



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

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Review

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

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31 **Abstract**

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

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53 Keywords: EURRECA, zinc intake, serum/plasma Zn status, infants

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66 Introduction

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68 Zinc (Zn) is an essential nutrient, present in all body tissues and fluids. The biologic role of Zn is
69 now recognized in the structure and function of proteins, including more than 300 enzymes,
70 transcription factors, hormonal receptor sites, and biologic membranes. Zn has numerous central
71 roles in DNA and RNA metabolism (MacDonald 2000), and it is involved in signal transduction,
72 gene expression, and apoptosis. Zn enzymes are involved in nucleic acid metabolism and cellular
73 proliferation, differentiation, and growth (Chesters 1978).

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

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

89

90 Infants have a relatively high requirement of Zn per unit body weight during a sensitive period of
91 rapid growth and development (Hermoso et al. 2010). Recommendations for Zn intake during
92 infancy vary widely across Europe, ranging from 1 mg/day up to 5 mg/day (Hermoso et al. 2010).
93 The EURRECA project attempts to consolidate the basis for the definition of micronutrient
94 requirements across Europe, taking into account relationships among intake, status and health
95 outcomes, in order to harmonise these recommendations (Ashwell et al. 2008). This paper presents
96 a systematic review of the data from all available randomized controlled trials (RCTs) meeting
97 EURRECA's quality standard (Matthys et al. 2011), which investigated Zn intake and biomarkers

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

100

101 **Materials and Methods**

102 **Search strategy**

103

104 This research was conducted within the framework of the European Micronutrient
105 Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the
106 micronutrient requirements for optimal health in European populations (www.eurreca.org). This
107 review was part of a wider review process to identify studies assessing the effect of Zn intake on
108 different outcomes (biomarkers of Zn status and health outcomes). The wider searches were
109 performed of literature published up to and including February 2010, and an updated search was
110 carried out in January 2013. The databases MEDLINE, EMBASE and Cochrane using search terms
111 for “study designs in humans” and “zinc” and “intake”. Both indexing and text terms were used and
112 languages included were restricted to those spoken in the EURRECA Network (English, Dutch,
113 French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian.). The Ovid
114 MEDLINE search strategy can be found in Table 1. Reference lists of retrieved articles and
115 published literature reviews were also checked for relevant studies. The procedure for the
116 identification, selection of articles and data extraction is illustrated in Figure 1.

117 **Selection of articles**

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

124 Exclusion criteria applied were: studies conducted in animals; combined interventions e.g. >1
125 micronutrient or micronutrient + lifestyle intervention which did not study the effect of the
126 micronutrient separately; non primary studies (e.g. letters & narrative literature reviews); duplicate
127 publications; studies where the Zn intake – status relationship was not reported or biomarkers of Zn
128 other than serum / plasma Zn were used.

129 Briefly, titles and abstracts of the 10% of the library were screened in duplicate for eligibility by
130 two reviewers and any discrepancies were discussed and resolved before screening the remaining
131 references. Only when both reviewers agreed that titles and abstracts met the inclusion criteria were
132 the articles included. When a title and abstract could not be included with certainty, the full text of
133 the article was obtained and then further evaluated. The remaining 90% was distributed among the
134 two reviewers in even parts. Following the initial screening process, full-text articles were obtained.
135 Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-
136 analysis if they were: randomised controlled trials; had an intervention duration of at least 2 weeks;
137 and reported baseline data for all outcome measures. Non-randomised controlled trials, uncontrolled
138 trials or trials reporting insufficient or unclear data were excluded. Data were extracted from each
139 study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA).

140

141 **Data synthesis**

142 When Zn status in serum/plasma was measured at different time points within the same population,
143 we used the measures as different estimations (Bates et al. 1993; Makonnen et al. 2003 I/II). One
144 study reported data from the total of infants included, between males and females separately, and
145 according to age (<11 months and > 11 months) (Sazawal et al. 1996; 2004) and it was treated as
146 five estimations within the meta-analysis. One study reported data from two groups of infants
147 (stunted and non stunted) and these were treated as two different estimations (Umeta et al. 2000).
148 One study reported data from two groups according to the form of Zn supplementation (tablets or
149 liquid) and these were treated as two estimations within the meta analysis (Wessells et al. 2012). Of
150 the selected studies, two RCTs were companion papers (Makonnen et al. 2003 I; Sazawal et al.
151 2004). If dietary intake of Zn (in addition to the intervention) was not reported in the RCTs, we
152 imputed a value of 1.3 mg/day, the mean dietary intake level of the RCTs that did report dietary Zn
153 intake. As mean baseline serum/plasma Zn concentrations were infrequently reported in the RCTs,
154 most of the RCTs assumed no differences in baseline serum/plasma Zn concentrations (n= 12).
155 Only one study, Bates et al. 1993, failed to report anything regarding baseline serum /plasma Zn
156 concentrations.

157

158 *Exposure and outcome and other covariates assessment:*

159

160 The influence of Zn intake on serum/plasma Zn concentrations was considered in the overall meta-
161 analysis. Other variables were also taken into account as possible effect modifiers. We considered
162 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

163 3 weeks, 4 to 20 weeks, and > 20 weeks), nutritional situation (healthy, nutritionally at risk, and
164 poor nutritional status) and risk of bias (low, moderate or high).

165

166 *Assessment of nutritional situation in included studies*

167

168 Nutritionally at risk was defined as infants who lived in low income families with a low
169 socioeconomic situation and poor nutritional status was defined as infants with protein energy
170 malnutrition (PEM) but without congenital abnormalities or cerebral palsy or heart disease or
171 infants with low birth weight during their first year. PEM occurs characteristically in children under
172 5 years of age in circumstances where the diet is poor in protein, calories and micronutrients, and
173 insufficient to satisfy the body's nutritional needs. It remains one of the most common causes of
174 morbidity and mortality among children worldwide (WHO, 1999).

175

176 *Assessment of risk of bias in included studies*

177 Risk of bias was assessed in order to evaluate the quality of the studies included. The following
178 indicators of internal validity specific to the RCT methodology were collected during data
179 extraction: 1) method of sequence generation and 2) adequate allocation, 3) blinding, 4) number of
180 participants at start, dropouts and dropout reasons, 5) outcome data complete, 6) funder adequate 7)
181 other potential funding bias . Based on these indicators, two reviewers assessed the overall risk of
182 bias. Disagreements were resolved by discussion. The criteria for judging these indicators were
183 adapted from the Cochrane Handbook for Systematic Reviews (Higgins & Green 2009) (Table 2).

184

185 **Statistical analyses**

186

187 Mean and standard deviation (SD) or standard errors (SE) of the outcome (serum/plasma Zn) were
188 assessed. From the mean and SD of each study beta values (β) and their SE were calculated because
189 the statistical model that we used to estimate the relation between Zn intake (x-variable) and
190 serum/plasma Zn (y- variable) is based on the assumption that this intake-serum/plasma Zn status
191 curve is a logarithmic function and that both intake and serum/plasma Zn status follow a log-normal
192 distribution (the natural logarithm of intake and serum/plasma Zn status have a normal distribution).

193 Thus, the expected value of the serum/plasma Zn status score is expressed as:

194 $\mu_y = \beta * \mu_x + \text{intercept}$, where μ_y represents the mean of the natural logarithm of the y-variable (=
195 serum/plasma Zn status score), β represents the regression coefficient, and μ_x represents the mean
196 of the natural logarithm of the x-variable (= Zn intake). The method used to systematically review

197 differences was a formal meta-analysis (Greenland 1998). A random-effects model was considered
198 to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's
199 (DerSimonian & Laird 1986) to pool the estimates of betas across studies. Under this model, the
200 pooled effect was the beta in the status parameter (serum / plasma), for an increment of 1 unit in Zn
201 intake. A pooled beta estimate was calculated as a weighted average of the beta reported in each
202 study.

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

$$204 \beta_{pooled} = \frac{\sum \beta_i w_i}{\sum w_i}$$

205 where β_{pooled} is the pooled estimate of the beta in status parameters; the weight (w_i) of each study
206 was computed as:

$$207 w_i = 1 / (V_i + \tau^2)$$

208 where V is the variance of each study and τ^2 is the inter study variance.

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

$$210 95\% \text{ CI} = \beta_{pooled} \pm (1.96 \times \text{SE}_{pooled})$$

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

212

213 A test of heterogeneity was calculated, estimating Q statistics, which follows a chi-square
214 distribution with degrees of freedom $n-1$, n being the number of studies included in the analysis.

215 The I^2 Index measures the extent of the heterogeneity. A low P value for this statistic (lower than
216 0.05) indicates the presence of heterogeneity, which somewhat compromises the validity of the
217 pooled estimates (Takkouche et al. 1999). Because significant heterogeneity was clearly evident in

218 the pooled beta estimates for all studies combined in each outcome, we evaluated potential sources
219 of heterogeneity by linear meta-regressions (Greenland 1998). We fitted a meta-regression using the
220 duration of the intervention, the doses of Zn intake, the risk of bias, and the nutritional situation as
221 independent variables. The betas of the different status parameters according to Zn intake were used
222 as the dependent variable. Statistical differences in multivariate adjusted mean beta values between
223 each possible heterogeneity sources were determined by ANCOVA. Additionally we carried out
224 additional meta-analyses by subgroups considering only those groups which provided significant
225 values in the meta-regression. Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review
226 Manager 5.1, were used to conduct the statistical analyses.

227

228 **Results**

229

230 Five thousand five hundred articles were identified in the initial search strategy. After applying the
231 exclusion / inclusion criteria, 344 articles from the search appeared to be potentially relevant. After

232 applying the additional eligibility criteria and grouping the studies by outcome, 9 randomized
233 controlled trials (17 estimations) were selected (Walravens et al. 1989; Bates et al. 1993; Sazawal et
234 al. 1996, 2004; Umeta et al. 2000; Osendarp et al. 2002; Lind et al. 2003; Makonnen et al. 2003;
235 Wasantwisut et al. 2006; Chang et al 2010). The 2013 update of the original search identified 4
236 additional articles (Berger et al. 2006; Mazariegos et al. 2010; Ba Lo et al. 2011; Wessells et al.
237 2012), providing a total of 13 articles (22 estimates) for meta-analysis (Figure 1).

238

239 Descriptive characteristics of the studies included in the meta-analysis are presented in Table 2. Of
240 the 13 studies included, only six comply strictly with the age infants (0 to 12 months) (Umeta et al.
241 2000; Osendarp et al. 2002; Lind et al. 2003; Berger et al. 2006; Wasantwisut et al. 2006;
242 Mazariegos et al. 2010). The other seven studies included this age among their sample, but did not
243 clarify how many are actually aged 0 to 12 months (Walravens et al. 1989; Bates et al. 1993;
244 Sazawal et al. 1996, 2004; Makonnen et al. 2003; Chang et al 2010; Ba Lo et al. 2011; Wessells et
245 al. 2012). None of the ages extended beyond 27 months, except Makonnen et al. 2003 which
246 included children up to 5 years. Thus the age range of the studies included was from 3 weeks to 60
247 months.

248

249 Six studies were conducted in Asia, one in North America, one in Latin America and the Caribbean
250 and five in Africa. The duration of the interventions ranged from 2 to 24 weeks. Doses of Zn intake
251 ranged from 2.5 to 20 mg per day. The nutritional situation of infants also varied between studies:
252 six studies were conducted in healthy infants (Bates et al. 1993; Umeta et al. 2000; Osendarp et al.
253 2002; Lind et al. 2003; Wasantwisut et al. 2006; Wessells et al. 2012), six studies were conducted
254 on infants who were nutritionally at risk (Walravens et al. 1989; Sazawal et al. 1996, 2004; Berger
255 et al. 2006; Chang et al 2010; Mazariegos et al. 2010; Ba Lo et al. 2011;), and one study was
256 conducted on infants with poor nutritional status (Makonnen et al. 2003).

257 Table 3 summaries the internal validity of the included studies, assessed as described in the data
258 synthesis section. The risk of bias was high in two studies (Bates et al. 1993; Umeta et al. 2000),
259 five had a moderate risk (Sazawal et al. 1996; 2004; Osendarp et al. 2002; Makonnen et al. 2003;
260 Berger et al. 2006; Wessells et al. 2012) and six had a low risk of bias (Walravens et al. 1989; Lind
261 et al 2003; Wasantwisut et al. 2006; Chang et al 2010; Mazariegos et al. 2010; Ba Lo et al. 2011).

262

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

266 1989; Bates et al. 1993; Makonnen et al. 2003; Wessells et al. 2012 (a) Tablets group). In order to
267 summarize the results we performed a formal meta-analysis (Figure 2).

268

269 Differences between serum/plasma Zn status measured according to the intervention group in each
270 particular study and in the pooled analysis are shown in Figure 2. The pooled β was 0.09 (95%CI
271 0.05, 0.12). However, a substantial heterogeneity was present in the analyses (I^2 for status = 98%).
272 In order to investigate which variables may be potential effect modifiers, we performed a meta-
273 regression (Table 4). The effect of Zn intake on serum/plasma Zn status changed depending on the
274 duration of the intervention, the dose of supplementation and the nutritional situation (p
275 ANCOVA= 0.054; <0.001 and <0.007) respectively. After stratifying the sample according to the
276 effect modifiers identified in the meta-regression (Table 5) the results by duration of intervention
277 showed no significant effect when the duration was short (1 to 3 weeks) (β = 0.02; CI 95% -0.03 to
278 0.07). Nevertheless, a positive effect was shown when Zn intake was provided over medium (4 to
279 20 weeks) (β = 0.09; CI 95% 0.06 to 0.13) and long periods of time (>20 weeks) (β = 0.12; CI 95%
280 0.07 to 0.16). However these pooled β still revealed high evidence of statistically significant
281 heterogeneity (I^2 = 91 and 96 %) respectively. When doses of Zn ranged from 4.1 to 8 mg/day, there
282 was no significant effect of Zn intake on the serum/plasma Zn; whereas a positive effect was seen
283 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
284 mg/day we found no effect. However high evidence of heterogeneity was observed (I^2 = from 77 to
285 96 %). When studies were categorised by nutritional situation, those studies based on healthy
286 infants and on infants at nutritional risk reported a positive association between Zn intake and
287 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)
288 respectively. However, no association was found when the nutritional situation was poor (β = 0.05;
289 CI 95% -0.02 to 0.12). Once again, the pooled β still showed high evidence of heterogeneity (I^2 =
290 from 95 to 99 %). Due to the high heterogeneity found in all the analyses, we decided to avoid
291 calculating the dose-response relationship between Zn intake and serum/plasma Zn status.

292

293 **Discussion**

294

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

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

302

303 The interpretation of these results should be carefully considered for a number of reasons. First, the
304 number of studies that were eligible for inclusion in this meta-analysis was small, which limited the
305 statistical power of the analyses to examine the relation between status responses to Zn
306 supplementation. Thus, the small effect size we found may be explained by the limited amount of
307 available information. Also, it is well acknowledged that when many statistical comparisons are
308 carried out, one or more might reach significance due to chance alone (Bland & Altman 1995). It is
309 also important to consider the scientific quality of included studies. Although meta-analyses are
310 increasingly used to consolidate results from multiple studies of the same topic and to develop
311 evidence-based policies for clinical practice and public health programmes, the reliability of
312 reached conclusions depend on the methodological quality of the original studies, the
313 appropriateness of the study inclusion criteria, and the thoroughness of the review and synthesis of
314 information (Brown et al. 2002). While strict systematic review protocols were followed adhering
315 to EURRECA's quality standards (Matthys et al 2011), an assessment of the risk of bias of included
316 studies revealed that the majority (n=7) had a high to moderate risk of bias.

317

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

326

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

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

338

339 Some confounders should be considered in evaluating the effect of Zn intake on infant
340 serum/plasma Zn status. Those confounders include low birth weight, breastfeeding, protein energy
341 malnutrition, poverty and social deprivation. The pre-existing serum/plasma Zn status of the study
342 subjects, the content and bioavailability of Zn in the local diets, and the incidence of common
343 infections that can affect individual’s serum/plasma Zn status are others important confounders to
344 take into account. Moreover, methodological aspects of these studies, such as variations in the dose,
345 chemical form, method of administration of Zn and duration of supplementation, may have
346 influenced their results (Brown et al. 2002). However, with the exception of Bates et al (1993), all
347 the RCTs included in the meta-analysis assumed no baseline differences in serum/plasma Zn. As all
348 the studies included in our meta-analysis are RCTs we may assume that the randomization has been
349 correct and these factors should not bias the results.

350

351 Age of the study populations considered in this meta-analysis was another important point. We
352 believe that there was no reason to exclude any study that did not adhere exclusively to the group of
353 0 to 12 months of age. For this reason, we took into account all the studies which included this age
354 group in the study, even if they were not analysed according to their age group (Walravens et al.
355 1989; Bates et al. 1993; Makonnen et al. 2003; Sazawal et al. 2004, 1996; Chang et al. 2010; Ba Lo
356 et al. 2011; Wessells et al. 2012) and assumed the consequences of this possible bias. Another
357 confounding factor that might explain the inconsistency in our findings is that serum Zn
358 concentrations vary according to the time of day, proximity of previously consumed meals, and
359 occurrence of recent physical activity or other forms of stress, fluctuating by as much as 20%
360 during a 24-hour period (Hambidge et al. 1989). The diurnal variation in circulating Zn
361 concentration is largely a result of metabolic changes after meal consumption, although some
362 variation may occur as a result of normal circadian variation in metabolism (Guillard et al. 1979;
363 Wallock et al. 1993). Meal consumption results in a decrease in serum/plasma Zn concentrations,
364 which add up following repeated meals (Goode 1991; Wallock et al. 1993), whereas overnight and
365 daytime fasting result in increased circulating Zn concentrations (Wallock et al. 1993). Of the
366 studies included in our meta-analyses, those conducted by Walravens et al. 1989, Umeta et al. 2000,
367 Osendarp et al. 2002, Berger et al. 2006, Wasantwisut et al. 2006, Ba lo et al. 2011 and Wessells et
368 al. 2012 reported the time of the day when the blood samples were collected (during the morning).

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

371

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

380

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

393

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

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404

405

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Figure 1: Flow diagram for the systematic review.

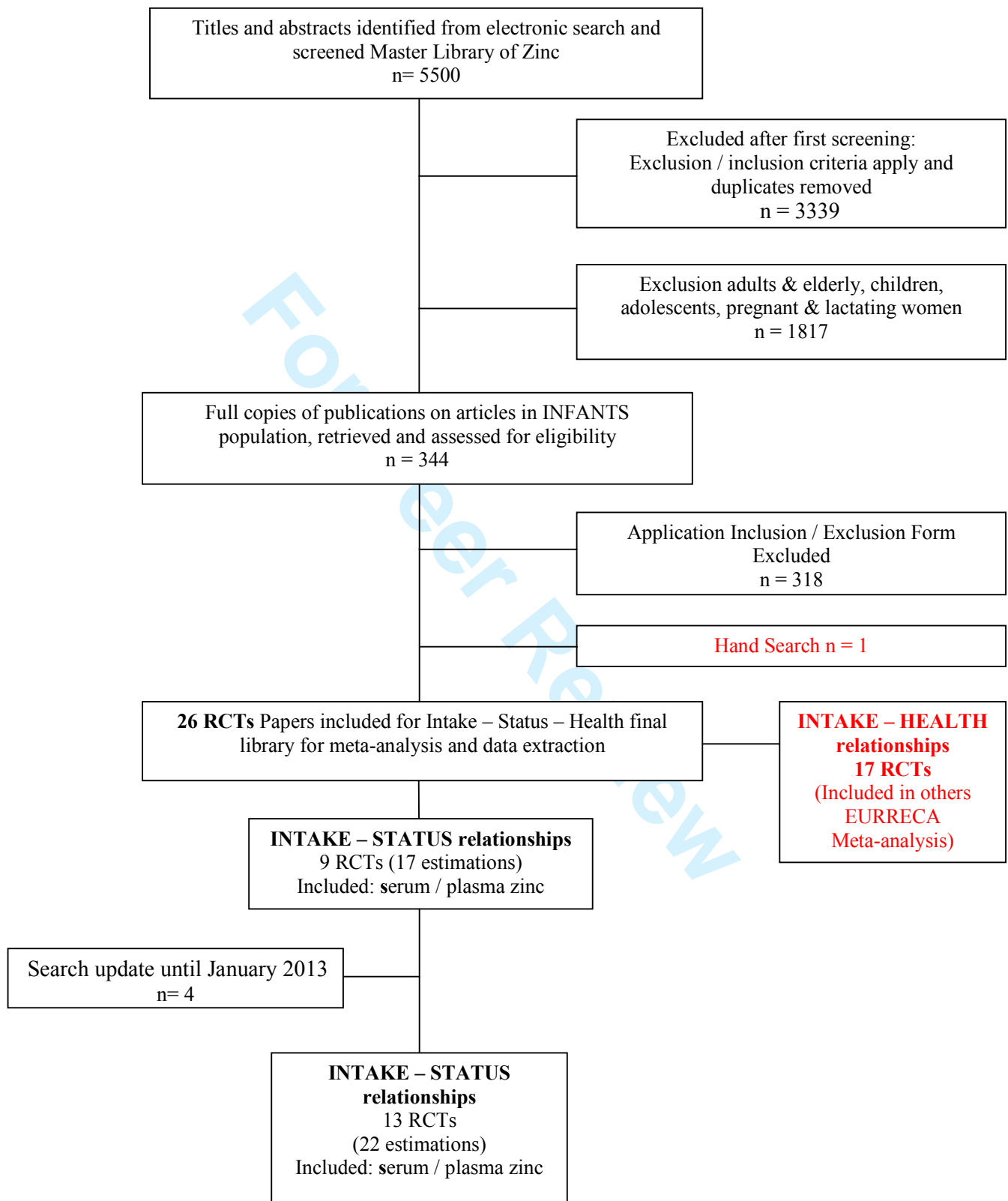


Figure 2: Forest Plot of RCTs evaluating the effect of zinc intake on serum/plasma zinc status in infants

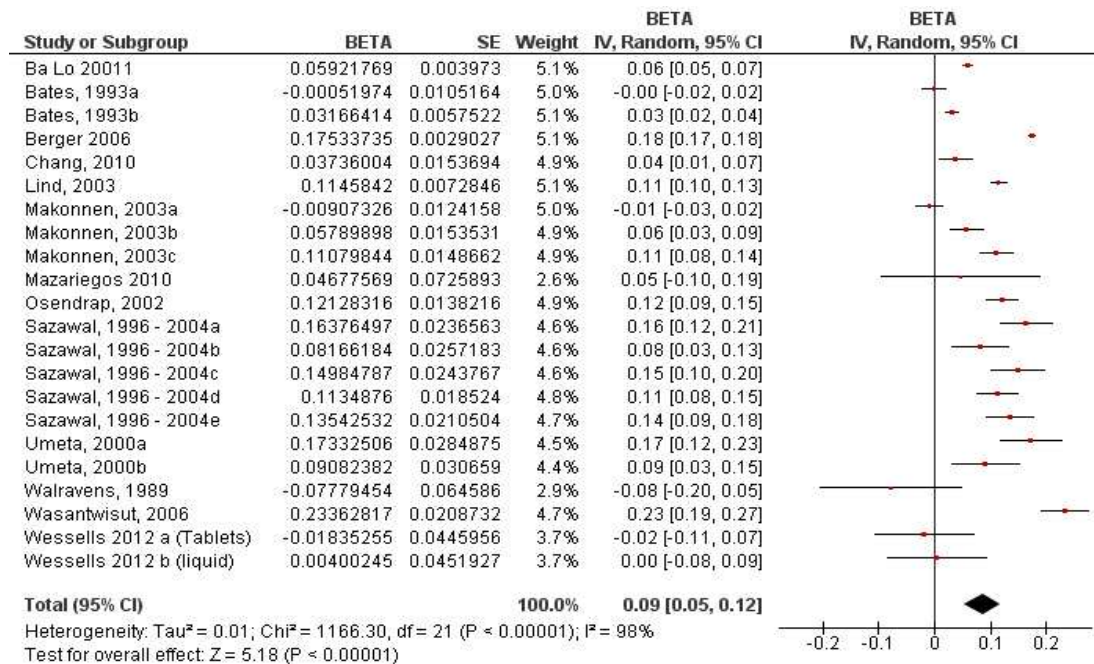


Table 1: Search strategy: MEDLINE February 2010(MEDLINE home page. Available online: <http://www.ncbi.nlm.nih.gov/pubmed/>)

<i>No.</i>	<i>Search term</i>	<i>Results</i>
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 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/	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

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) (b)	1993	Gambia	5.7 to 27 months	30	28	20 mg	2 weeks	Status (plasma)	Healthy	High risk
				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) Makonnen (b) (c)	2003	Lesotho	6 to 60 months	142	121	10 mg	4 weeks	Status (serum)	Poor nutritional status	Moderate risk
				141	119		8 weeks			
				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) (b) (c) (d) (e)	1996-	India	6 to 35 months	223	224	10 mg	16 weeks	Status (plasma)	Nutritionally at risk	Moderate risk
	2004 ³		6 to 11 months	78	78					
	> 11 months		69	73						
	Females		115	106						
	Males		108	118						

(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

¹Zn: Zinc group / ¹C: 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)

³Companion paper

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

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				
<i>By duration of the intervention</i>				
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
<i>By Dose</i>				
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
<i>By Nutritional situation</i>				
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
<i>By Risk of Bias</i>				
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

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%
<i>By duration of the intervention</i>			
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%
<i>By dose</i>			
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%
<i>By Nutritional Situation</i>			
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