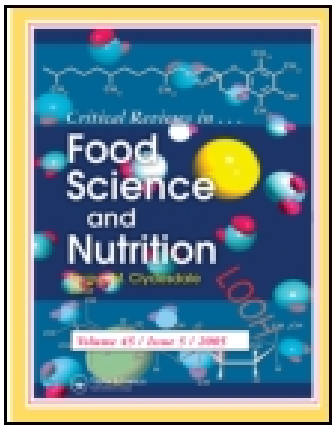


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# EURRECA—Estimating Zinc Requirements for Deriving Dietary Reference Values

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# EURRECA—Estimating Zinc Requirements for Deriving Dietary Reference Values

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*Zinc was selected as a priority micronutrient for EURRECA, because there is significant heterogeneity in the Dietary Reference Values (DRVs) across Europe. In addition, the prevalence of inadequate zinc intakes was thought to be high among all population groups worldwide, and the public health concern is considerable. In accordance with the EURRECA consortium principles and protocols, a series of literature reviews were undertaken in order to develop best practice guidelines for assessing dietary zinc intake and zinc status. These were incorporated into subsequent literature search strategies and protocols for studies investigating the relationships between zinc intake, status and health, as well as studies relating to the factorial approach (including bioavailability) for setting dietary recommendations. EMBASE (Ovid), Cochrane Library CENTRAL, and MEDLINE (Ovid) databases were searched for studies published up to February 2010 and collated into a series of Endnote databases that are available for the use of future DRV panels. Meta-analyses of data extracted from these publications were performed where possible in order to address specific questions relating to factors affecting dietary recommendations. This review has highlighted the need for more high quality studies to address gaps in current knowledge, in particular the continued search for a reliable biomarker of zinc status and the influence of genetic polymorphisms on individual dietary requirements. In addition, there is a need to further develop models of the effect of dietary inhibitors of zinc absorption and their impact on population dietary zinc requirements.*

**Keywords** Zinc, dietary recommendations, zinc intake, systematic review, zinc status, zinc bioavailability, zinc requirements

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## *INTRODUCTION*

Zinc is well established as an essential micronutrient for human health, having numerous structural and biochemical functions at the cellular and subcellular level, including enzyme function, DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation, and cell-mediated immunity. Inadequate zinc intake has profound consequences at all points of the human lifecycle from the point of conception through to old age. Zinc was selected as a priority micronutrient for EURRECA, because the prevalence of inadequate zinc intakes was thought to be high among all population groups, and the public health concern is considerable. In addition, new scientific evidence has recently become available that demonstrates a large heterogeneity among current recommendations on zinc intake across Europe (Doets et al., 2008; Cavelaars et al., 2010) [see Activity 1 in (Dhonukshe-Rutten et al., 2013)].

The total amount of zinc present in the adult human body ranges from 1.5 to 2.5 mg, most of which is intracellular, within skeletal muscle tissue (57%), bone (29%), and other tissues including skin and organs (Jackson, 1989). The zinc located within these tissues has a relatively slow turnover rate and is not readily responsive to changes in dietary zinc intake. Kinetic studies suggest that only a small proportion of total body zinc (approximately 10%) represents the "functional pool" of zinc, which is comprised of zinc, located within the liver and other tissues, that exchanges rapidly with the plasma, and when this functional pool is depleted zinc deficiency ensues (King, 1990). Zinc deficiency in adults can lead to dermatitis, hair loss, diarrhea, loss of appetite, reproductive failure, hypogeusia, loss of cognitive function, susceptibility to infections, and depressed immune function (Shankar and Prasad, 1998), delayed wound healing and depression (Andrews and Gallagher-Allred, 1999). Zinc deficiency has also been associated with three major health diseases prevalent in Europe: Diabetes, cancer, and coronary heart disease (Singh et al., 1998). Zinc deficiency may be a serious public health problem that compromises the development of millions of children (Sandstead and Smith, 1996). The recent Lancet series on maternal and child under nutrition concluded that zinc deficiency is responsible for about 4% of child mortality and disability-adjusted life years (Black et al., 2008). The consequences and manifestations of severe zinc deficiency in infants and children and adolescents can be retardation of linear growth and development, poor appetite, delayed sexual maturation and hypogonadism, frequent infections (Maret and Sandstead, 2008), alopecia, dermatitis, delayed wound healing, diarrhea, pneumonia, malaria (Fischer Walker et al., 2009), limitation on the senses of taste and smell, and night blindness (Christian et al., 2001; Seiler et al., 2002). Zn deficiency may be associated with deficits in activity, attention, and motor development (Bhatnagar and Taneja, 2001). Results of several studies indicated that supplementation with zinc can significantly reduce the rates of diarrhea and pneumonia in young children and increase the growth rate of stunted children (Brown et al., 2002; Brooks et al., 2005a; Brooks et al., 2005b). During the acute diarrhea, zinc supplementation reduces the duration and severity of the disease, so that now the World Health Organization (WHO) recommends zinc supplementation as an adjunct to rehydration therapy, to replace the excessive losses of zinc during periods of diarrhea (WHO). The involvement of maternal zinc status in pregnancy outcome is still unclear, animal models have shown that severe maternal zinc deficiency results in impaired implantation, abortions, and fetal malformations (Keen et al., 2003). The consideration that zinc deficiency is a teratogenic risk in humans may be supported by the correlation of low plasma zinc concentrations in the first and third trimesters of pregnancy with an increased risk for malformations and low birth weight, respectively. Zinc deficiency is thought to influence embryonic and fetal development through reduced cell proliferation, or reduced protein synthesis, or reductions in rates of tubulin polymerization rather than increased rates of cellular oxidative damage or increased rates of apoptosis and reduced binding of hormones and transcription factors dependent on zinc-finger regions (Jankowski-Hennig et al., 2000; Mackenzie et al., 2002; WHO).

## *CURRENT DIETARY RECOMMENDATIONS*

Dietary recommendations for zinc intake have been mainly based on balance studies focusing on the prevention of deficiency and use the factorial approach which assumes that the zinc requirement is the lowest intake which replaces obligatory zinc endogenous loss. The method computes the dietary zinc requirement by dividing the endogenous zinc loss by the fractional zinc absorption (King, 1986). In Europe and in other non-European countries, zinc recommendations for infants are generally set either based on zinc concentration of breast milk, using a factorial approach, or extrapolating values from those given for adults.

There is significant heterogeneity among current recommendations on zinc intake across Europe and worldwide (Doets et al., 2008). Table 1 illustrates the range of dietary zinc recommendations for various countries worldwide. This heterogeneity is due to a number of factors including the data used to derive the value, and differences in expert opinion between panels convened to review the data.

## *CURRENT EUROPEAN INTAKES*

As part of the EURRECA programme of work, Roman-Vinas et al. undertook an analysis of population dietary surveys from across Europe in order to determine the prevalence of inadequate nutrient intake in Europe using the Nordic Nutritional Recommendations as the standard (Roman-Vinas et al., 2010). This study revealed that the failure to meet the estimated average requirement (EAR) of 6.4 mg/d or 5.7 mg/d for adult males and female, respectively, was greatest in Ireland with dietary zinc intakes falling below this cut off value in 11.9% men and

**Table 1** Selected recommended intake levels for zinc (mg)[†]

| | | | Population group | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Data source | Gender | Special conditions | Infants | Children | Adolescents | Adults | Elderly | Lactation | Pregnancy |
| WHO/FAO | *Male* | High bioavailability | *0–6 m* 1.1 *7–12 m* 0.8 | *1–3 y*, 2.4; *4–6 y*, 2.9; *7–9 y*, 3.3 | *10–18 y*, 5.1 | *19–65 y*, 4.2 | *>65 y, 4.2* | — | — |
| | *Female* | Low bioavailability | *0–6 m*, 6.6; *7–12 m*, 8.4 | *1–3 y*, 8.3; *4–6 y*, 9.6; *7–9 y*, 11.2 | *10–18 y*, 14.4 | *19–50 y*, 9.8 | *65 y*, 9.8 | *0–3 m*, 19 | *< 3 m*, 11 |
| Nordic | *Male* | — | *<6 m*, NULL; *6–11 m*, 5 | *2–5 y*, 6; *6–9 y*, 7; *10–13 y*, 11 | *14–17 y*, 12 | *18–30 y*, 9; *31–60 y*, 9 | *≥75 y*, 9 | — | — |
| | *Female* | — | *<6 m*, NULL; *6–11 m*, 5 | *2–5 y*, 6; *6–9 y*, 7; *10–13 y*, 8 | *14–17 y*, 9 | *18–30 y*, 7; *31–60 y*, 7 | *≥75 y*, 7 | — | — |
| Australia/NZ | *Male* | — | *0–6 m*, 2; *7–12 m*, 3 | *1–3 y*, 3; *4–8 y*, 4; *9–13 y*, 6 | *14–18 y;* 13 | *19–30 y*, 14; *31–50 y*, 14 | *>70 y*, 14 | — | — |
| | *Female* | — | *0–6 m*, 2; *7–12 m, 3* | *1–3 y*, 3; *4–8 y*, 4; *9–13 y*, 6 | *14–18 y*, 7 | *19–30 y*, 8; *31–50 y*, 8 | *>70 y*, 8 | *14–18 y*, 11; *19–50 y*, 12 | *14–18 y*, 10; *19–30 y*, 11 |
| DACH | *Male* | — | *0–3 m*, 1; *4–11 m*, 2 | *1–3 y*, 3; *4–6 y*, 5; *7–9 y*, 7; *10–12 y*, 7 | *13–14 y*, 9.5; *15–18 y*, 10 | *19–64 y*, 10 | *>65 y*, 10 | — | — |
| | *Female* | — | *0–3 m*, 1; *4–11 m*, 2 | *1–3 y*, 3; *4–6 y*, 5; *7–9 y*, 7; *10–12 y*, 7 | *13–14 y*, 7; *15–18 y*, 7 | *19–64 y*, 7 | *>65 y*, 7 | 10 | *>4 m*, 10 |
| EC | *Male* | — | *6–11 m*, 4 | *1–3 y*, 4; *4–6 y*, 6; *7–10 y*, 7 | *11–14 y*, 9; *15–17 y*, 9 | *≥18 y*, 9.5 | *≥18 y*, 9.5 | — | — |
| | *Female* | — | *6–11 m*, 4 | *1–3 y*, 4; *4–6 y*, 6; *7–10 y*, 7 | *11–14 y*, 9; *15–17 y*, 7 | *≥18 y*, 7 | *≥18 y*, 7 | 12 | 7 |
| IOM (US/Canada) | *Male* | — | *0–6 m*, 2; *7–12 m*, 3 | *1–3 y*, 3; *4–8 y*, 5; *9–13 y*, 8 | *14–18 y*, 11 | *19–70 y*, 11 | *≥70 y*, 11 | — | — |
| | *Female* | — | *0–6 m*, 2; *7–12 m*, 3 | *1–3 y*, 3; *4–8 y*, 5; *9–13 y*, 8 | *14–18 y*, 9 | *19–70 y*, 8 | *>70 y*, 8 | *14–18 y*, 12; *19–30 y*, 12 | *19–30 y*, 11; *31–50 y*, 11 |
| United Kingdom | *Male* | — | *0–3 m*, 4; *4–6 m*, 4 | *1–3 y*, 5; *4–6 y*, 6.5; *7–10 y, 7* | *11–14 y*, 9 | *19–50 y*, 9.5 | *>50 y*, 9.5 | — | — |
| | *Female* | — | *0–3 m*, 4; *4–6 m*, 4 | *1–3 y*, 5; *4–6 y*, 6.5; *7–10 y*, 7 | *14–18 y*, 9 | *19–50 y*, 7 | *>50 y*, 7 | — | — |

Data presented in the table was obtained from online EURRECA web resource *NutriRecQuest*: http://www.serbianfood.info/eurreca/ (Cavelaars et al., 2010) from original source documents (Australian Government Department of Health and Aging New Zealand Ministry of Health and National Health and Medical Research Council, 2005; DACH, 2008; European Commission, 1993; UK Department of Health, 1991; Institute of Medicine, 2000; World Health Organization, 2004).

[†]RDA, Recommended Dietary Allowance (USA, Canada); equivalent to RNI, Reference Nutrient Intake (UK); PRI, Population Reference Intake (EFSA); RNI, Recommended Nutrient Intake (WHO/FAO); RDI, Recommended Dietary Intake (AU/NZ); RI, Recommended Intake (DACH, Nordic).

"DACH" refers to reference intakes for Germany, Austria, and Switzerland.

"Nordic countries" refers to Denmark, Finland, Norway, and Sweden.

"EC" refers to European Commission.

28.8% women. A similar picture emerged for elderly people (aged >64 years), with those living in Ireland having the highest percentage failing to meet the EAR, 13.6% and 13.1% of elderly men and women, respectively (Vinas et al., 2011)

A review of available micronutrient intake and status data in Europe (Novakovic et al. 2012a) showed that data on intake of zinc were very limited for all life stages, so no cross country comparison could have been made. However, available data for zinc status (based on serum/plasma zinc concentrations) in children, adolescents, and adults showed no regional differences when Central and Eastern Europe, Scandinavia, Western, and Mediterranean countries were compared. All levels were within the optimal range indicating adequacy in zinc status (Novakovic et al. 2012a).

A systematic review of the relationship between micronutrient intake and socioeconomic determinants in Europe revealed that there were almost no differences in zinc intake between different socioeconomic status (SES) groups. On the other hand, status data in adults showed 5% higher serum zinc level in the low SES group. In comparison to reference values (Nordic nutrient recommendations for intake and the WHO for status), all observed intake and status levels were within the optimal range, with the exception of levels of the low SES group in UK children (Novakovic et al. 2012b).

On an individual basis, an inadequate dietary intake of zinc could be the result of a strict vegan diet, a diet which is primarily based on grain products (Solomons and Slavin, 2001) or through a restrictive diet due, for example, to anorexia or alcohol or drug addiction. Certain disease states such as acrodermatitis enteropathica, Celiac disease, Crohn's disease, and ulcerative colitis may disrupt the absorption of zinc (Solomons and Slavin, 2001). Other health states may increase zinc losses primarily through diarrhea, or increase requirements, such as the postoperative state (Solomons and Slavin, 2001).

The purpose of this review is to provide a summary of the methods used, and the results obtained from the systematic literature searches and subsequent meta-analysis of the data retrieved that were performed by partners in the EURRECA network of excellence. These activities were designed to answer specific questions regarding zinc-intake-status relationships. In addition, a comprehensive review of the factorial approach to setting zinc recommendations was undertaken. The overall aim of these activities was to generate new data and approaches that could assist future panels to derive dietary zinc recommendations using robust and transparent methodology.

## *METHODS*

### *Assessing Dietary Zinc Intake*

One of the initial activities in the EURRECA process was to establish the most robust methodology of assessing zinc intake and status (Matthys et al., 2010) and Activity 3 in (Dhonukshe-Rutten et al., 2013). The accurate determination of dietary micronutrient intake is notoriously problematic. Following a series of reviews of the methods used to assess micronutrient intake in Europe (Serra-Majem and Roman-Vinas, 2009), best practice guidelines were developed and adopted by the EURRECA network for all subsequent nutrient review activities. These are described in detail in "RA1.1 Best practice guidelines" in www.eurreca.org. In summary, only studies that used the following methodologies were included in the systematic reviews:

(1) validated FFQ/Dietary History
(2) validated 24 h recall/food records/diary measurements for at least 3 days
(3) validated 24 h recall/food records/diary measurements < 3 days with adjustment for intraindividual variability.

Since interventions commonly involve supplements, these were considered, taking into account the possible differences in bioavailability.

### *Assessing Zinc Status*

The assessment of zinc status is also problematic and it is generally accepted that there is currently no specific, reliable biomarker of zinc status. A systematic review and meta-analysis of the literature examining the efficacy of potential biomarkers of zinc status was undertaken (Lowe et al., 2009). This review presented an analysis of data from over 32 potential biomarkers, however, for many there was insufficient evidence to assess their reliability (Table 2).

Plasma/serum zinc concentration was the most commonly used marker of zinc status and, therefore, the biomarker for which there were most data. It was found to respond to both increases and decreases in zinc intake, and was identified as being a useful biomarker however there are considerable reservations due to the effect of multiple confounders, such as infection, inflammatory status, and time of last meal. Urine and hair were also considered useful biomarkers.

### *Health Outcomes Associated with Inadequate Zinc Intake*

Important health problems related to zinc intake in adults and elderly people were identified by a literature search. These include: compromised immunity, dermatitis, hypogeusia, impaired cognitive functioning (dementia), depression, diabetes (reduced glucose tolerance), ischemic heart disease, carcinogenesis, and anorexia. These were discussed and prioritized by the experts in zinc research within the RA2 team (Matthys, 2011). Prioritization was based on the strength of evidence of the deficiency and role of zinc on the health outcome, the relevance of the health outcome to the European population groups and the amount of evidence-based research literature available based on a pilot literature search. The health outcomes that were

**Table 2** Biomarkers identified in systematic review (Lowe et al., 2009)

| | |
|---|---|
| Potentially useful | • Plasma/serum zinc concentration |
| | • Hair zinc concentration |
| | • Urinary zinc excretion |
| Not useful | • Erythrocyte zinc concentration |
| | • Mononuclear cell zinc |
| | • Concentration |
| | • Polymorphonuclear cell zinc |
| | • Concentration |
| | • Platelet zinc concentration |
| | • Plasma alkaline phosphatise activity |
| Inconclusive due to lack of data | • Aminolevulinic acid dehydratase |
| | • Erythrocyte metallothionein |
| | • Monocyte metallothionein cDNA |
| | • Salivary zinc |
| | • Salivary-sediment zinc |
| | • Mixed saliva zinc |
| | • Plasma extracellular superoxide dismutase |
| | • Lymphocyte zinc |
| | • Lymphocyte ecto-5′-nucleotidase |
| | • Nail zinc |
| | • Plasma ACE |
| | • Neutrophil zinc |
| | • T lymphocyte metallothionein-2A mRNA |
| | • Plasma 5′-nucleotidase |
| | • Endogenous zinc excretion |
| | • Plasma zinc flux |
| | • Exchangeable zinc pool |
| | • Carbonic anhydrase |
| | • Fecal zinc |
| | • Neutrophil α-D-mannosidase |
| | • Neutrophil alkaline phosphatase |
| | • Erythrocyte membrane zinc |
| | • Erythrocyte membrane alkaline phosphatase |
| | • Erythrocyte membrane neutral phosphatase |

identified for each population group are shown in Table 3, and are listed in order of priority.

The best practice guidelines were then used to design the search protocols for the subsequent systematic reviews of the zinc intake-status-health relationships, the factorial approach for assessing dietary zinc requirements, zinc bioavailability, and the influence of polymorphisms on zinc requirements. [Details of the search protocols can be found at: www.eurreca.org.]

## *RESULTS*

### *Factorial Approach and Bioavailability*

A technique commonly used when setting dietary zinc recommendations is the factorial approach which combines zinc required to replace obligatory losses with additional needs for zinc during different stages in the life cycle and makes adjustments for the bioavailability of zinc in the diet [see Table 3 in (Dhonukshe-Rutten et al., 2013)]. Additional needs for zinc during different stages of life include that required for fetal growth during pregnancy, lactation, and growth through infancy to adulthood. Literature searches were, therefore, designed to answer the following research questions: What are the key factors that affect zinc losses in all population groups? What are the key factors that affect zinc gains in all population groups? What are the additional needs for zinc during pregnancy, lactation, and for growth? How well is zinc absorbed from meals and whole diets?

All titles and abstracts were screened for potential relevance and sorted into the population groups; infants, children and adolescents, pregnant and lactating women, adults, and elderly people as defined by the EURRECA consortium [see Activity 1 in (Dhonukshe-Rutten et al., 2013)]. An EndNote library was created compiling all the papers that met the inclusion criteria. The data regarding zinc losses and gains were extracted and collated using Excel (Microsoft Office Excel 2003). The quality and the risk of bias were assessed as indicators of validity. The studies included in this review were checked for a minimum quality score system developed by the EURRECA consortium which was adapted from The Cochrane Handbook for Systematic Reviews (Higgins and Green, 2008).

### *Factors Affecting Zinc Losses and Gains*

From a total of 491 abstracts retrieved from electronic and hand searches, 105 appeared potentially relevant studies and were assessed for inclusion once the full paper had been obtained. Seventy-two papers were finally considered relevant across all population groups (adults and elderly, infants, children

**Table 3** Priority health outcomes associated with inadequate zinc intake for each population group

| Infants | Children and adolescent | Pregnant and lactating women | Adults/elderly |
|---|---|---|---|
| Growth | Growth | Fetus | Immune function |
| Immune response to vaccination | Immune function | Fetal growth | Cognitive function |
| Neurodevelopment | Cognitive functions and psychomotor development | Fetal malformation | Dermatitis |
| | Dermatitis | Mother | Anorexia |
| | | Preeclampsia | Hypogeusia |
| | | Preterm delivery | Ischemic heart disease |
| | | | Depression |
| | | | Diabetes mellitus |
| | | | Carcinogenesis |

and adolescents, and pregnant and lactating women, adults, and elderly). Despite the relatively stringent inclusion criteria, the included studies displayed a broad variety of methodological approaches, and were, therefore, unsuitable for meta-analysis. Therefore, the data extracted from the papers were tabulated and summarized narratively (Silvia Bel-Serrat et al., in progress). Overall, balance studies have shown that zinc losses and gains are a function of the initial zinc status of an individual, the amount of bioavailable zinc in the diet and are modulated by homeostatic mechanisms. That means that dietary zinc recommendations should be estimated on the basis of the target population diet making difficult the possibility of establishing a value valid for the entire population. In addition, age, physical activity level, malabsorption syndromes, and disease status can all affect zinc losses and gains. Moreover, interactions among nutrients should be also taken into account as they may also play an important role by means of affecting zinc utilization. As suggested by Taylor et al. (1991), the interaction between these homeostatic changes and zinc availability from different dietary sources should be better characterized to improve the accuracy of dietary zinc recommendations.

### *Factors Affecting Zinc Bioavailability*

The systematic review identified 120 studies as relevant to the research question of which 87 studies were conducted in adults and elderly, 2 in pregnancy and lactating women, 14 in children and adolescents, and 17 in infants. Potential modifiers of zinc bioavailability were identified as illustrated in Figure 1. Phytate was the most frequently investigated modifier of zinc absorption. Twenty four estimates from 17 studies that investigated the effect of dietary phytate level on zinc absorption were combined in a random effects meta-analysis. A forest plot showing the overall effect size of high versus low dietary phytate intake on zinc absorption is shown in Figure 2. The mean difference in fractional zinc absorption between low and high phytate diets was 0.11 (95% CI: 0.07–0.16), however, there was a high degree of between study heterogeneity ($I^2$ 94%, $P < 0.0001$). Further analysis of this data set is underway to examine the factors that contribute to this heterogeneity and the overall effect of phytate:zinc molar ratio on zinc absorption.

### *The Influence of Gene Polymorphisms Zinc Metabolism*

The primary aim of this activity was to generate a database containing relevant information related to the impact of functional gene polymorphisms on zinc metabolism. Specifically, this involved identifying data assessing the impact of functional polymorphisms (e.g., single nucleotide polymorphisms, or SNPs) on micronutrient status biomarkers and associated health outcomes. The research questions used to develop the search protocol were: How do genetic polymorphisms affect zinc status? Are there any interactions between functional polymorphisms which affect zinc status and various health outcomes? Information was collated from studies of individuals who are either homozygous, heterozygous, or wild type for specific polymorphisms. Where data exist for zinc status, functional polymorphisms, and linked health outcome, this information was also recorded. Data were collated from all population groups including infants, children, adolescents, and adults including the elderly into a database that is available at www.eurreca.org.

Of the 167 papers identified by the systematic search of the literature databases, 12 papers met the inclusion criteria and reported statistically significant results for altered zinc biomarker status in groups of people with differing gene variants. These are summarized in Table 5. The gene interleukin 6 which regulates the amount of circulating proteins involved in inflammatory responses associated with hyperglycemia and noninsulin-dependent diabetes mellitus and coronary artery disease is thought to be influenced by zinc status (Giacconi et al., 2005; Giacconi et al., 2006). Two papers reported a relationship between SNPs of the Interleukin 6 gene and plasma zinc concentration and health outcomes, including perceived stress scale, geriatric depression scale, and mini mental state examination (Mariani et al., 2008; Mocchegiani et al., 2008).

Another gene angiotensin-converting enzyme (ACE) was also reported. An impaired zinc status can alter enzyme activity, with adverse effects on angiotensin conversion from I to II affecting vasoconstriction and hypertension and subjects with the DD genotype polymorphism in the *ACE* gene have been shown to have the highest enzyme activity increasing the risk of hypertension (Tamura et al., 1996). Tamura et al. found a significant correlation between plasma zinc concentration and ACE activity in pregnant women at 33 weeks of gestation. However, as this was the only significant correlation found in this study, it was stated that the significant result may have occurred by chance.

The metallothionine gene has been included in the zinc database. Some polymorphisms of the metallothionein gene have been correlated to chronic inflammation and may affect zinc release (Richards et al., 2002; Mocchegiani et al., 2006). Another gene reported in the zinc database was apolipoprotein E (ApoE). Gonzalez et al. (1999), reported that serum zinc concentrations in epsilon 4 ApoE carriers were significantly higher in patients with Alzheimer's disease than in healthy control patients.

The TP53 mutation in exon 5 through to 8, found in esophageal squamous cell carcinoma tumors was reported in a paper by Dar et al. (2008). There is a notion that an imbalance of copper and zinc levels may lead to a higher prevalence of TP53 tumor mutations. Dar et al. found that cancer patients with the TP53 tumor mutation had lower plasma zinc levels than those with no mutation.

The glutathione-*S*-transferase (GST) gene was reported by 3 papers (Reszka et al., 2005; Reszka et al., 2007; Jin et al., 2011). The detoxifying enzyme GST metabolizes tobacco smoke derived compounds; zinc deficiency, therefore, can increase the risk of mutations occurring and can decrease the activity of the antioxidant GST enzyme increasing the risk of some cancers

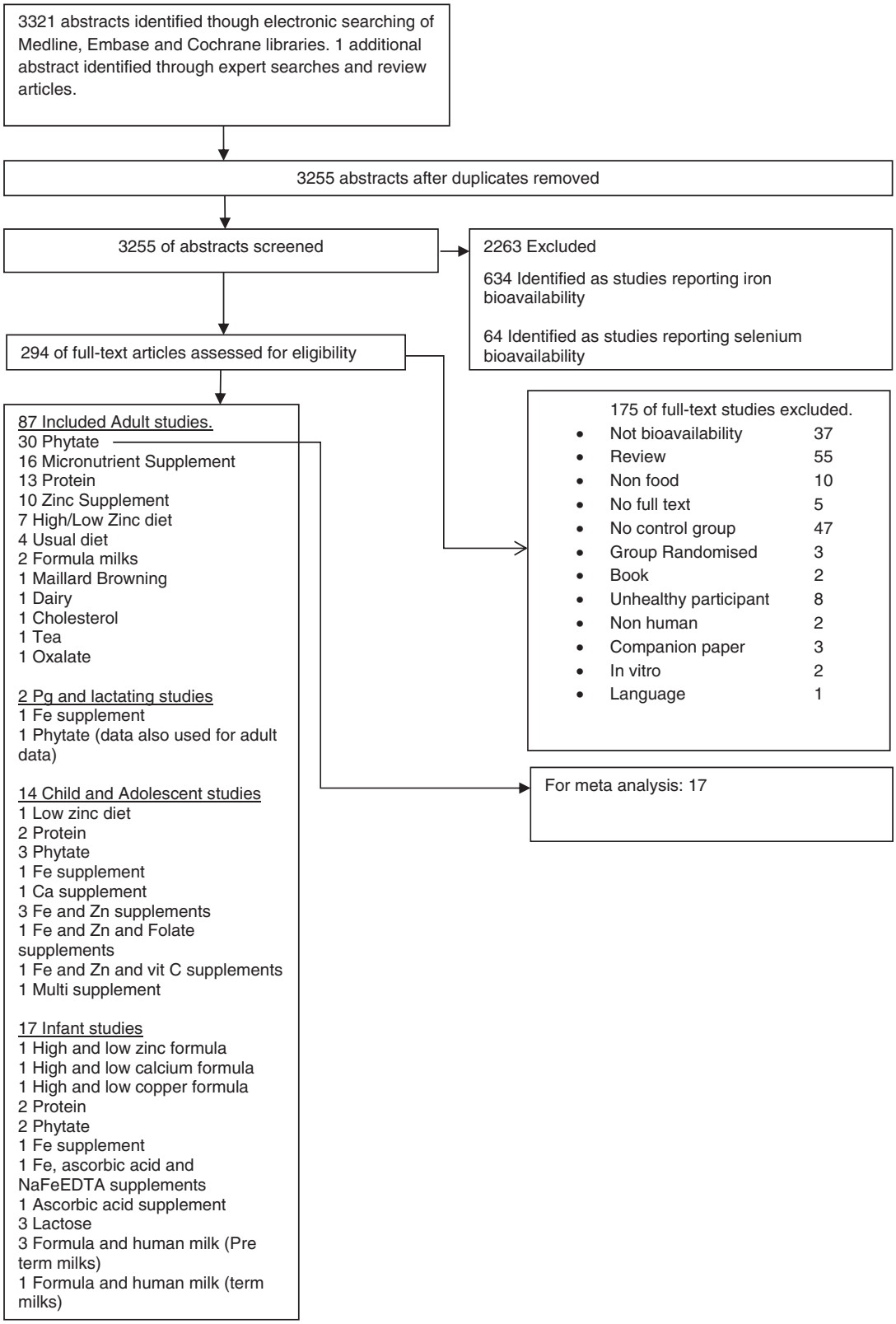


**Figure 1** Flow diagram showing the results of the systematic review of studies investigating zinc bioavailability.

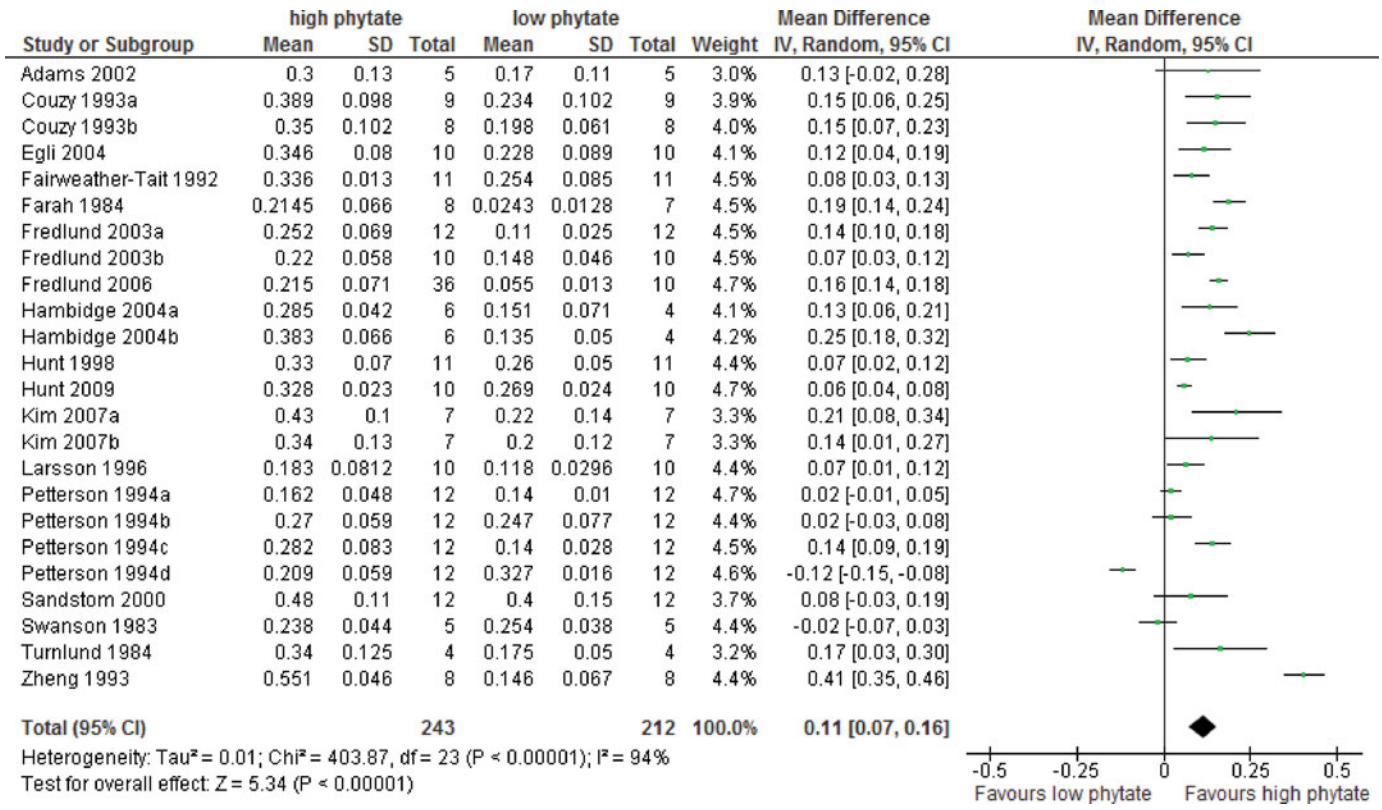


**Figure 2** Forest plot of high and low phytate meals and the impact on fractional zinc absorption. (Color figure available online.)

including lung cancer. Some polymorphisms in the GST gene may be associated with an elevated risk of lung cancer and therefore the effect of zinc status on each variant allele needs to be investigated.

The gene CYP1A1 was also investigated by Jin et al. (2011). The CYP1A1 gene has a polymorphism at exon 7 where a new Mspl restriction site is introduced. The CYP1A1 gene is thought to influence metabolic activation and detoxification of some toxins and, therefore, can increase susceptibility to increasing risk of lung cancer. Jin et al. reported that the risk of lung cancer decreased with a zinc level >1200 ng/ml for both CYP1A1 variants and CYP1A1 carriers suggesting that a higher concentration of serum zinc may protect against lung cancer.

Hemoglobin H disease (HbH) was also included in the database and was thought to influence zinc status. Zinc deficiency is thought to be involved with impaired growth and hypogonadism traits observed in patients with polymorphic diseases such as the thalassemic diseases (Ajayi, 1997; Kajanachumpol et al., 1997) and cystic fibrosis (Van Biervliet et al., 2007).

The final gene reported as having a significant association with blood biomarker zinc status is SLC30A4 gene with a polymorphism on exon 5 915 T-C. The SLC30A4 gene encodes one of the zinc transport proteins and, therefore, it is thought that a polymorphism in this gene may affect zinc absorption and fetal development. Akar et al. (2006) studied this gene and zinc status association and found that 3 h after a zinc tolerance test, there was a significant difference in plasma zinc level for TT (homozygous for T allele) and CC (homozygous for C allele) carriers, indicating a functional property of this polymorphism.

### *Intake-Status-Health Relationships*

EURRECA is developing a method for the quantitative integration of evidence for deriving nutrient intake recommendations using bivariate dose–response relationships for intake–health (I–H) as well as intake–status (I–S) and status–health (S–H) relationships. These data will be combined in a new integrated trivariate intake–status–health (I–S–H) dose–response model with data from classical nutrition studies and bioavailability factors (see Activity 6 in (Dhonukshe-Rutten et al., 2013). Search protocols were, therefore, designed to answer the following questions: What is the effect of zinc intake on functional or clinical outcomes (I–H) and what factors affect this relationship? What is the effect of zinc intake on indicators of exposure or body stores/biomarkers (I–S) and what factors affect this relationship? What is the effect of indicators of exposure or body stores (i.e., biomarkers) on functional or clinical outcome (S–H) and what factors affect this relationship?

The result of the systematic search for studies addressing zinc intake status health relationships yielded over 1000 articles that were obtained in full text for eligibility evaluation. Due to the heterogeneity of the methodological approaches and outcome

**Table 4** Results of the meta-analysis of I–S in all populations groups

| Population group | Overall beta | 95% CIs | $I^2$ | *P* |
|---|---|---|---|---|
| Adults and elderly | 0.09 | 0.07–0.12 | 79.1% | $< 0.0001$ |
| Pregnant women | 0.04 | 0.02–0.07 | 55% | $= 0.002$ |
| Lactating women | 0.02 | −0.01–0.05 | 0% | $= 0.28$ |
| Children and adolescents | 0.12 | 0.04–0.20 | 97.6% | $< 0.005$ |
| Infants | 0.09 | 0.06–0.12 | 95% | $< 0.00001$ |

measures used in these studies, it is unlikely that meta-analysis of the data will be possible.

*Intake-Status Relationships*

Sufficient high-quality randomized controlled trial (RCT) studies were identified to enable a meta-analysis of data describing the relationship between plasma zinc intake and plasma zinc concentration in each of the population categories. Units of measurement were converted to a standard form to facilitate comparison across studies. I–S regression coefficients ($\hat{\beta}$) were estimated for each individual study as described in detail elsewhere (Souverein et al., 2012). An overall pooled $\hat{\beta}$ and SE($\hat{\beta}$) was calculated using random effects meta-analysis. The statistical transformations to obtain ($\hat{\beta}$)s and SE($\hat{\beta}$)s were performed using GenStat version 13-SP2 (VSN International Ltd., http://www.vsni.co.uk/) and the meta-analysis was performed using STATA version 11.0 (College Station, TX), with statistical significance defined as $P < 0.05$.

For all population groups, with the exception of lactating women, the I–S analyses revealed a positive and significant relationship between zinc intake and plasma zinc concentration; however, a high degree of heterogeneity between the studies was observed (Table 4).

## *DISCUSSION*

The determination of dietary zinc recommendations has relied primarily on the factorial approach, with extrapolation to population groups for which data are limited or missing. A complementary approach involves examining the associations between dietary intake, status, and health, to arrive at intakes that result in optimal status levels and are sufficient to prevent disease due to deficiency at one end of the spectrum, or toxic effects due to excess dietary zinc at the other end of the spectrum. The difficulty of this approach for zinc is the lack of a reliable and sensitive marker of zinc status, and the nonspecific nature of the diseases symptoms associated with suboptimal zinc intake. However, one of the overarching aims guiding the work described in this review was to gather the best-quality data using the most robust methodology to provide a database for future panels to use when setting recommendations. This included both a comprehensive review of the data available using both the classical factorial approach and a more novel I–S–H association approach for zinc.

Regarding the factorial approach, a key factor that has been highlighted in this review and in discussion with experts is the need to consider bioavailability more closely as a modifier of the amount of dietary zinc required to meet requirements (Hambidge, 2010). This review identified a broad range of dietary components that may impact on the amount of dietary zinc that is absorbed and utilized, the majority of which had a deleterious effect on zinc bioavailability (Figure 1). Many of these food components require further studies to generate sufficient high-quality data to enable conclusive evaluation of their effect at different levels of intake; however, the most widely studied modifier of zinc absorption is dietary phytate. This systematic review and meta-analysis confirms that phytate is a potent

**Table 5** Results of the systematic search for studies of the effect of gene polymorphisms on zinc metabolism

| Author | Year | Reference | Gene with significant interaction with Zn |
|---|---|---|---|
| (Tamura et al.) | 1996 | Obstet Gynecol 88:497–502 | ACE insertion/deletion DD, DI, and II |
| (Mariani et al.) | 2008 | Experimental Gerontology 43:462–471 | MT1a +674 A/C transition and Interleukin 6 IL-6 +174C/G transition |
| (Gonzalez et al.) | 1999 | Eur J Clin Investigation 29:637–642 | Epsilon 4 ApoE |
| (Giacconi et al.) | 2005 | Biogerontology 6:407–413 | MT2A rs1610216 AA and AG, 246bp, 131, and 115bp |
| (Dar et al.) | 2008 | Nutrition and Cancer 60(5):585–591 | TP53 mutation at exon 5–8 |
| (Reszka et al.) | 2005 | Trace Elements and Electrolytes 22(1):23–32 | GSTP1, GSTT1, and GSTM1 |
| (Jin et al.) | 2011 | Cancer Epidemiology 32:182–187 | CYP1A1 mspl Aa or aa and GSTM1null |
| (Reszka et al.) | 2007 | Genes Nutr 2:287–294 | GSTM1 and GSTT1 |
| (Mocchegiani et al.) | 2008 | Experimental Gerontology 43:433–444 | IL-6 +174G/C |
| (Kajanachumpol et al.) | 1997 | Southeast Asian J Trop Med Public Health 28(4):877–880 | HbH, ß-thal/HbE, ß-thal major |
| (Ajayi) | 1997 | Trace Elements and Electrolytes 14(2):69–71 | HbAA, HbSS, HbAS, and HbAC |
| (Akar et al.) | 2006 | Trace Elements and Electrolytes 23(4):266–269 | SLC30A4 ZNT4 915T-C at exon 5 |

modifier of zinc absorption and should be taken into consideration when using the factorial approach to setting dietary zinc recommendations for any given population. Mathematical models that combine the effects of varying levels of phytate and zinc intake on true zinc absorption are potentially valuable tools in this process. A trivariate model (zinc intake-absorption-phytate intake), published by Hambidge et al. in 2010 has helped to explain much of the variability of zinc absorption from human diets (Hambidge et al., 2010). This mathematical model is based on the accepted view that zinc absorption is a carrier-mediated process, phytate inhibits absorption by binding with zinc in the gut to form an insoluble complex, and that dietary zinc and phytate are the primary dietary factors determining zinc absorption. The model predicts that the quantity of zinc absorbed from 40 mg dietary zinc at zero phytate intake is 6.4 mg Zn/d and that the dietary zinc intake required to meet the requirements for zinc doubles with every 1000 mg phytate consumed in the diet per day (Hambidge et al., 2010).

Another potential modifier of the amount of dietary zinc needed to meet the requirement is genotype (Hambidge, 2010). This has been shown to have profound effects on the bioavailability of some micronutrients such as folate and iron (Casgrain et al., 2010). Most ZnT and Zip families show evidence of polymorphisms, which could produce structurally different proteins and, hence, transporter activity and/or specificity for zinc. Such polymorphisms could influence the amount of dietary zinc needed to meet the requirements and alter zinc metabolism (Liuzzi and Cousins, 2004; Cousins et al., 2006). The systematic search for studies on micronutrient metabolism yielded a very small number of relevant studies. This is clearly an important area for future research development.

Investigation of the zinc I–S relationships in some population groups yielded some potentially useful new data. A dose–response curve was constructed from the extracted data, where the slope was based on the pooled from the meta-analysis of the RCTs expressed on a $\log_e$ scale. Reported means and standard deviations of zinc intake and zinc status were extracted from the observational studies and was used to estimate the intercept of this curve [see Activity 6 in (Dhonukshe-Rutten et al., 2013)]. The dose–response curves for the adult and elderly, and the pregnant and lactating women population groups are shown in Figures 3 and 4, respectively. These data can be used as complementary evidence for underpinning zinc reference values; however, the limitations of serum/plasma zinc concentration as a biomarker for zinc status should be acknowledged. Serum/plasma zinc is recognized as being a relatively insensitive index of zinc nutritional status due to efficient homeostatic regulation which responds to alterations in zinc intake, upregulating absorption, and conserving losses via the gastrointestinal tract and kidneys when intakes fall. In addition, whilst all studies included in the analysis were undertaken in apparently healthy individuals, factors such as stress, infection, and inflammation, which are known to affect plasma zinc concentrations, may have gone unreported. Unfortunately, more sensitive indexes of zinc status have yet to be identified and plasma serum zinc remains

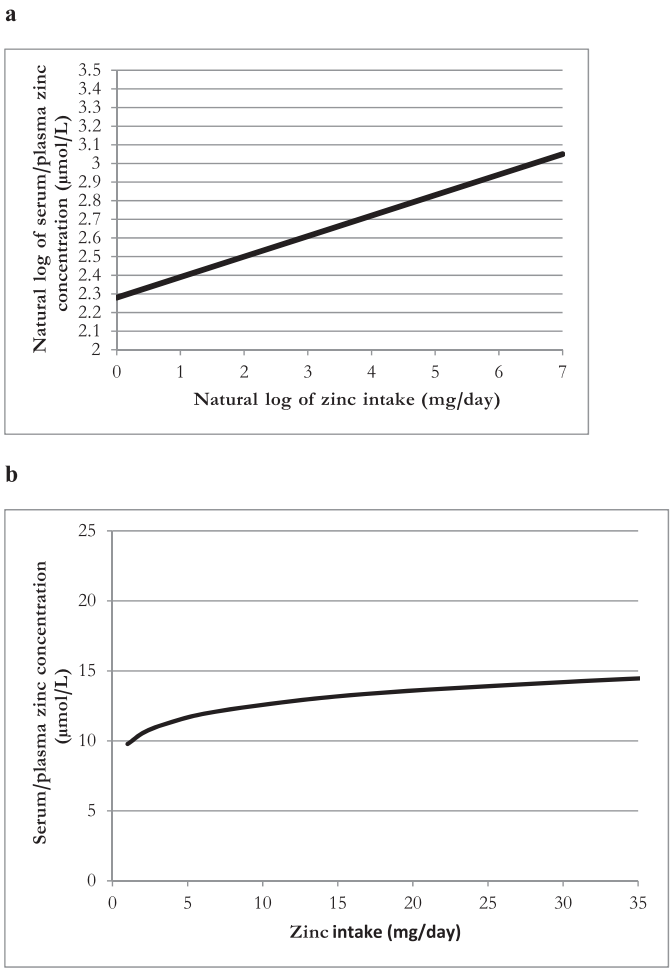


**Figure 3** Serum/plasma zinc concentration ($\mu$mol/L) as a function of dietary zinc intake (mg/d), estimated by random-effects meta-analyses of RCTs. In Figure 3a the data are presented on natural log scale, where Y = 0.11*x+2.28.

by far the most commonly used biomarker of zinc status (Lowe et al., 2009). It is anticipated that this approach may be used to model the relationships between zinc intake or status with the health outcomes for zinc, and that these can be combined with the intake status relationships described above to form a trivariate model of intake status and health [see Activity 6 in (Dhonukshe-Rutten et al., 2013)]. It is unclear at the moment whether or not our systematic searches have yielded sufficient data to enable this but it is likely that further studies are required.

This process has highlighted the need for more high quality studies to address gaps in current knowledge. Some of the key issues that came out of EURRECA workshops through discussion with experts external to the EURRECA Network focused around zinc bioavailability and the need to model the effect of inhibitors of zinc absorption such as phytate, calcium, and iron (Casgrain et al., 2010). Most current knowledge of zinc homeostasis is based on research in healthy adult males. In order to avoid scaling efforts should be made to obtain data on both genders at all ages, including pregnancy and lactation. In particular there is a paucity of data from studies in young children which

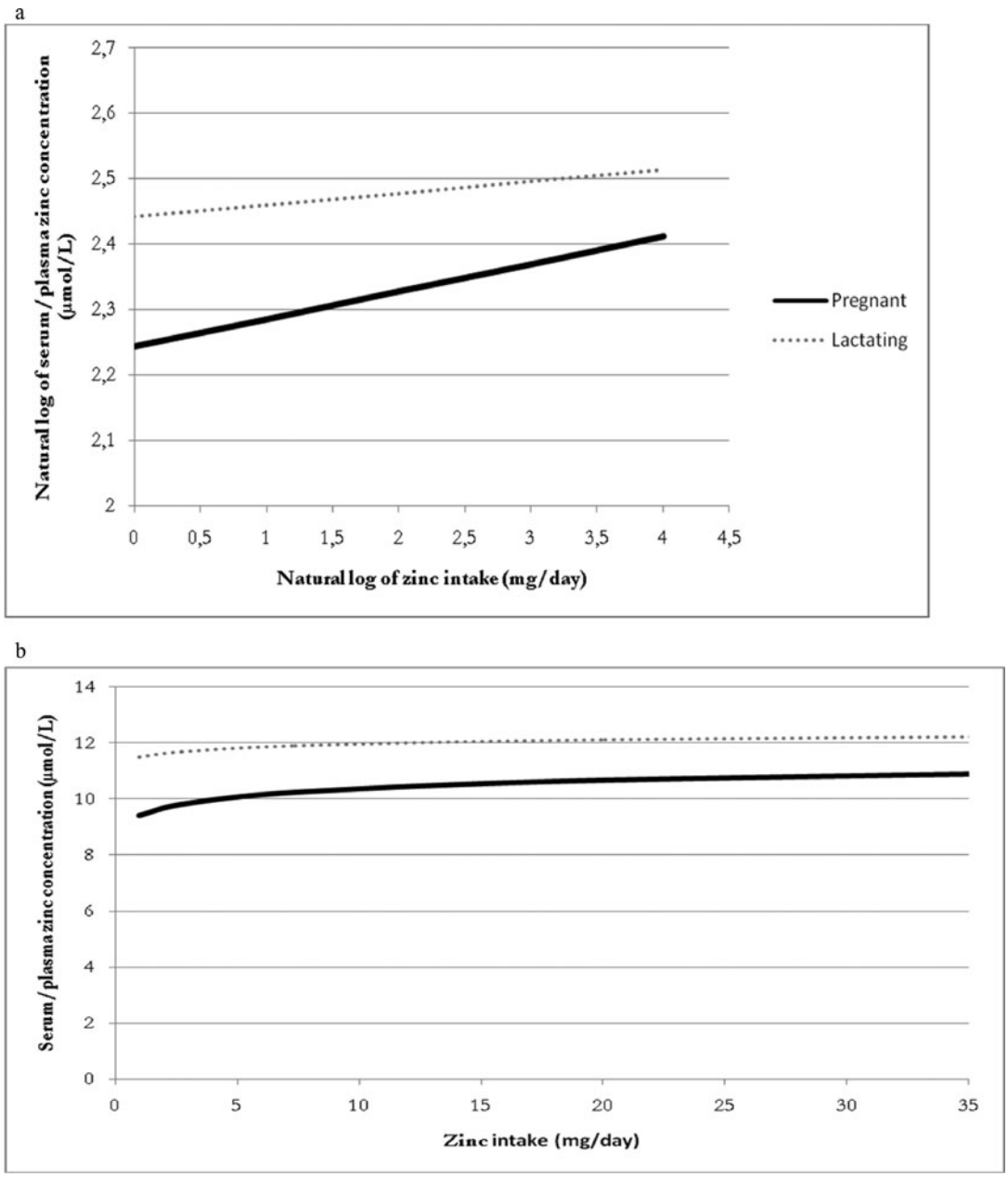


**Figure 4** Serum/plasma zinc concentration ($\mu$mol/L) as a function of dietary zinc intake (mg/d), estimated by random-effects meta-analyses of RCTs of pregnant (solid line) and lactating (dashed line) women. In Figure 4a the data are presented on natural log scale.

necessitates the use of scaling to arrive at dietary recommendations, where this is the only option, there needs to be consensus regarding which growth/weight data to use.

In summary, a series of systematic reviews and meta-analyses were conducted in accordance with the protocols and procedures developed by the EURRECA consortium. This process has gathered together information relating to the setting dietary zinc recommendations which will be available as a valuable resource for future Dietary Reference Value panels. It has also generated new I–S–H association data that may be used in combination with the classical factorial approach to model dietary zincs necessary to meet physiological requirements. This process has also highlighted the key areas for further research, in particular, the urgent need for a reliable biomarker of zinc status, the further development of models of the impact of dietary factors on zinc bioavailability and the influence of genetic polymorphisms on individual dietary requirements.

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## *ABBREVIATIONS*

ACE = Angiotensin-converting enzyme
ApoE = Apolipoprotein E
EAR = Estimated Average Requirement
EURRECA = European Micronutrient Recommendations Alligned
DNA = Deoxyribonucleic acid
FFQ = Food frequency questionnaire
GST = Glutathione-*S*-transferase
HbH = Haemoglobin H
RA = Research activity
RCT = Randomised controlled trials
RNA = Ribonucleic acid
SES = Socioeconomic status
SNP = Single nucleotide polymorphism
WHO = World Health Organisation

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