The relationship between zinc intake and serum/plasma zinc concentration in adults. A systematic review and dose-response meta-analysis by the EURRECA Network

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Short title: Zinc intake and plasma zinc concentration

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Abstract

Dietary zinc recommendations vary widely across Europe due to the heterogeneity of approaches used by expert panels. Under the EURRECA consortium a protocol was designed to systematically review and undertake meta-analyses of research data to create a database that includes "best practice" guidelines which can be used as a resource by future panels when setting micronutrient recommendations. As part of this process, the objective of the present study was to undertake a systematic review and meta-analysis of previously published data describing the relationship between zinc intake and status in adults. Searches were performed of literature published up to February 2010 using MEDLINE, Embase, and Cochrane Library. Data extracted included population characteristics, dose of zinc, duration of study, dietary intake of zinc, and mean concentration of zinc in plasma or serum at the end of the intervention period. An intake-status regression coefficient ($\hat{\beta}$) was estimated for each individual study, and pooled meta-analysis undertaken. The overall pooled $\hat{\beta}$ for zinc supplementation on serum/plasma zinc concentrations from RCTs and observational studies was 0.08 (95% CI 0.05, 0.11; $p<0.0001$; I^2 84.5%). An overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0.08}$ = 1.06), which is 6%. Whether the dose-response relationship, as provided in this paper, could be used as either qualitative or quantitative evidence to substantiate the daily zinc intake dose necessary to achieve normal or optimal levels of biomarkers for zinc status, remains a matter of discussion.

Introduction

Dietary zinc recommendations vary widely across Europe due to the heterogeneity of approaches used by expert panels [1]. There is a need for a harmonised approach that is transparent and based on the best quality data and methods available. Traditionally, the factorial approach is used in the determination of zinc requirements. This method seeks to estimate the zinc intake required to meet physiological requirements for growth, metabolism and tissue repair while replacing obligatory losses. An alternative approach is to examine the dose-response relationship between intake and biomarkers of status and also between intake and health outcomes. This information could then be integrated using a mathematical model to provide an insight into the level of zinc intake required for optimal health based on a range of parameters and indices of health that are known to be dependent upon dietary zinc intake [2]. To this end, the members of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence have undertaken a series of systematic reviews of zinc-intake-status relationships, according to rigorous protocols defined by consortium members and external experts [2] . This paper presents the results of the systematic review and meta-analysis of the dose response relationship between dietary zinc intake and zinc status using novel methodology developed by members the EURRECA consortium.

The assessment of zinc status is notoriously problematic for zinc, as a sensitive, specific biomarker for zinc has not yet been identified [3]. A systematic review and meta-analysis of biomarkers of zinc status was undertaken in 2009 [4]. For many putative biomarkers (such as the zinc concentrations found in the cellular components of whole blood) there were insufficient data to arrive at a definitive conclusion regarding their efficacy as a biomarker of zinc status, however plasma (or serum) zinc concentration was responsive to both zinc supplementation and zinc depletion and is the most widely reported biomarker for zinc. Hair and urine zinc concentrations were also considered to be potentially useful biomarkers in response to zinc supplementation.

The purpose of this study was to systematically and quantitatively assess the dose response relationships relevant to deriving zinc recommendations based on intervention studies, cohort (nested case control) studies and cross-sectional studies. The specific questions to be addressed were; what is the effect of intake on indicators of exposure or body stores (i.e. biomarkers)? What factors affect this relationship?

The data used in this meta-analysis were extracted from published studies (RCTs, prospective cohort studies, nested case-control studies and cross-sectional), performed in healthy adult and elderly populations, reporting the relationship between zinc status (plasma or serum zinc, hair or urine zinc concentration) and intake from supplements, fortified diets or natural food diets.

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 research was part of a wider review process to identify studies assessing the effect of zinc intake on different outcomes (biomarkers of zinc status and health outcomes). The wider searches were performed of literature published up to and including February 2010 using Ovid MEDLINE, Embase (Ovid), and the Cochrane Library (CENTRAL) using search terms for ('study designs in humans') AND (zinc) AND (intake OR status). 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 full 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. Authors were contacted to request missing data or clarify methods or results. The search process is illustrated in Figure 1.

Criteria for the consideration of studies for this review

Included studies were RCTs, prospective cohort studies, nested case-control studies and cross-sectional studies in healthy human populations that supplied zinc supplementation (RCTs) or measured dietary zinc intake with either a validated food frequency questionnaire, a dietary history method, a 24-hour recall method for at least 3 days, or a food record/diary for at least 3 days (observational studies). Studies had to be conducted in apparently healthy adult and elderly (human) populations aged ≥18 years and supplied zinc supplementation either as capsules or part of a fortified meal. If supplemental zinc was provided as a component of a fortified meal, studies were only considered acceptable if zinc was the only

constituent that was different between treatment groups. Biomarkers of zinc status included plasma/serum, urine and hair zinc concentrations. Only studies that reported sufficient data or had sufficient data obtainable from the authors to estimate $\hat{\beta}$ and SE($\hat{\beta}$) for the assumed linear relation on the $\log_e$-$\log_e$ scale were included. Studies were excluded if they were a group RCT (community trial), or were commentaries, reviews, or duplicate publications from the same study. Studies were excluded if adults were hospitalised, had a chronic disease or if supplemental zinc was provided for less than 2 weeks.

Selection of articles

Of 4719 identified articles in the wider search on zinc intake, status and priority health outcomes in all populations, 2557 were excluded based upon screening of the title and abstract. Two independent reviewers screened 10% of the abstracts in duplicate and any discrepancies were discussed before screening the remaining references. Following subdivision into appropriate population groups the full texts of the 1231 manuscripts were assessed to determine inclusion and exclusion by two independent reviewers and disagreements rectified through discussion. 1147 studies were excluded because they did not meet the inclusion criteria. Of the remaining 84 studies, 54 studies were excluded as they related either zinc intake or status directly to a health endpoint, but they had not investigated the relationship between zinc intake and zinc related to biomarkers. A further 17 studies were excluded from the meta-analysis because study participants were not healthy, insufficient data was reported, data was duplicated, or the dosage and duration was unclear. For the purpose of this meta-analysis, 10 RCTs and 3 observational studies remained. The characteristics of the included studies are presented in Table 2 and Table 3 respectively.

Data extraction

For each of the identified manuscripts, data was extracted independently by two reviewers into a standardized database. Extracted data included population characteristics, dose of zinc in intervention and placebo supplements, duration of the study, dietary intake of zinc, and mean concentration of zinc in plasma or serum at the end of the intervention period. Serum/plasma zinc concentrations were converted to µmol/L when applicable.

Data synthesis

Two RCTs that reported data for two zinc-treated groups and two control groups were treated as two independent estimates in the analysis [5; 6]. Where RCTs provided outcome data for two or more zinc-treated group, they were included as separate estimates in the meta-analysis [7; 8; 9; 10; 11]. Where zinc status was measured at different time points within the same population only the final measure was used in the analysis [12; 13]. One observational study reported data from males and females and these were treated as two estimates in the meta-anaysis [14]. If dietary intake of zinc (in addition to the intervention) was not reported in the RCTs, a value of 9.7 mg/day was imputed, which was the mean dietary intake level of the RCTs that did report dietary zinc intake. As mean baseline serum/plasma zinc concentrations were infrequently reported in the RCTs, the serum/plasma zinc concentrations in the control group were used as a proxy of the baseline serum/plasma zinc concentrations for our analyses.

Statistical analyses

A stratified random effects meta-analysis was conducted using STATA version 11 (College Station, TX), with one subgroup combining the evidence from RCTs and the other subgroup combining the evidence from observational studies. As serum/plasma zinc levels have been reported to decline with age [15], a separate stratified random effects meta-analysis compared zinc intake and status according to age in RCTs (< 55 years and ≥ 55 years). In addition, stratified meta-analyses were also conducted on dose of zinc (<35 mg/day and ≥ 35 mg/day) and trial duration (in weeks). It was not possible to perform a stratified meta-analysis for gender, because most studies included both men and women and data were not available at the individual level.

The transformations used to derive coherent single-study estimates from the available summary statistics per study have been described elsewhere [16] . In short, an intake-status regression coefficient (β) for each individual study was estimated from the mean serum/plasma zinc concentrations, based on the assumption of a linear relation on the $\log_e$-$\log_e$-scale (natural logarithm of intake versus natural logarithm of status). Algebraically deriving an estimate from each study of the regression coefficient ($\hat{\beta}$) and its standard error (SE($\hat{\beta}$)) enabled a comparison of the results from studies with heterogeneously reported associations and effects. The overall pooled $\hat{\beta}$ and SE($\hat{\beta}$) was calculated using random effects meta-analysis, which estimates the between-study variance using the method of DerSimonian and Laird [17]. This was then used to modify the weights used to calculate the summary

estimate. Residual heterogeneity between studies was evaluated using the I^2 statistic. To evaluate potential sources of heterogeneity, the variables study duration, age, gender and zinc dose were added simultaneously to a meta-regression model as continuous variables. The statistical transformations to obtain $\hat{\beta}$'s and SE($\hat{\beta}$)'s were performed using GenStat version 13-SP2 (VSN International Ltd. Hemel Hemptead, UK) and the meta-analysis was performed using STATA version 11.0, with statistical significance defined as $P<0.05$.

Assessment of risk of bias in included studies

In order to assess the quality of the included studies and the risk of bias, indicators of internal validity were collected during data extraction (Table 3). Based on the indicators two independent reviewers assessed the overall risk of bias and disagreements resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook for Systematic Reviews [18].

Results

Twenty estimates of zinc intake and serum/plasma zinc status in 10 RCTs and four estimates in 3 observational studies were eligible for meta-analysis. All studies were published between 1979 and 2010. Although plasma/serum, urine and hair zinc concentrations were included as markers of status in the systematic review protocol, only plasma/serum zinc concentration was reported universally and sufficiently frequently to be used in the meta-analysis. Most studies included, but did not differentiate between, males and females, but three studies included only females [19; 9; 20], two included only males [13; 8] and one provided both male and female data [14]. Studies were conducted in Europe (n=7), North America (n=3), South Asia (n=1), East Asia (n=1) and Australasia (n=1) and ages of participants ranged from 18 to 106 years.

All but one RCTs used a parallel design. Boukaïba and colleagues employed a cross-over RCT design [6]. The RCTs included 1285 participants in total with sample sizes ranging from 5-201. The median duration of the trials was 25 weeks (range 2-52 weeks). In 9 studies zinc was supplemented alone at doses ranging from 15-135.3 mg/day and in 1 study zinc was provided within a multi-micronutrient supplement [12]. Most studies (n=7) provided the zinc supplements in the form of zinc gluconate, but others used zinc sulphate [21], zinc acetate [7], or zinc carnosine [11]. Habitual zinc intakes ranged from 5.4-10.8 mg/day (where data was provided).

The observational studies included 1184 participants in total with sample sizes ranging from 170-500. Zinc intake was measured using a combination of FFQ and 24 hour recall, or 24 hour recall alone and values ranged from 8.6-12.2 mg/day. The meta-analysis of available studies suggested that zinc supplementation was associated with increased serum/plasma zinc concentrations. The estimated effect for zinc supplementation on serum/plasma zinc concentrations from RCTs and observational studies was 0.08 (95% CI 0.05, 0.11; $p<0.0001$; I^2 84.5%) (Fig 2). When data sets were grouped according to study design, only the RCTs showed a significant effect size (0.09 95% CI 0.07, 0.120; $p<0.0001$; I^2 79.1%).

Since a base-*e* logarithmic transformation was applied to the zinc intake and serum/plasma zinc concentration before calculation of the study-specific $\hat{\beta}$'s, the overall $\hat{\beta}$ represents the difference in the $\log_e$transformed predicted value of serum/plasma zinc status for each one-unit difference in the $\log_e$transformed value in zinc intake. Therefore, an overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0.08} = 1.06$), which is 6%. This means that a person with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 6% higher than a person who has a zinc intake of 7 mg/day (Fig 3).

As plasma/serum zinc concentrations have been reported to decline with age [15], a separate subgroup analysis compared zinc intake and status according to age in RCTs (< 55 years and ≥ 55 years). Two studies for which mean serum/plasma zinc values were given for adults whose ages spanned both age groups were excluded from this analysis [12; 11]. A stronger effect size was found in adults aged under 55 years (0.14 95% CI 0.04, 0.24; $p<0.005$; I^2 92.1%) compared to adults aged 55 years and over (0.09 95% CI 0.07, 0.11; $p<0.0001$; I^2 32.8%), although care should be taken with interpreting this finding as the younger age group analysis is based on only three estimates in two studies. Stratifying the analysis for dose of zinc (<35 mg/day and ≥ 35 mg/day) revealed a stronger effect size for a zinc dose ≥ 35mg/d (0.14 95% CI 0.08, 0.21; $p<0.0001$; I^2 85.2%) compared to <35mg/d (0.09 95% CI 0.07, 0.10; $p<0.005$; I^2 27.6%). Similar effect sizes were demonstrated for study duration (0-12 weeks 0.13 CI 0.05, 0.20 I^2 92.4% and > 12 weeks 0.10 CI 0.07, 0.12 I^2 75.8%).

To evaluate potential sources of heterogeneity, the variables duration, age, gender and dose were added simultaneously to a meta-regression model as continuous variables. The analysis revealed that only zinc dose was a statistically significant determinant of the overall

beta. The model explained 50% of between-study variance and the residual variation due to heterogeneity was reduced to 48.2%.

Table 4 summarises the internal validity of the included studies, assessed as described in the methods section. The risk of bias was high in 5 out of the 10 papers [21; 6; 22; 23; 11]. Papers were given a high risk of bias rating due to insufficient information provided on sequence generation and/or allocation, drop-outs and funding bodies.

Discussion

The current study is unique in providing an estimate of the dose-response relationship of zinc intake and serum/plasma zinc concentrations in adults. A meta-analysis of 20 estimates in 10 RCTs and 4 estimates in 3 observational studies found that zinc supplementation produced a statistically significant increase in serum/plasma zinc concentrations and provided an estimate of the dose-response relationship between zinc intake and serum/plasma concentrations. An overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is 6%. In other words, an adult with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 6% higher than a person who has a zinc intake of 7 mg/day. This association was slightly stronger when considering only the RCTs, as no observational studies found a significant association between zinc intake and plasma zinc concentrations. The intake-status regression coefficient for the observational studies is likely to be attenuated by random and intake-related errors in assessing dietary zinc intake [24], whereas in RCTs zinc intake can be considered as fixed at each level of dosage and random errors arise only through assessment of biomarkers.

The studies included in this meta-analysis were different in a number of aspects, such as using various designs, follow-up times, zinc doses, and populations. Therefore, it is no surprise that, when combining these studies in a meta-analysis, a large heterogeneity is observed between the studies (I^2 = 84.5% p=0.0001). This between-study heterogeneity may be caused by methodological factors, such as differences in study population characteristics (age, socio-economic status) or differences in doses of provided zinc (amount, one or more doses per day, study duration). When considering some key variables (study duration, zinc dose, age, and gender) in a meta-regression model, only dose explained some between-study heterogeneity. An individual participant data meta-analysis may have provided a more conclusive explanation of the between-study heterogeneity in this meta-analysis. However,

this type of analysis would involve the input of raw individual participant data provided by the original study investigators for re-analysis and combination in a pooled analysis and as such would be a major undertaking in terms of time, costs, and collaboration. Moreover, an inability to include individual participant data from all relevant studies could introduce selection bias. The meta-analytic approach used in this paper is not an attempt to accurately describe the biological relation between actual zinc intake and zinc concentrations in blood under strict experimental conditions and on an individual level, but rather to simulate a dose-response relationship between zinc intake and status that is useful for surveillance studies with a public health point of view and, as such, deliberately incorporates the differences between dietary assessment methods, laboratory assessment methods and participant characteristics to ensure a broad external validity. Thus, the heterogeneity reflects the lack of standardization of methods and the true heterogeneity between study populations and necessarily enters as uncertainty into the application of such data for public health purposes [25].

To conduct this meta-analysis some assumptions related to the availability of the required data or related to statistical issues had to be made. First, when two or more intervention groups were compared to the same control group (5 RCTs), independence of estimates was assumed. As a consequence bias may have been introduced, by either increasing the estimates of the intervention effect (if the control group values were in fact lower), or decreasing the estimates of the intervention effect (if the control group values were higher). Second, the meta-analysis required transformations of the intake and biomarker data to a common scale, as the studies included in this meta-analyses had different ways of reporting the relation between zinc and serum/plasma zinc concentration . The different ways of reporting by transformation of both the intake and biomarker data were standardized to double $\log_e$-scale, which allowed the derivation of a standardized estimate from each study of the regression coefficient and its standard error as a basis for comparing these heterogeneously reported results. A linear relationship on the double $\log_e$-scale was also assumed. This transformation allowed the pooling of beta values and enable these to be reported as a dose-response relationship between zinc intake and serum/plasma zinc concentrations [16].

The meta-analyses were conducted within the context of the EURRECA project as a means to provide additional evidence for underpinning reference values for zinc intake of populations. This dose-response relationship methodology may be used as either qualitative or quantitative evidence to substantiate the daily zinc intake dose necessary to achieve normal

or optimal levels of biomarkers for zinc status. The dose-response relationship between zinc intake and plasma zinc concentration is of course subject to the debate around the usefulness of plasma/serum zinc concentration as a biomarker of zinc status, and the it's predictive value for relevant functional health outcomes, such as markers of immune function.

The relationship observed between serum/plasma zinc concentration and zinc intake may have been weakened by the limitation of this particular biomarker for zinc status. It is well established that plasma zinc concentration can fall in response to factors unrelated to zinc status or dietary zinc intake, such as infection, inflammation, exercise, stress or trauma [26]. Conversely, tissue catabolism during starvation can release zinc into the circulation, causing a transient increase in circulating zinc levels. Six studies used non-fasted blood samples in their analyses [5; 7; 27; 20; 11; 14]. As postprandial plasma zinc concentrations have been reported to fall up to 19% [28], the inclusion of these studies may have weakened the observed relationship between zinc intake and status. Whilst all studies included in the analysis were undertaken in individuals without chronic disease or severe protein-energy malnutrition, other factors such as stress, infection and inflammation may also have gone unreported. In addition, serum zinc concentration has been reported to decrease with age [15]. Clearly such confounders have a strong influence on the interpretation of plasma zinc concentrations. However, as more sensitive indices of zinc status have yet to be identified, plasma serum zinc remains by far the most commonly used biomarker of zinc status [4].

In conclusion, the current study presents the application of a novel technique to analyse data from 10 RCT's and 3 observational studies reporting the relationship between zinc intake and serum/plasma zinc concentration. This meta-analysis has provided an estimate of the dose-response relationship between zinc intake and serum/plasma zinc concentration in adults and elderly populations. Based on 24 estimates among 2469 participants, the results indicate that a doubling of zinc intake increases plasma/serum levels by 6%. There is a high level of heterogeneity in the data obtained from the studies included in this meta-analysis. Analysis of the factors that may contribute to this, namely study duration, zinc dose, age, and gender, indicated that zinc dose was able to explain 50% of this heterogeneity. This novel method of analyzing intake/biomarker relationships may be useful for the setting of future dietary zinc recommendations.

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

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References

1. Doets EL, de Wit LS, Dhonukshe-Rutten RAM *et al.* (2008) Current micronutrient recommendations in Europe: towards understanding their differences and similarities. *European Journal of Nutrition* **47**, 17-40.

2. Matthys C vTVP, de Groot L, Hooper L, Cavelaars AE, Collings R, Donutske-Rutten R, Harvey LJ, Casgrain A, Rollin F, Contor L. (2011) EURRECA's Approach for Estimating Micronutrient Requirements. *Int J Vitam Nutr Res.* **81**, 256-263.

3. King JC (2011) Zinc: an essential but elusive nutrient. *Am J Clin Nutr* **94**, 679S-684S.

4. Lowe NM, Fekete K & Decsi T (2009) Methods of assessment of zinc status in humans: a systematic review. *American Journal of Clinical Nutrition* **89**, 2040S-2051S.

5. Abdulla M & Svensson S (1979) Effect of oral zinc intake on delta-aminolaevulinic acid dehydratase in red blood cells. *Scandinavian Journal of Clinical & Laboratory Investigation* **39**, 31-36.

6. Boukaiba N, Flament C, Acher S *et al.* (1993) A physiological amount of zinc supplementation: Effects on nutritional, lipid, and thymic status in an elderly population. *American Journal of Clinical Nutrition* **57**, 566-572.

7. Bogden JD, Oleske JM, Lavenhar MA *et al.* (1988) Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. *The American journal of clinical nutrition* **48***, 655-663.*

8. Feillet-Coudray C, Meunier N, Rambeau M *et al.* (2005) Long-term moderate zinc supplementation increases exchangeable zinc pool masses in late-middle-aged men: The Zenith Study. *American Journal of Clinical Nutrition* **82**, 103-110.

9. Feillet-Coudray C, Meunier N, Bayle D *et al.* (2006) Effect of zinc supplementation on in vitro copper-induced oxidation of low-density lipoproteins in healthy French subjects aged 55-70 years: The Zenith study. *British Journal of Nutrition* **95**, 1134-1142.

10. Hininger-Favier I, Andriollo-Sanchez M, Arnaud J *et al.* (2007) Age- and sex-dependent effects of long-term zinc supplementation on essential trace element status and lipid metabolism in European subjects: The Zenith Study. *British Journal of Nutrition* **97**, 569-578.

11. Sakagami M, Ikeda M, Tomita H *et al.* (2009) A zinc-containing compound, Polaprezinc, is effective for patients with taste disorders: Randomized, double-blind, placebo-controlled, multi-center study. *Acta Oto-Laryngologica* **129**, 1115-1120.

12. Preziosi P, Galan P, Herbeth B *et al.* (1998) Effects of supplementation with a combination of antioxidant vitamins and trace elements, at nutritional doses, on biochemical indicators and markers of the antioxidant system in adult subjects. *Journal of the American College of Nutrition* **17**, 244-249.

13. Sullivan VK, Burnett FR & Cousins RJ (1998) Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. *Journal of Nutrition* **128**, 707-713.

14. Sanchez C, Lopez-Jurado M, Planells E *et al.* (2009) Assessment of iron and zinc intake and related biochemical parameters in an adult Mediterranean population from southern Spain: influence of lifestyle factors. *Journal of Nutritional Biochemistry* **20**, 125-131.

15. Rea IM (1989) Sex and age-changes in serum zinc levels. *Nutrition Research* **9**, 121-125.

16. Souverein OW, Dullemeijer C, van TVP *et al.* (2012) Transformations of summary statistics as input in meta-analysis for linear dose-response models on a logarithmic scale: a methodology developed within EURRECA. *BMC Med Res Methodol* **12**, 57.

17. DerSimonian R & Laird N (1986) Meta-analysis in clinical trials. *Control Clin Trials* **7**, 177-188.

18. Higgins J & Green Se (2009) *Cochrane Handbook for Systematic Reviews for Interventions, version 5.0.2 (updated September 2009)*

19. Gibson RS, Heath ALM, Limbaga MLS *et al.* (2001) Are changes in food consumption patterns associated with lower biochemical zinc status among women from Dunedin, New Zealand? *British Journal of Nutrition* **86**, 71-80.

20. Chandyo RK, Strand TA, Mathisen M *et al.* (2009) Zinc deficiency is common among healthy women of reproductive age in Bhaktapur, Nepal. *Journal of Nutrition* **139**, 594-597.

21. Abdulla M, Behbehani A & Dashti H (1989) Dietary intake and bioavailability of trace elements. *Biological Trace Element Research* **21**, 173-178.

22. Preziosi P, Galan P, Herbeth B *et al.* (1998) Effects of supplementation with a combination of antioxidant vitamins and trace elements, at nutritional doses, on biochemical indicators and markers of the antioxidant system in adult subjects. Journal of the American College of Nutrition 17, 244-249.

23. Sullivan VK, Burnett FR & Cousins RJ (1998) Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. *Journal of Nutrition* **128**, 707-713

24. Kipnis V, Subar AF, Midthune D *et al.* (2003) Structure of dietary measurement error: Results of the OPEN biomarker study. *American Journal of Epidemiology* **158**, 14-21.

25. Dullemeijer C, Souverein O, Doets E *et al.* (Under review) Systematic review with dose-response meta-analyses between vitamin B12 intake and EURRECA's prioritized biomarkers of vitamin B12 including randomized controlled trials and observational studies in adults and elderly.

26. King JC (1990) Assessment of zinc status. *Journal of Nutrition* **120**, 1474-9.

27. Prasad AS, Beck FW, Bao B *et al.* (2007) Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *The American journal of clinical nutrition* **85**, 837-44

28. Lowe NM, Woodhouse LR & King JC (1998) A comparison of the short-term kinetics of zinc metabolism in women during fasting and following a breakfast meal. *British Journal of Nutrition* **80**, 363-370.

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454 **Figure Legends**

455 **Figure 1** Study selection process for systematic review

456 **Figure 2** Random effects meta-analyses of RCTs and observational studies evaluating the
457 pooled effect of dietary zinc on serum/plasma zinc in adults. Beta values (♦) represent the
458 regression coefficients for the linear association between $\log_e$ transformed zinc intake and
459 $\log_e$ transformed serum/plasma zinc status.

460 **Figure 3** Serum/plasma zinc concentration (μmol/L) as a function of dietary zinc intake
461 (mg/day), estimated by random-effects meta-analyses of RCTs of adults

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Table 1. Ovid MEDLINE search strategy.

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

Table 2: Randomised controlled trials (n=10) reporting the effect of dietary zinc intake on serum/plasma zinc status in adults.

First author, year, country	Gender, age	Treatment groups	Micronutrient type	Duration	Status marker reported [analytic method]
Abdulla, 1979 Sweden (5)	Mean age 25 y. SD, age range, gender not reported	*Study 1* Placebo (n=5) 135.3mg/d Zn (n=7) *Study 2* Placebo (n=8) 45mg/d Zn (n=7)	Zinc sulphate	12 wk	Plasma Zn [AAS]
Bodgen, 1988 USA (6)	Males and females aged 60- 89 y	Placebo (n=36) 15 mg/d Zn (n=36) 100 mg/d Zn (n=31)	Zinc acetate	3 mo	Plasma Zn [AAS]
Boukaïba, 1993	Males and females aged 73-106 y	*BMI ≤ 21* Placebo (n=21)	Zinc gluconate	8 wk	Serum Zn [AAS]

France (4)		20mg/d Zn (n=21) *BMI ≥ 24* Placebo (n=23) 20mg/d Zn (n=23)			
Preziosi, 1998 France (12)	Males and females aged 35-60 y	Placebo (n=200) Multi-micronutrient supplement (20mg/d Zn) (n=201)	Zinc gluconate	3 & 6 mo	Serum Zn [AAS]
Sullivan, 1998 USA (11)	Males aged 19- 35 y	Placebo (n=13) 50mg/d Zn (n=13)	Zinc gluconate	15 d	Plasma Zn [AAS]
Feillet-Coudray, 2005 France (8)	Males aged 58-68 y	Placebo (n=16) 15 mg/d Zn (n=16) 30 mg/d Zn (n=16)	Zinc gluconate	6 mo	Plasma Zn [ICP-MS]
Feillet-Coudray, 2006 France (7)	Females aged 55-70 y	Placebo (n=16) 15 mg/d Zn (n=16) 30 mg/d Zn (n=15)	Zinc gluconate	6 mo	Serum Zn [ICP-MS]

Hininger-Favier, 2007 France, UK, Italy (9)	Males and females aged 55-85 y	*Age 55-70y* Placebo (n=63) 15mg/d Zn (n=60) 30mg/d Zn (n=65) *Age >70 y* Placebo (n=67) 15 mg/d Zn (n=66) 30 mg/d Zn (n=66)	Zinc gluconate	6 mo	Serum Zn [AAS]
Prasad, 2007 USA (21)	Males and females aged 55-87 y	Placebo (n=25) 45 mg/d Zn (n=24)	Zinc gluconate	12 mo	Plasma Zn [AAS]
Sakagami, 2009 Japan (10)	Males and females aged 21-77 y	Placebo (n=28) 17 mg/d Zn (n=27) 34 mg/d Zn (n=26) 68 mg/d Zn (n=28)	Zinc carnosine	12 wk	Serum Zn [AAS]

AAS atomic absorption spectroscopy; ICP-MS inductively coupled plasma mass spectrometry

Table 3: Observational studies (n=3) reporting the association between dietary zinc intake and serum/plasma zinc status in adults.

First author, year, country	**N**	**Mean (SD) zinc intake (mg/day)**	**Mean (SD) plasma/serum zinc (µmol/L)**	**Zinc intake (source)**	**Zinc intake (assessment)**	**Zinc status biomarker [analytical method]**
Gibson 2001 (New Zealand) (17)	330 females aged 18-40 y	10.44 (3.51)	12.00 (1.36)	Diet	FFQ & 24 hr recall	Serum zinc [AAS]
Chandyo, 2009 (Nepal) (16)	500 females aged 13-35 y	8.6 (3.3)	8.5 (2.4)	Diet	FFQ & 24 hr recall (2 days)	Plasma zinc [ICP-AES]
Sánchez 2009 (Spain) (13)	170 males aged	12.24 (7.16)	17.48 (6.68)	Diet	24 hr recall (2	Plasma zinc [AAS]

	25-60 y			days)
	184 females aged 25-60 y	9.07 (4.40)	16.32 (6.21)	

AAS atomic absorption spectroscopy; ICP-MS inductively coupled plasma mass spectrometry

Table 4 Assessment of validity of included RCTs reporting zinc intake and serum/plasma zinc in adults

Study	Adequate sequence generation	Adequate Blinding	Dropouts adequate and outcome date complete	Funder adequate	Compliance check & results	Dose check & results	Dietary intake data reported & results	Status reproducibility reported	Similarity of most & least exposed groups at baseline	Lack of other potential threats to validity	Overall risk of bias
Abdulla 1979	no	no	unclear	no	unclear	unclear	nr	No	yes	no	High
Bodgen 1988	yes	yes	yes	yes	nr	yes	yes	no	yes	yes	Low
Boukaiba 1993	unclear	yes	yes	unclear	yes	nr	yes	nr	yes	yes	High
Preziosi 1998	yes	yes	yes	unclear	yes	yes	nr	yes	yes	yes	High
Sullivan 1998	unclear	unclear	yes	yes	yes	nr	nr	no	yes	yes	High
Feillet-Coudray 2005	unclear	yes	yes	yes	yes	yes	unclear	yes	yes	yes	Low
Feillet-Coudray	unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes	Low

2006											
Hininger-Favier 2007	unclear	yes	unclear	yes	yes	yes	yes	yes	yes	yes	Low
Prasad 2007	yes	yes	yes	yes	yes	yes	nr	yes	yes	yes	Low
Sakagami 2009	unclear	yes	yes	unclear	nr	yes	nr	yes	yes	unclear	High

nr: not reported

Figure 1 Study selection process for systematic review

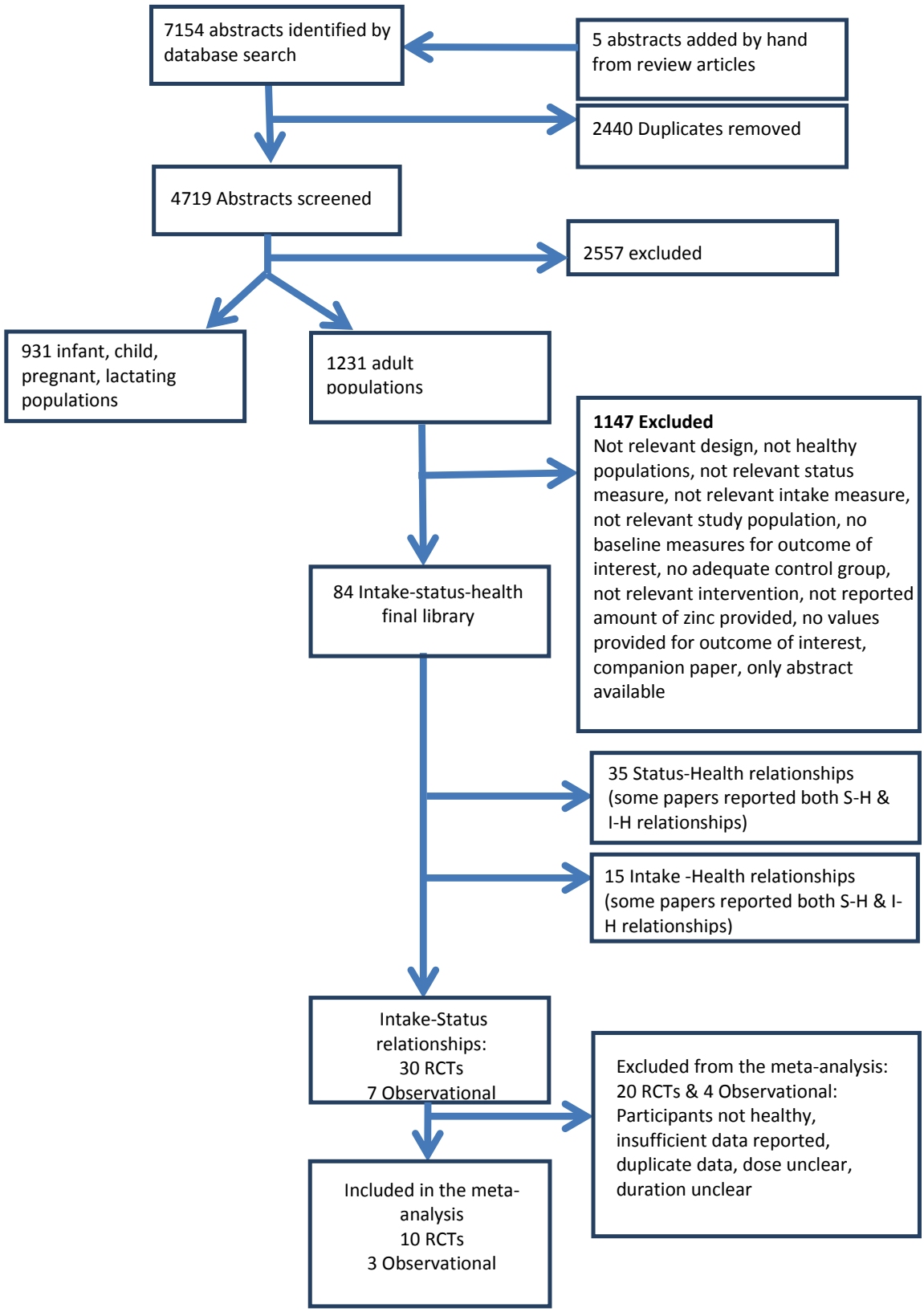
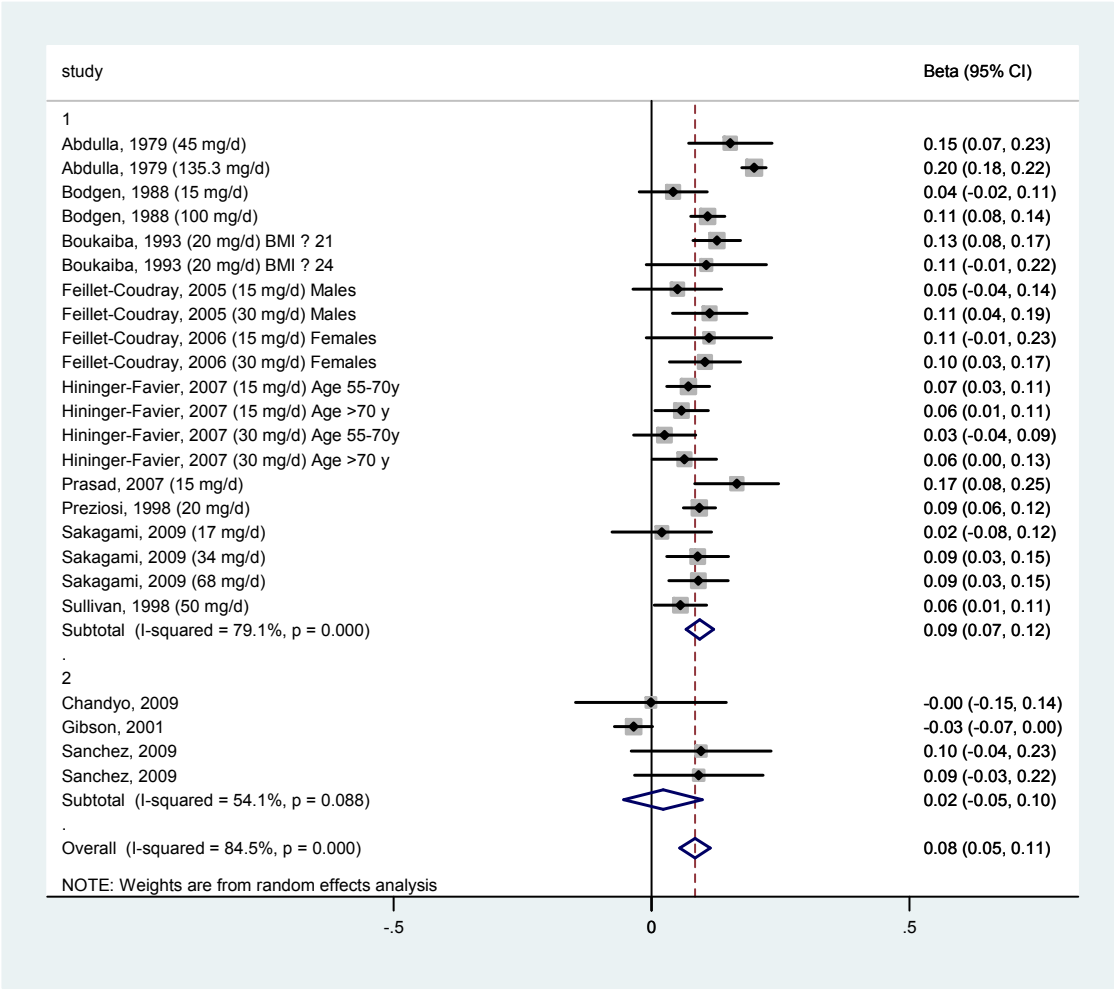


Figure 2 Random effects meta-analyses of RCTs and observational studies evaluating the pooled effect of dietary zinc on serum/plasma zinc in adults. Beta values (♦) represent the regression coefficients for the linear association between $\log_e$ transformed zinc intake and $\log_e$ transformed serum/plasma zinc status.



1: RCTs; 2: Observational studies

Figure 3 Serum/plasma zinc concentration (μmol/L) as a function of dietary zinc intake (mg/day), estimated by random-effects meta-analyses of RCTs of adults