Systematic Review with Meta-analysis

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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Abstract

Dietary Zn recommendations vary widely across Europe due to the heterogeneity of approaches used by expert panels. Under the EURopean micronutrient RECommendations Aligned (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 Zn intake and status in adults. Searches were performed of literature published up to February 2010 using MEDLINE, Embase and the Cochrane Library. Data extracted included population characteristics, dose of Zn, duration of study, dietary intake of Zn, and mean concentration of Zn 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 Zn supplementation on serum/plasma Zn concentrations from randomised controlled trials 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 Zn intake, the difference in Zn serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0·08} = 1·06$), which is 6 %. Whether the dose–response relationship, as provided in the present paper, could be used as either qualitative or quantitative evidence to substantiate the daily Zn intake dose necessary to achieve normal or optimal levels of biomarkers for Zn status remains a matter of discussion.

Key words: EURRECA: Zinc: Dose–response relationships: Systematic reviews: Meta-analyses

Dietary Zn 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 Zn requirements. This method seeks to estimate the Zn 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 Zn intake required for optimal health based on a range of parameters and indices of health that are known to be dependent upon dietary Zn intake[2]. To this end, the members of the

EURopean micronutrient RECommendations Aligned (EUR-RECA) Network of Excellence have undertaken a series of systematic reviews of Zn intake–status relationships, according to rigorous protocols defined by consortium members and external experts[2]. We present the results of the systematic review and meta-analysis of the dose–response relationship between dietary Zn intake and Zn status using novel methodology developed by members of the EURRECA consortium.

The assessment of Zn status is notoriously problematic, as a sensitive, specific biomarker for Zn has not yet been identified[3]. A systematic review and meta-analysis of biomarkers of Zn status was undertaken in 2009[4]. For many putative biomarkers (such as the Zn 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 Zn status; however, plasma (or serum) Zn concentration was responsive to both Zn supplementation and Zn depletion and is the most widely reported biomarker for Zn. Hair and urine Zn concentrations were also considered to be potentially useful biomarkers in response to Zn supplementation.

The purpose of the present review was to systematically and quantitatively assess the dose–response relationships relevant to deriving Zn 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 the present meta-analysis were extracted from published studies (randomised controlled trials (RCT), prospective cohort studies, nested case–control studies and cross-sectional studies), performed in healthy adult and elderly populations, reporting the relationship between Zn status (plasma or serum Zn, hair or urine Zn concentration) and intake from supplements, fortified diets or natural food diets.

Methods

Search strategy

The present 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 research was part of a wider review process to identify studies assessing the effect of Zn intake on different outcomes (biomarkers of Zn status and health outcomes). The wider searches were performed of literature published up to and including February 2010 using Ovid MEDLINE, Embase (Ovid) and the Cochrane Library (CENTRAL) using search terms for ('study designs in humans') AND (Zn) 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,

Table 1. Ovid MEDLINE search strategy

No.	Search term	Results
1	randomized controlled trial.pt	280 821
2	controlled clinical trial.pt	79 998
3	randomized.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

pt, Publication type; ab, abstract; sh, subject heading; mp, multiple posting; exp, explode; adj3, words have to appear within three words of each other; ti, title.

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 Fig. 1.

Criteria for the consideration of studies for the present review

Included studies were RCT, prospective cohort studies, nested case–control studies and cross-sectional studies in healthy human populations that supplied Zn supplementation (RCT) or measured dietary Zn intake with either a validated FFQ, a
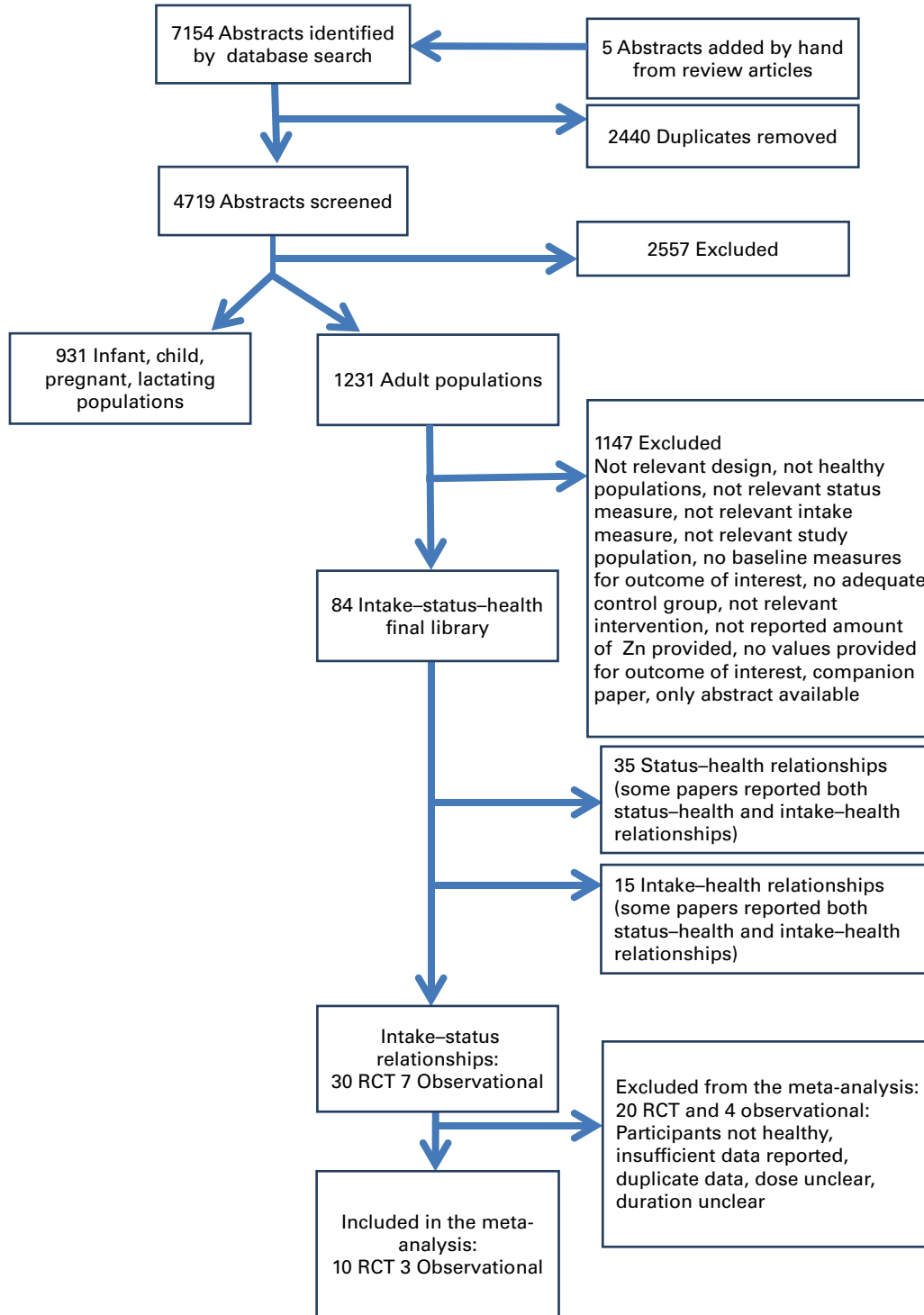

Fig. 1. Study selection process for systematic review. RCT, randomised controlled trial (a colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

dietary history method, a 24 h recall method for at least 3 d, or a food record/diary for at least 3 d (observational studies). Studies had to be conducted in apparently healthy adult and elderly (human) populations aged ≥18 years and supplied Zn supplementation either as capsules or part of a fortified meal. If supplemental Zn was provided as a component of a fortified meal, studies were only considered acceptable if Zn was the only constituent that was different between treatment groups. Biomarkers of Zn status included plasma/serum, urine and hair Zn concentrations. Only studies that reported sufficient data or had sufficient data obtainable from the authors to estimate $\hat{\beta}$ and $\mathrm{SE}(\hat{\beta})$ for the assumed linear relationship 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 Zn was provided for less than 2 weeks.

Selection of articles

Of 4719 identified articles in the wider search on Zn 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. A total of 1147 studies were excluded because they did not meet the inclusion criteria. Of the remaining eighty-four studies, fifty-four were excluded as they related either Zn intake or status directly to a health endpoint, but they had not investigated the relationship between Zn intake and Zn related to biomarkers. A further seventeen studies were excluded from the meta-analysis because study participants were not healthy, insufficient data were reported, data were duplicated, or the dosage and duration were unclear. For the purpose of the present meta-analysis, ten RCT and three observational studies remained. The characteristics of the included studies are presented in Tables 2 and 3, respectively.

Data extraction

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

Data synthesis

Of the RCT, two that reported data for two Zn-treated groups and two control groups were treated as two independent estimates in the analysis[5,6]. Where RCT provided outcome data for two or more Zn-treated groups, they were included

as separate estimates in the meta-analysis[7–11]. Where Zn 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-analysis[14]. If dietary intake of Zn (in addition to the intervention) was not reported in the RCT, a value of 9·7 mg/d was imputed, which was the mean dietary intake level of the RCT that did report dietary Zn intake. As mean baseline serum/plasma Zn concentrations were infrequently reported in the RCT, the serum/plasma Zn concentrations in the control group were used as a proxy of the baseline serum/plasma Zn concentrations for our analyses.

Statistical analyses

A stratified random-effects meta-analysis was conducted using STATA (version 11; StataCorp LP), with one subgroup combining the evidence from RCT and the other subgroup combining the evidence from observational studies. As serum/plasma Zn levels have been reported to decline with age[15], a separate stratified random-effects meta-analysis compared Zn intake and status according to age in RCT (<55 years and ≥55 years). In addition, stratified meta-analyses were also conducted on dose of Zn (<35 mg/d and ≥35 mg/d) and trial duration (in weeks). It was not possible to perform a stratified meta-analysis for sex, 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 ($\hat{\beta}$) for each individual study was estimated from the mean serum/plasma Zn concentrations, based on the assumption of a linear relationship on the $\log_e$–$\log_e$ scale (natural logarithm of intake $v.$ natural logarithm of status). Algebraically deriving an estimate from each study of the regression coefficient ($\hat{\beta}$) and its standard error ($\mathrm{SE}(\hat{\beta})$) enabled a comparison of the results from studies with heterogeneously reported associations and effects. The overall pooled $\hat{\beta}$ and $\mathrm{SE}(\hat{\beta})$ were calculated using random-effects meta-analysis, which estimates the between-study variance using the method of DerSimonian & 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, sex and Zn dose were added simultaneously to a meta-regression model as continuous variables. The statistical transformations to obtain $\hat{\beta}$ and $\mathrm{SE}(\hat{\beta})$ were performed using GenStat version 13-SP2 (VSN International Ltd) and the meta-analysis was performed using STATA (version 11.0; StataCorp LP), 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

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

Study, year, country	Sex, age	Treatment groups	Micronutrient type	Duration	Status marker reported and analytic method
Abdulla & Svensson (1979)[5], Sweden	Mean age 25 years SD, age range and sex not reported	Study 1 Placebo (*n* 5) 135·3 mg Zn/d (*n* 7) Study 2 Placebo (*n* 8) 45 mg Zn/d (*n* 7)	Zinc sulfate	12 weeks	Plasma Zn, AAS
Bogden *et al.* (1988)[7], USA	Males and females aged 60–89 years	Placebo (*n* 36) 15 mg Zn/d (*n* 36) 100 mg Zn/d (*n* 31)	Zinc acetate	3 months	Plasma Zn, AAS
Boukaïba *et al.* (1993)[6], France	Males and females aged 73–106 years	BMI ≤ 21 kg/m² Placebo (*n* 21) 20 mg Zn/d (*n* 21) BMI ≥ 24 kg/m² Placebo (*n* 23) 20 mg Zn/d (*n* 23)	Zinc gluconate	8 weeks	Serum Zn, AAS
Preziosi *et al.* (1998)[12], France	Males and females aged 35–60 years	Placebo (*n* 200) Multi-micronutrient supplement (20 mg Zn/d) (*n* 201)	Zinc gluconate	3 and 6 months	Serum Zn, AAS
Sullivan *et al.* (1998)[13], USA	Males aged 19–35 years	Placebo (*n* 13) 50 mg Zn/d (*n* 13)	Zinc gluconate	15 d	Plasma Zn, AAS
Feillet-Coudray *et al.* (2005)[8], France	Males aged 58–68 years	Placebo (*n* 16) 15 mg Zn/d (*n* 16) 30 mg Zn/d (*n* 16)	Zinc gluconate	6 months	Plasma Zn, ICP-MS
Feillet-Coudray *et al.* (2006)[9], France	Females aged 55–70 years	Placebo (*n* 16) 15 mg Zn/d (*n* 16) 30 mg Zn/d (*n* 15)	Zinc gluconate	6 months	Serum Zn, ICP-MS
Hininger-Favier *et al.* (2007)[10], France, UK, Italy	Males and females aged 55–85 years	Age 55–70 years Placebo (*n* 63) 15 mg Zn/d (*n* 60) 30 mg Zn/d (*n* 65) Age >70 years Placebo (*n* 67) 15 mg Zn/d (*n* 66) 30 mg Zn/d (*n* 66)	Zinc gluconate	6 months	Serum Zn, AAS
Prasad *et al.* (2007)[24], USA	Males and females aged 55–87 years	Placebo (*n* 25) 45 mg Zn/d (*n* 24)	Zinc gluconate	12 months	Plasma Zn, AAS
Sakagami *et al.* (2009)[11], Japan	Males and females aged 21–77 years	Placebo (*n* 28) 17 mg Zn/d (*n* 27) 34 mg Zn/d (*n* 26) 68 mg Zn/d (*n* 28)	Zinc carnosine	12 weeks	Serum Zn, AAS

AAS, atomic absorption spectroscopy; ICP-MS, inductively coupled plasma MS.

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

A total of twenty estimates of Zn intake and serum/plasma Zn status in ten RCT and four estimates in three observational studies were eligible for meta-analysis. All studies were published between 1979 and 2010. Although plasma/serum, urine and hair Zn concentrations were included as markers of status in the systematic review protocol, only plasma/serum Zn 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[9,19,20], two included only males[8,13] 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 RCT used a parallel design. Boukaïba *et al.*[6] employed a cross-over RCT design. The RCT included 1285 participants in total with sample sizes ranging from five to 201. The median duration of the trials was 25 weeks (range 2–52 weeks). In nine studies Zn was supplemented alone at doses ranging from 15 to 135·3 mg/d and in one study Zn was provided within a multi-micronutrient supplement[12]. Most studies (*n* 7) provided the Zn supplements in the form of zinc gluconate, but others used zinc sulfate[5], zinc acetate[7] or zinc carnosine[11]. Habitual Zn intakes ranged from 5·4 to 10·8 mg/d (where data were provided).

The observational studies included 1184 participants in total with sample sizes in the range of 170–500. Zn intake was measured using a combination of FFQ and 24 h recall, or

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

Study, year, country	Subjects and ages	Mean Zn intake (mg/d)	Mean plasma/serum Zn (μmol/l)	Zn intake (source)	Zn intake (assessment)	Zn status biomarker and analytical method
Gibson et al. (2001)[19], New Zealand	330 females aged 18–40 years	10·44 (SD 3·51)	12·00 (SD 1·36)	Diet	FFQ and 24 h recall	Serum Zn, AAS
Chandyo et al. (2009)[20], Nepal	500 females aged 13–35 years	8·6 (SD 3·3)	8·5 (SD 2·4)	Diet	FFQ and 24 h recall (2 d)	Plasma Zn, ICP-AES
Sánchez et al. (2009)[14], Spain	170 males aged 25–60 years	12·24 (SD 7·16)	17·48 (SD 6·68)	Diet	24 h recall (2 d)	Plasma Zn, AAS
	184 females aged 25–60 years	9·07 (SD 4·40)	16·32 (SD 6·21)			

AAS, atomic absorption spectroscopy; ICP-MS, inductively coupled plasma MS.

24 h recall alone and values ranged from 8·6 to 12·2 mg/d. The meta-analysis of available studies suggested that Zn supplementation was associated with increased serum/plasma Zn concentrations. The estimated effect for Zn supplementation on serum/plasma Zn concentrations from RCT and observational studies was 0·08 (95 % CI 0·05, 0·11; $P < 0·0001$; I^2 84·5 %) (Fig. 2). When datasets were grouped according to study design, only the RCT 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 Zn intake and serum/plasma Zn concentration before calculation of the study-specific $\hat{\beta}$, the overall $\hat{\beta}$ represents the difference in the $\log_e$-transformed predicted value of serum/plasma Zn status for each one-unit difference in the $\log_e$-transformed value in Zn intake. Therefore, an overall $\hat{\beta}$ of 0·08 means that for every doubling in Zn intake, the difference in Zn serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0·08} = 1·06$), which is 6 %. This means that an individual with a Zn intake of 14 mg/d has a Zn serum/plasma concentration that is 6 % higher than an individual who has a Zn intake of 7 mg/d (Fig. 3).

As plasma/serum Zn concentrations have been reported to decline with age[15], a separate subgroup analysis compared Zn intake and status according to age in RCT (< 55 years and ≥ 55 years). Of the studies, two for which mean serum/plasma Zn values were given for adults whose ages spanned both age groups were excluded from this analysis[11,12]. 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 with 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 Zn (< 35 mg/d and ≥ 35 mg/d) revealed a stronger effect size for a Zn dose ≥ 35 mg/d (0·14; 95 % CI 0·08, 0·21; $P < 0·0001$; I^2 85·2 %) compared with < 35 mg/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; 95 % CI 0·05, 0·20; I^2 92·4 %; > 12 weeks: 0·10; 95 % CI 0·07, 0·12; I^2 75·8 %).

To evaluate potential sources of heterogeneity, the variables duration, age, sex and dose were added simultaneously to a meta-regression model as continuous variables. The analysis revealed that only Zn dose was a statistically significant determinant of the overall β. 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 five out of the ten papers[5,6,11–13]. 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 present review is unique in providing an estimate of the dose–response relationship of Zn intake and serum/plasma
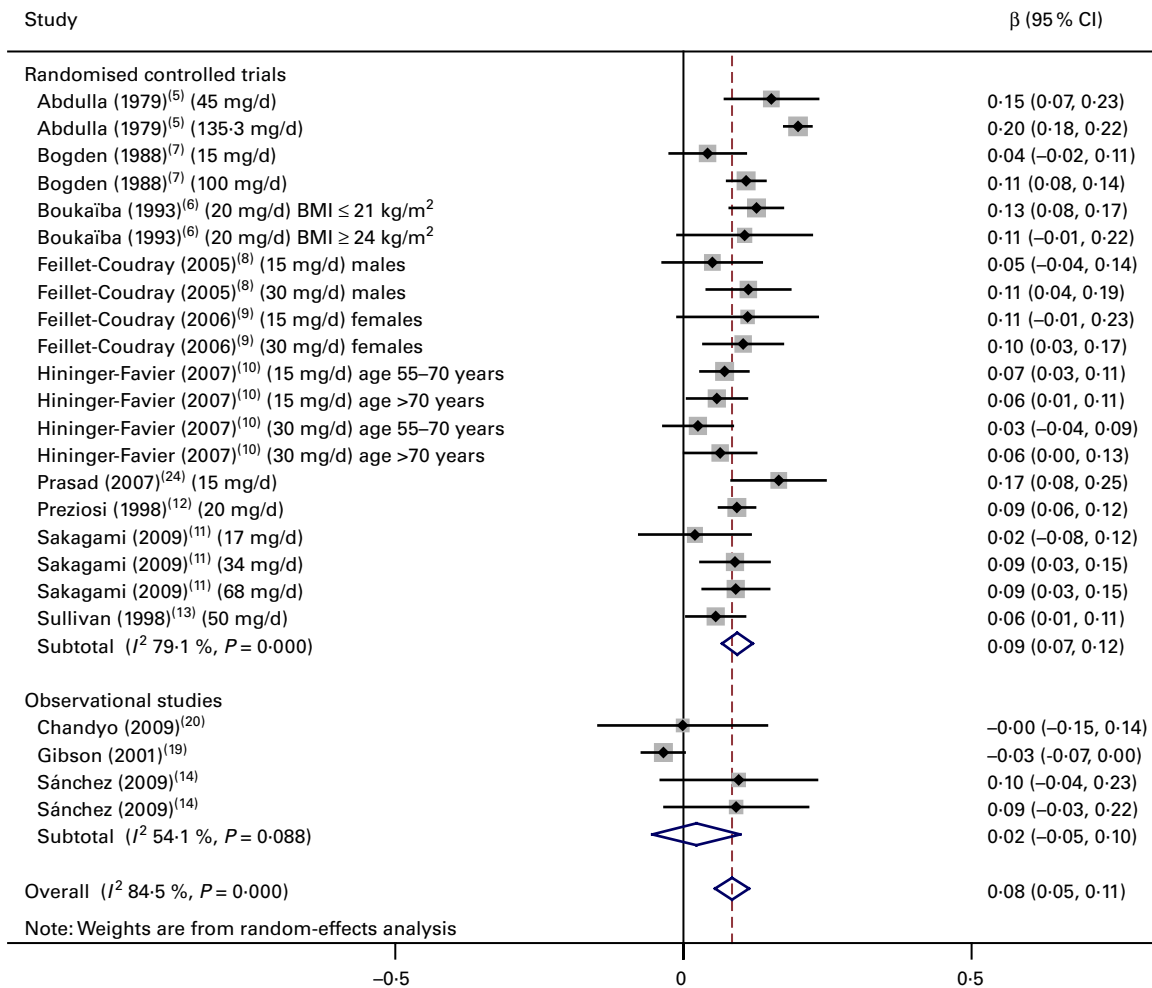
Study	β (95 % CI)

Fig. 2. Random-effects meta-analyses of randomised controlled trials and observational studies evaluating the pooled effect of dietary zinc on serum/plasma zinc in adults. β Values (♦) represent the regression coefficients for the linear association between log_e-transformed zinc intake and log_e-transformed serum/plasma zinc status (a colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Zn concentrations in adults. A meta-analysis of twenty estimates in ten RCT and four estimates in three observational studies found that Zn supplementation produced a statistically significant increase in serum/plasma Zn concentrations and provided an estimate of the dose–response relationship between Zn intake and serum/plasma concentrations. An overall $\hat{\beta}$ of 0·08 means that for every doubling in Zn intake, the difference in Zn serum or plasma concentration is 6 %. In other words, an adult with a Zn intake of 14 mg/d has a Zn serum/plasma concentration that is 6 % higher than an individual who has a Zn intake of 7 mg/d. This association was slightly stronger when considering only the RCT, as no observational studies found a significant association between Zn intake and plasma Zn concentrations. The intake–status regression coefficient for the observational studies is likely to be attenuated by random and intake-related errors in assessing dietary Zn intake[21], whereas in RCT Zn intake can be considered as fixed at each level of dosage and random errors arise only through assessment of biomarkers.

The studies included in the present meta-analysis were different in a number of aspects, such as using various designs, follow-up times, Zn doses and populations. Therefore, it is no surprise that, when combining these studies in a meta-analysis, 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 Zn (amount, one or more doses per d, study duration). When considering some key variables (study duration, Zn dose, age and sex) 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 the present meta-analysis. However, this type of analysis would involve the input of raw individual participant data provided by the original study investigators for reanalysis 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 the present paper is not an attempt to
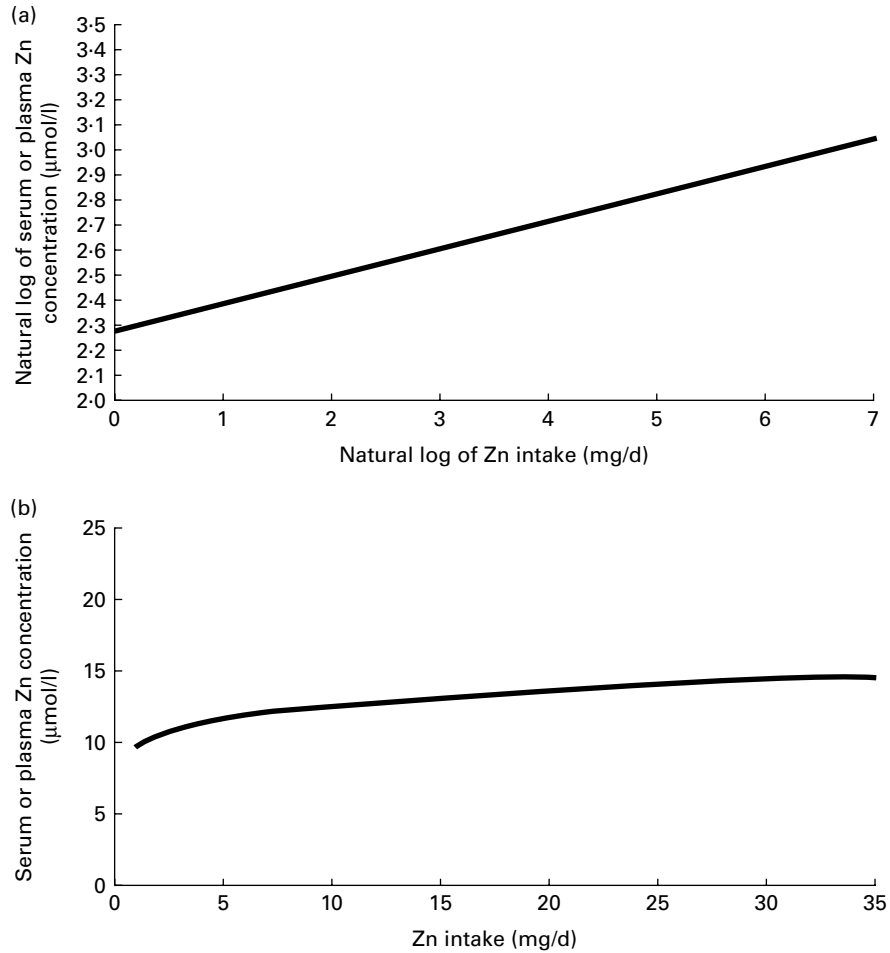
Fig. 3. Serum/plasma zinc concentration (μmol/l) as a function of dietary zinc intake (mg/d), estimated by random-effects meta-analyses of randomised controlled trials of adults: natural log-transformed data (a); untransformed data (b).

accurately describe the biological relationship between actual Zn intake and Zn concentrations in blood under strict experimental conditions and on an individual level, but rather to simulate a dose–response relationship between Zn 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 standardisation of methods and the true heterogeneity between study populations and necessarily enters as uncertainty into the application of such data for public health purposes[22].

To conduct the present 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 with the same control group (five RCT), 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 the present meta-analyses had different ways of reporting the relationship between Zn and serum/plasma Zn concentration. The different ways of reporting by transformation of both the intake and biomarker data were standardised to double $\log_e$ scale, which allowed the derivation of a standardised 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 β values and enable these to be reported as a dose–response relationship between Zn intake and serum/plasma Zn 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 Zn intake of populations. This dose–response relationship methodology may be used as either qualitative or quantitative evidence to substantiate the daily Zn intake dose necessary to achieve normal or optimal levels of biomarkers for Zn status. The dose–response relationship between Zn intake and plasma Zn concentration is of course subject to the debate around the usefulness of plasma/serum Zn concentration as a biomarker

Table 4. Assessment of validity of included randomised controlled trials reporting zinc intake and serum/plasma zinc in adults

Study	Adequate sequence generation	Adequate blinding	Drop-outs adequate and outcome data complete	Funder adequate	Compliance check and results	Dose check and results	Dietary intake data reported and results	Status reproducibility reported	Similarity of most and least exposed groups at baseline	Lack of other potential threats to validity	Overall risk of bias
Abdulla & Svensson (1979)[5]	No	No	Unclear	No	Unclear	Unclear	NR	No	Yes	No	High
Bogden et al. (1988)[7]	Yes	Yes	Yes	Yes	NR	Yes	Yes	No	Yes	Yes	Low
Boukaïba et al. (1993)[6]	Unclear	Yes	Yes	Unclear	Yes	NR	Yes	NR	Yes	Yes	High
Preziosi et al. (1998)[12]	Yes	Yes	Yes	Unclear	Yes	Yes	NR	Yes	Yes	Yes	High
Sullivan et al. (1998)[13]	Unclear	Unclear	Yes	Yes	Yes	NR	NR	No	Yes	Yes	High
Feillet-Coudray et al. (2005)[8]	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Low
Feillet-Coudray et al. (2006)[9]	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low
Hininger-Favier et al. (2007)[10]	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low
Prasad et al. (2007)[24]	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	Low
Sakagami et al. (2009)[11]	Unclear	Yes	Yes	Unclear	NR	Yes	NR	Yes	Yes	Unclear	High

NR, not reported.

of Zn status, and its predictive value for relevant functional health outcomes, such as markers of immune function.

The relationship observed between serum/plasma Zn concentration and Zn intake may have been weakened by the limitation of this particular biomarker for Zn status. It is well established that plasma Zn concentration can fall in response to factors unrelated to Zn status or dietary Zn intake, such as infection, inflammation, exercise, stress or trauma[23]. Conversely, tissue catabolism during starvation can release Zn into the circulation, causing a transient increase in circulating Zn levels. A total of six studies used non-fasted blood samples in their analyses[5,7,11,14,20,24]. As postprandial plasma Zn concentrations have been reported to fall up to 19 %[25], the inclusion of these studies may have weakened the observed relationship between Zn 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 Zn concentration has been reported to decrease with age[15]. Clearly, such confounders have a strong influence on the interpretation of plasma Zn concentrations. However, as more sensitive indices of Zn status have yet to be identified, plasma serum Zn remains by far the most commonly used biomarker of Zn status[4].

In conclusion, the present review presents the application of a novel technique to analyse data from ten RCT and three observational studies reporting the relationship between Zn intake and serum/plasma Zn concentration. The present meta-analysis has provided an estimate of the dose–response relationship between Zn intake and serum/plasma Zn concentration in adult and elderly populations. Based on twenty-four estimates among 2469 participants, the results indicate that a doubling of Zn 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, Zn dose, age and sex, indicated that Zn dose was able to explain 50 % of this heterogeneity. This novel method of analysing intake–biomarker relationships may be useful for the setting of future dietary Zn recommendations.

Acknowledgements

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