 (–0,03 to 0,02)	283.42 (10, < 0,00001)	96 %
By duration of the intervention			
4 to 20 weeks (n = 2)	–0.05 (–0.06 to –0.04)	0.40 (1, 0,52)	0 %
> 20 weeks (n = 9)	0.01 (–0.01 to 0.03)	76.42 (8, < 0,00001)	90 %
By dose			
1 to 4 mg (n = 2)	–0.02 (–0.08 to 0.03)	1.49 (1, 0.22)	33 %
4,1 to 8 mg (n = 3)	–0.01 (–0.02 to 0.01)	1.63 (2, 0.44)	0 %
8,1 to 12 mg (n = 6)	0 (–0.03 to 0.04)	268.60 (5, < 0,00001)	98 %
By Nutritional situation			
Healthy (n = 3)	0 (–0.02 to 0.02)	4.36 (2, 0.11)	54 %
Poor nutritional situation (n = 8)	0 (–0.03 to 0.03)	274.11 (7, < 0,00001)	97 %
By Risk of Bias			
Low (n = 6)	0 (–0.03 to 0.04)	268.60 (5, < 0,00001)	98 %
Moderate (n = 2)	–0.01 (–0.03 to 0.01)	1.26 (1, 0,26)	21 %
High (n = 3)	–0.01 (–0.04 to 0.02)	2.51 (2, 0,29)	20 %

I^2 Index measures the extent of the heterogeneity

Table V: Pooled beta (95 % confidence intervals) in mental and motor development according to the intervention group. Sensitivity Analyses.

	Pooled estimates (β)	Chi² (d.f., P)	I²
MDI			
All studies (n = 11)	–0.01 (–0.02 to 0)	55.73 (10, < 0.00001)	82 %
All Studies excluding (n = 1)	–0.01 (–0.02 to 0)	43.33 (9, < 0.00001)	79 %
Ashworth et al. 1998 b	–0.04 (–0.11 to 0.04)		
PDI			
All studies (n = 11)	0 (–0.03 to 0.02)	283.42 (10, < 0.00001)	96 %
All Studies excluding (n = 2)	0 (–0.02 to 0.02)	278.70 (8, < 0.00001)	97 %
Ashworth et al. 1998 a	–0.05 (–0.10 to 0.01)		
Ashworth et al. 1998 b	0.01 (–0.07 to 0.09)		

I^2 Index measures the extent of the heterogeneity

Table IV shows the results after stratifying the sample according to the effect modifiers identified in the meta-regression. After stratifying by dose of supplementation, the pooled β for MDI still showed evidence of heterogeneity (I^2= 87 %) for high doses of Zn (8.1 to 12 mg/day), but this heterogeneity disappeared within some strata such as low and medium doses of Zn (1 to 4 mg/day and 4.1 to 8 mg/day). However, there was no significant effect on Zn supplementation at these dosages.

In the case of the pooled β for PDI, the heterogeneity disappeared when we grouped the studies with an intervention period of 4 to 20 weeks, with low and medium doses of Zn (1 to 4 mg/day and 4.1 to 8 mg/day) and in studies with moderate or high risk of bias. Heterogeneity was maintained for the following studies: for >20 weeks of intervention (I^2 = 90 %); for a high dose of Zn (8.1 to 12 mg/day) (I^2 = 98 %), for healthy and poor nutritional status (I^2 = 54 and 97 %), and for low bias risk (I^2 = 98 %).

Zn supplementation showed a negative, weak and significant effect on PDI score in those studies with a length of 4 to 20 weeks (β= –0.05; CI 95 % –0.06 to –0.04).

The results of the sensitivity analyses are shown in Table V. We did not find a significant association between Zn intake and MDI and between Zn intake and PDI.

The study by Ashworth *et al.* (b) was considered as an outlier in the analysis of MDI because the limits of beta were very wide (from –0.11 to 0.04). When we excluded this study, the null association previously seen persisted, as did the degree of heterogeneity. The same was true when the Ashworth *et al.* studies (a) and (b) were removed from the PDI meta-analysis.

Due to the high heterogeneity found in most of the analyses, we decided to avoid calculating the dose-response relationship between Zn intake and mental and motor development.

Discussion

Our results indicated that Zn supplementation does not appear to influence mental or psychomotor neurodevelopment of infants. Only a negative effect on infant motor development (PDI) was found when the time of Zn supplementation ranged between 4 to 20 weeks. Nonetheless, interpretation of these results should be carefully considered for a number of reasons. First of all, the magnitude of effect was rather small. Also, it is well acknowledged that when many statistical comparisons are carried out, one or more might reach significance due to chance alone [18]. Furthermore, significant heterogeneity existed in the methods across pooled studies, and in general, after stratifying on several factors, heterogeneity persisted through various analyses and finally the few number of articles included in each sub-analysis. Anyway, sometimes the most important mission of a meta-analysis is not to create a unifying synthesis. On the contrary, occasionally, the meta-analysis shows and highlights the differences between the results of the studies available, in order to clarify that it is not possible to combine what is very different [19].

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 programs, the reliability of reached conclusions depend 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 [4]. While strict systematic review protocols were followed adhering to EURRECA's quality standards [5], an assessment of the risk of bias of included studies revealed that the majority (n = 3) had a high-to-moderate risk of bias.

For the interpretation of our results, one should bear in mind that sample size was considerably small. Information on the mean Zn intake and mental and motor development was available only from 5 studies, which limited the statistical power of the analyses to examine the relation between mental and motor development responses to Zn supplementation. Thus, the lack of significance in the current analysis may be due to the limited amount of available information. Moreover, we have to assume that maybe effect size could be rather unstable because of the inclusion of only 5 articles in the meta-analysis. More studies will be needed to resolve this issue.

It is also important to keep in mind that all of the included studies in this meta-analysis used Bayley Scales for the measurement of mental and motor development. This is the most commonly used instrument to test psychomotor development. Although the Bayley Scales of Infant Development were standardized in the United States, they have been used in many other countries, mostly in research focused on nutrition and development. It is possible that slight differences in administration of the test occur in different countries. Additionally, infants in different cultures are exposed to very different environments, which would be expected to affect their development. Therefore, it is difficult to interpret the differences infant's scores

across cultures [20]. Hence, although all the included studies used the same instrument for measuring mental and motor development, this might be a source of heterogeneity by itself and another factor contributing to the lack of effect of Zn supplementation upon mental and motor development.

Some confounders should be considered in evaluating the effect of Zn intake on mental and motor development in infancy. Those confounders include low birth-weight, breastfeeding, protein energy malnutrition, infectious morbidity, poverty, and social deprivation. For instance, in our meta-analysis, Ashworth *et al.* [7] performed an adjustment of initial differences, whilst others [8, 10, 11] assumed no initial differences or failed to mention anything regarding this matter [9]. However, all the studies included in our meta-analysis are RCTs. So, if we assume that the randomization has been correct, none of these factors should bias the results.

In some studies the difference in mental and motor development scores for Zn supplementation was rather small [10]. However the infants included in their study were undernourished and required more nutrients other than Zn. The same observation could be made regarding Ashworth *et al.* and Jimenez *et al.* conducted in low birth weight (LBW) infants. However, the first group of authors concluded that the duration of their intervention was insufficient and in the second study they found some benefit of Zn on PDI but not in MDI. It is very important to consider the duration of the intervention in studies conducted in LBW infants because the low weight and the immaturity associated with premature infants requires adjustment of gestational age with chronological age for proper assessment of catch-up growth [21]. The study by Jimenez *et al.* [9], also conducted in LBW infants showed an increment in development after following Zn supplementation. This increment was more evident in motor than in mental development, especially from 3 to 6 months of age. However, these authors do not assure that time of supplementation could be responsible for the reported effect. New studies are required to analyze the long-term effects on psychomotor development of Zn deficiency.

Other factors to consider are the influence of social context, the caregiving environment, and developmental stimulation, all of which play very important roles in the evolution of cognitive development in infants [3]. It is clear that all these factors play a relevant role for neuropsychological functioning, activity and motor development, and all of the studies included in our meta-analysis account for these variables, except Jimenez *et al.* [9].

In infants of 6 to 8 months of age, special considerations need to be acknowledged since they are particularly vulnerable to suffering from Zn deficiency while they are in the feeding transition period, while gradual introduction of solids is still taking place. This problem is particularly relevant to underdeveloped countries, but there is growing evidence that suggests that this is present also in adequately nourished populations [22]. More studies that examine the response to Zn supplementation or fortification in populations that are Zn deficient in the absence of poverty need to be conducted to clarify the relationship between Zn and mental and motor development. Zn deficiency poses a serious public health problem that compromises the adequate development of millions of children worldwide in both developing and developed countries [23].

Our results were in accordance with those reported by Black [24]. However this author carried out a narrative review. Thus, our study is a new step in the knowledge of the association between Zn supplementation and mental and motor development in infants.

In conclusion, no association was found between Zn supplementation and mental and motor development in infants. The magnitude of effect was small in all cases. Based on this limited group of studies and their heterogeneity, we found insufficient information to suggest that Zn supplementation has a positive effect on mental and motor development of infants. Further standardized research is urgently needed to clarify the role of Zn supplementation upon infant mental and motor development, mainly in Western populations.

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Conflicts of Interest

Authors declare no conflicts of interest.

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Mariela Nissensohn

Department of Clinical Sciences
University of Las Palmas de Gran Canaria
Las Palmas de Gran Canaria
Spain
Tel.: +34 928 459816
Fax: +34 928 451416
mnissensohn@acciones.ulpgc.es