Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Trace Elements in Medicine and Biology 20 (2006) 3–18
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

INVITED REVIEW

# Zinc requirements and the risks and benefits of zinc supplementation

Wolfgang Maret[a,b,*], Harold H. Sandstead[a]

[a]*Department of Preventive Medicine and Community Health, Division of Human Nutrition, University of Texas Medical Branch, 700 Harborside Drive, Galveston, TX 77555, USA*
[b]*Department of Anesthesiology, University of Texas Medical Branch, 700 Harborside Drive, Galveston, TX 77555, USA*

Received 8 December 2005; accepted 28 January 2006

## Abstract

The adult human contains 2–3 g of zinc, about 0.1% of which are replenished daily. On this basis and based on estimates of bioavailability of zinc, dietary recommendations are made for apparently healthy individuals. Absent chemical, functional, and/or physical signs of zinc deficiency are assumed indicative of adequacy. More specific data are seldom available. Changing food preferences and availability, and new food preparation, preservation, and processing technologies may require re-evaluation of past data. Conservative estimates suggest that $\geqslant$25% of the world's population is at risk of zinc deficiency. Most of the affected are poor, and rarely consume foods rich in highly bioavailable zinc, while subsisting on foods that are rich in inhibitors of zinc absorption and/or contain relatively small amounts of bioavailable zinc. In contrast, among the relatively affluent, food choice is a major factor affecting risk of zinc deficiency. An additional problem, especially among the relatively affluent, is risk of chronic zinc toxicity caused by excessive consumption of zinc supplements. High intakes of zinc relative to copper can cause copper deficiency. A major challenge that has not been resolved for maximum health benefit is the proximity of the recommended dietary allowance (RDA) and the reference dose (RfD) for safe intake of zinc. Present recommendations do not consider the numerous dietary factors that influence the bioavailability of zinc and copper, and the likelihood of toxicity from zinc supplements. Thus the current assumed range between safe and unsafe intakes of zinc is relatively narrow. At present, assessment of zinc nutriture is complex, involving a number of chemical and functional measurements that have limitations in sensitivity and specificity. This approach needs to be enhanced so that zinc deficiency or excess can be detected early. An increasing number of associations between diseases and zinc status and apparently normal states of health, where additional zinc might be efficacious to prevent certain conditions, point at the pharmacology of zinc compounds as a promising area. For example, relationships between zinc and diabetes mellitus are an area where research might prove fruitful. In our opinion, a multidisciplinary approach will most likely result in success in this fertile area for translational research.


***Keywords:*** Zinc; Requirements; Supplementation

## Introduction

The ranges of intake for nutritionally essential elements are usually discussed in the framework of a simple model of adverse health effects if intake is either too low (deficiency) or too high (toxicity). As discussed

*Corresponding author. Tel.: +409 772 4661; fax: +409 772 6287.
*E-mail address:* womaret@utmb.edu (W. Maret).

0946-672X/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2006.01.006

here, defining the range where the intake of zinc is only beneficial (acceptable range of oral intake, AROI) is a multidimensional problem and a major challenge.

Zinc occurs in hundreds of zinc enzymes and in thousands of protein domains. Enumerating and discussing the catalytic, structural, and regulatory functions of zinc in these proteins is far beyond the scope of this article. However, one needs to be aware of the great number of zinc-dependent biological processes and interactions in order to appreciate the significance and implications that dietary imbalances of this element will have.

Zinc is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis. Examples of functions that require zinc include immunity, intermediary metabolism, DNA metabolism and repair, reproduction, vision, taste, and cognition/behavior [1–11]. In addition, zinc is essential for neurogenesis, synaptogenesis, neuronal growth, and neurotransmission [12–15]. It is stored in specific synaptic vesicles by a class of glutaminergic neurons and released as a neuro-modulator in an activity-dependent manner [16].

One of the major advances in the last decade has been the recognition of a homeostatic system of proteins that control cellular zinc by coordinating zinc import and export, distribution, and sensing of zinc status. The involvement of so many proteins in homeostatic control increases the potential for variations of zinc metabolism due to mutations in these proteins. For example, acrodermatitis enteropathica, a genetic disorder of zinc absorption in humans and a fatal disease if untreated with zinc, is caused by a mutation in the zinc transporter hZip4 [17,18].

In spite of knowledge about so many functions of zinc, it is not understood whether or not these functions are hierarchical in terms of zinc utilization. If zinc becomes increasingly limiting, are all zinc-dependent functions affected to the same extent or are some functions compromised for the sake of preserving homeostasis? Without an answer to this question it is impossible to evaluate the relative significance of different clinical or functional tests for zinc deficiency.

## Zinc intake

Recommendations are based on measured requirements. Mean daily dietary zinc intakes of populations from several countries range from 4.7 to 18.6 mg [19]. In the United States, the third National Health and Nutrition Examination Survey (NHANES-III) reported median zinc intakes for whites, blacks, and Hispanics of different ages and gender (Table 1) [20]. Elderly aged $>69$ yr are apparently at increased risk of zinc deficiency. According to the US Department of Agriculture 1994–1996 Continuing Survey of Food Intakes by Individuals, mean daily zinc intakes of men and women $>20$ yr were 13.5 and 9.0 mg, respectively [21]; those of subjects $\geqslant 60$ yr were 12.0 mg in men and 8.0 mg in women [22], and in children, aged $<1$ yr, 1–3 yr, and 4–5 yr, they were 6.6, 7.6, and 9.1 mg, respectively [23]. The 2000–2001 United Kingdom National Diet and Nutrition Survey of adults, aged 19–64 yr, found zinc intakes of $10.7 \pm 5.7$ mg (males) and $7.9 \pm 3.5$ mg (females) [24]. British children aged 15–18 yr had zinc intakes that were similar to adults [25], while in children aged 11–14 yr intakes were 7.7 mg in boys and 6.7 mg in girls.

Consumption of nutritional supplements can substantially increase zinc intake. Supplement consumption is common in the USA. The third National Health and Nutrition Examination Survey found that about 40% of the population takes supplements. Among adults $\geqslant 60$ yr, 35–41% of men and 36–45% of women consumed dietary zinc that is inadequate by current standards, and supplements improved intakes [26]. Among US infants, aged 24 m, nearly 32% were given supplements while the majority had diets adequate in most vitamins and minerals including zinc [27]. In contrast, about 6% of young Germans aged 2–18 yr consumed supplemental minerals [28]. Effects of supplemental zinc when the dietary intake is adequate are incompletely understood; they are discussed below.

While average intakes are adequate in many countries, all populations have groups at risk of deficiency. Some of the factors that contribute to risk include poverty, limited food availability, and food preferences. Widespread zinc deficiency has serious implications for health and productivity. Prevention of zinc deficiency is a major challenge.

## Zinc in food

Zinc nutriture is based on the quantity and bioavailability of zinc in food. The zinc content of some common US foods varies by at least one order of magnitude (Table 2) [29]. Worldwide, pulses and cereals are the major sources of zinc for most people [30]. In the US, pulses and cereals provide about 30%, meat about 50%, and dairy products about 20% of dietary zinc [31]. Usually, pulses are richer in zinc than refined cereals.

Flesh foods are the most important dietary sources of readily bioavailable zinc. Red meat is the richest common source of zinc while fowl and fish usually provide substantially less zinc (Table 2). Avoidance of red meat by young women was partly responsible for their being both zinc and iron deficient [32–34].

**Table 1.** Median zinc intake (mg/d) of participants in NHANES-III [20]

| | White | | Hispanic | | Black | |
|---|---|---|---|---|---|---|
| | *N* | Median | *N* | Median | *N* | Median |
| *Male* | | | | | | |
| 2–11 months | 241 | 5.59 | 89 | 6.32 | 78 | 6.28 |
| 1–2 yr | 202 | 6.67 | 186 | 5.81 | 182 | 6.74 |
| 3–5 yr | 219 | 7.28 | 281 | 7.80 | 210 | 8.13 |
| 6–11 yr | 252 | 9.02 | 344 | 9.27 | 239 | 9.17 |
| 12–15 yr | 98 | 11.62 | 129 | 10.48 | 95 | 8.91 |
| 16–19 yr | 112 | 13.43 | 139 | 12.04 | 103 | 12.28 |
| 20–29 yr | 216 | 13.14 | 349 | 13.27 | 245 | 12.90 |
| 30–39 yr | 271 | 13.88 | 225 | 13.19 | 213 | 10.77 |
| 40–49 yr | 243 | 12.25 | 181 | 12.35 | 178 | 10.55 |
| 50–59 yr | 251 | 12.27 | 96 | 9.73 | 105 | 8.40 |
| 60–69 yr | 247 | 11.52 | 152 | 8.71 | 141 | 8.77 |
| 70–79 yr | 285 | 10.34 | 60 | 8.13 | 93 | 7.84 |
| 80 yr + | 250 | 9.06 | 19 | 7.74 | 21 | 7.04 |
| *Female* | | | | | | |
| 2–11 months | 232 | 5.55 | 74 | 5.89 | 84 | 5.80 |
| 1–2 yr | 222 | 5.65 | 216 | 5.72 | 173 | 5.69 |
| 3–5 yr | 206 | 6.48 | 328 | 6.79 | 244 | 7.47 |
| 6–11 yr | 259 | 7.70 | 383 | 8.07 | 213 | 8.12 |
| 12–15 yr | 123 | 8.07 | 140 | 8.65 | 96 | 8.53 |
| 16–19 yr | 133 | 8.38 | 131 | 8.62 | 114 | 9.37 |
| 20–29 yr | 244 | 8.41 | 317 | 8.80 | 254 | 8.86 |
| 30–39 yr | 279 | 8.78 | 247 | 8.39 | 241 | 7.55 |
| 40–49 yr | 224 | 8.55 | 185 | 7.80 | 160 | 7.16 |
| 50–59 yr | 221 | 7.94 | 100 | 8.13 | 125 | 7.01 |
| 60–69 yr | 246 | 7.71 | 153 | 6.80 | 148 | 6.93 |
| 70–79 yr | 253 | 7.18 | 51 | 6.42 | 93 | 6.37 |
| 80 yr + | 251 | 6.59 | 23 | 5.26 | 35 | 5.92 |

**Table 2.** Zinc content of foods commonly consumed in the United States; per common measure in milligrams [29]

| >15 | 5–10 | 4–5 | 3–4 | 2–3 | 1–2 | <1 |
|---|---|---|---|---|---|---|
| Oyster | Beef | Beef liver | Lamb | Lamb | Pork loin | Chicken breast |
| Peanut Butter Crunch® | Lamb | Beef | Pork | Pork | Chicken dark meat | Chicken liver |
| Product 19® | Duck | Lamb | Veal | Lobster | Sword fish | Salmon |
| Total® | King Crab | Pork | Turkey dark meat | Clam | Shrimp | Tuna |
| | Wheaties® | Capitan Crunch® | Blue Crab | Yogurt | Mushroom | Other finfish |
| | | Quaker Oats® | Rice Chex® | Skim milk | White wheat flour | Vegetables |
| | | | Corn Chex® | White bean | Navy bean | White rice |
| | | | Cheerios® | Chick pea | Black bean | Egg |
| | | | Whole wheat flour | Lentil | Pinto bean | Tofu |
| | | | | Corn meal | All Bran® | Cheddar cheese |
| | | | | | Nuts | Blue cheese |
| | | | | | | Cottage cheese |
| | | | | | | Nuts |

Individuals who shun certain foods increase their risk of zinc deficiency. For example, a preference for poultry, fish and dairy products instead of red meat increases the risk of zinc deficiency [35] as does a purely vegetarian diet. Thus, in affluent societies, food selection is a major influence on zinc nutriture. For example, a constant omnivorous diet based on white poultry meat and finfish provided insufficient zinc for adult men [36].

Bioavailability is influenced by other food constituents. Non-digestible plant ligands such as phytate, some dietary fibers, lignin, and products of Maillard reactions bind zinc, inhibit its absorption, and thus affect risk of dietary (primary) zinc deficiency [37]. Calcium can also inhibit zinc absorption, and augments the inhibition of zinc absorption by phytate [38,39]. Thus the dietary phytate:zinc, and phytate × calcium:zinc molar ratios are predictive of the risk of zinc deficiency [39,40]. Other factors that affect zinc bioavailability include high concentrations of ferrous iron in iron supplements [41], and pharmacological intakes of folic acid [42,43]. Bioavailability of zinc from supplements ranges from very low, e.g. zinc oxide, to relatively high, e.g. zinc salts such as zinc acetate. The bioavailability of zinc from mixed omnivorous Western diets of common foods is about 20–30% [44].

In this article, we focus on food as the primary source of zinc. We will not address the effects of zinc exposure in the work place including inhalation of metallic zinc and solid zinc compounds, or topical applications of zinc compounds.

## Zinc deficiency

### Dietary (primary) deficiency

Zinc deficiency was first described in Iranian and Egyptian farmers [45,46]. According to an analysis of data from the Food and Agricultural Organization, the prevalence might be as high as 40% worldwide [47].

### Conditioned (secondary) deficiency

Zinc deficiency also occurs secondary to diseases that impair intestinal absorption and/or increase intestinal loss of zinc, e.g., acrodermatitis enteropathica, sprue, cystic fibrosis, other intestinal malabsorption syndromes [48], inflammatory bowel diseases [49] such as Crohn's disease [50,51], hemolytic anemias such as sickle cell disease [52,53] and thalassemia [54], chronic bleeding from hookworm and other intestinal parasites [55], menorrhagia [32,33], chronic increased urinary zinc loss as may occur in some renal diseases [56], cirrhosis of the liver [57–60], alcoholism [61], stress [62], catabolism [63], and chronic inflammatory diseases that increase interleukin-1 [64–66].

## Physical signs of zinc deficiency

In children and adolescents, poor growth and retarded development may be evident long before other signs of zinc deficiency are recognized. It seems that one of the early manifestations of zinc deficiency is suppression of aspects of cell-mediated immunity [67–69]. In contrast, dermatitis appears to be a later manifestation as the severity of zinc deficiency increases. The severe dermatitis affects the peri–oral–facial, peri–anal–perineal–scrotal, and peri–ungual areas as is characteristic for "acrodermatitis" in acrodermatitis enteropathica [70,71]. Any or all of these surfaces may be infected [70–73]. Atrophy of lingual papillae, which is commonly associated with severe iron deficiency [74], may also occur. Patients may display poor healing of cutaneous wounds [75–79] without other obvious signs of zinc deficiency; hair may be easily plucked and alopecia evident; black hair may be changed to a reddish brown. Effects on the nervous system may include decreased nerve conduction [80], ataxia, disorientation [81], and impaired neuropsychological performance [82]. Initial manifestations of zinc deficiency are non-specific and unlikely to suggest zinc deficiency unless the history of the patient alerts the caregiver to the possibility. After deficiency has been present for some time other manifestations may be recognized. They include retarded genital development and hypogonadism [46,83–86], poor pregnancy outcomes and teratology [87–93], high morbidity and death from diarrhea, pneumonia, and other infections [94], and impaired brain function [71,95–96]. No sign is pathognomonic.

## Biomarkers of zinc status

### Plasma (serum) zinc

Plasma or serum zinc is the most frequently used index for evaluating the likelihood of zinc deficiency [97–100]. Values vary diurnally, decrease after meals, and appear related to gender and age. The lower limit of normal (morning) fasting plasma zinc has been set at 10.7 μmol/L (700 mg/L). The relation of this value to diet history, zinc kinetics, and physiological function in premenopausal women [33] suggests that a cutoff value of 11.5 μmol/L (750 mg/L) might be a more reliable index of status. Plasma and serum zinc concentrations are convenient indices, but physiologically insensitive [36,45,101–102] as they may not necessarily reflect the cellular zinc status. Plasma concentrations were maintained within the accepted normal range for several weeks to months even though diets provided only 2.6–3.6 mg/d (40–55 μmol/d) [36,103], amounts of zinc that are inadequate for neurobiological function [104].

### White blood cell zinc and immunological markers

Zinc in leukocytes or lymphocytes is substantially more reflective of zinc status and associated functions, e.g., growth at all stages of the life cycle and immunity, than plasma zinc [105]. Thus leukocyte, but not plasma

zinc, was reflective of fetal growth and related to maternal muscle zinc concentration [87]. Lymphocyte ecto 5′-nucleotidase activity is more sensitive to zinc status than plasma 5′-nucleotidase activity or plasma zinc [106–108]. A conceptually different approach employs gene expression of zinc-dependent genes in lymphocytes as a bioassay for zinc status [109]. The authors observed that expression of the cellular zinc transporter hZip1 decreased by 17% when humans were supplemented with 22 mg/d zinc gluconate for 27 days.

The CD4+/CD8+ T-lymphocyte ratio was advanced as a robust immunological test for zinc deficiency [110]. Also, very mild zinc deficiency inactivates the peptide hormone thymulin by making zinc unavailable for binding to the hormone, and thus impairing immunity without causing thymic atrophy [111], which is a manifestation of zinc deficiency [112]. Thymulin mediates T-cell differentiation and various T-cell subset functions [113]. Low plasma thymulin activity was found in elderly subjects with normal plasma and low leukocyte zinc [114].

### Red blood cells

Human erythrocyte metallothionein responds to zinc supplementation (50 mg/d) and dietary restriction [115].

### Clinical vs. functional markers

Zinc deficiency can also be assessed by demonstrating effects of supplemental zinc on physiological functions. In laboratory medicine, abnormalities of chemical values often precede functional and physical signs of illness. This is *not* true for plasma (or serum) zinc, the most commonly measured index of zinc status. Functional effects may be observed in the absence of usual laboratory indices, thereby indicating the inadequacy of such indices. Parameters of physiological function include growth, body composition, cell-mediated immunity, neurobiological performance/cognition, neuromotor function, dark adaptation, taste and smell acuity, and conduction of gustatory nerves. However, these indices are not specific for zinc deficiency and therefore they are of limited diagnostic value in the absence of controlled observation. In the natural world, single nutrient deficiencies are rare. Zinc, iron and other micronutrient deficiencies often occur together. Demonstration of functional effects of zinc deficiency in humans is best achieved through prospective controlled dietary studies or treatment trials in which *all* other nutrients and energy intake are adequate.

In conclusion, experiments and clinical observations have shown serum/plasma zinc to be insensitive of zinc status. Effects of zinc deficiency on specific functions are often apparent before plasma zinc decreases. More specific markers of zinc status are needed, and their relationships to zinc-dependent cellular functions and the distribution and allocation of zinc to the different organ systems need to be clarified.

## Zinc excess

In as much as zinc has multiple essential functions, it also has the potential to interact with at least as many biological functions to induce adverse effects.

Concentrations of zinc in blood plasma or serum, urine, and hair may increase when exposures are high, but their measurement is not a standardized procedure to confirm exposure.

In rats, the oral $LD_{50}$ for zinc salts is 237–623 mg/kg, the intraperitoneal injection $LD_{50}$ is 28–73 mg/kg [19,116], and the inhalation $LD_{50}$ for zinc chloride is 2000 mg/m$^3$ [19,117]. In humans, these doses for acute toxicity may be achieved only under the most unusual circumstances. High concentrations of zinc in drinks, up to 2500 mg/L with an estimated dose of 325–650 mg, have been linked to poisoning of individuals, causing nausea, abdominal cramping, vomiting, tenesmus, and diarrhea with or without bleeding [19,118]. Acute toxicity from consumption of contaminated drink or food is unusual. We found no scientific reports that clearly implicate natural or anthropogenic sources of environmental zinc in food and drink as human health hazards.

Excess zinc during embryogenesis can be teratogenic or lethal [19]. However, much more subtle effects are suggested by recent investigations that reach back to classic work in nutrition by Bacon F. Chow and colleagues. They demonstrated that nutritional deficiencies in the maternal diet can have permanent negative effects on the growth of offspring [119]. Rehabilitation is possible only if begun in utero, and even then, only to a certain degree. The studies of David Barker and colleagues have brought to the attention of many scientists relationships between low birth weight and chronic diseases later in adult life [120]. Molecular sciences have now uncovered that such generational effects are expressed by epigenetic mechanisms such as DNA methylation. Even after zinc repletion in the offspring of rats, altered immune functions persist in subsequent generations when a marginally zinc-deficient diet is fed during gestation [121–123], clearly showing that maternal nutrition alters the programming of fetal genes. Recent studies of maternal epigenetics and methyl supplements, including diets that contain zinc supplements [124,125], are ‘‘the first report of an effect of dietary methyl supplements on gene imprinting and specific gene expression’’ and it was concluded that the investigation ‘‘demonstrates that the diet influences mechanisms of epigenetic regulation, imprinting, and development.’’ The authors suggest that ‘‘dietary

supplementation, long presumed to be purely beneficial, may have unintended deleterious influences on the establishment of epigenetic gene regulation in humans.'' These data clearly suggest that intrauterine and postnatal environment influences health in adulthood, and they serve as a warning that both zinc deficiency and excessive zinc supplementation might have adverse long-term effects on the epigenome.

Zinc is apparently neither a mutagen nor a carcinogen [19,116]. However, what should be of great concern is that studies in rats found that zinc deficiency causes precancerous esophageal epithelial hyperkeratosis, parakeratosis, acanthosis and hyperplasia of basal cells [126–128]. Zinc deficiency also facilitates induction of *N*-nitrosomethylbenzylamine-induced esophageal cancers [129] that were prevented by zinc administration. While zinc deficiency also increases susceptibility of fore-stomach and tongue to cancer induced by the carcinogen 4-nitroquinoline-1-oxide [130], an increase in cancer in other tissues has not been reported.

One of the established effects of chronic zinc toxicity is that oral zinc intakes disproportionately high relative to copper are a conditioning factor to induce copper deficiency. In humans, multiple adverse effects include decreases in copper-dependent enzymes such as superoxide dismutase, ceruloplasmin, and cytochrome c oxidase, and changes in immunological parameters, cholesterol, and its lipoprotein distribution (Table 3). The hematological complications of copper deficiency are well documented. More recent work calls attention to neurological manifestations of copper deficiency secondary to high intakes of zinc (Table 3). The thresholds for the observed effects are unknown from these studies, emphasizing the need for additional

**Table 3.** Zinc/copper interactions affecting the nutritional copper status in humans

| *N* | Sex | Time days | Cu intake (mg/d) | Zn intake (mg/d) | Zn:Cu | Diet | Response[a] | Ref. |
|---|---|---|---|---|---|---|---|---|
| 7 | F | 165 | 1.25 ± 0.20 | ? | | | Nil | [131] |
| 24 | M | 77 | 1.03/2850 kcal | ~25 | 24.3 | 20% Fructose vs 20% Starch | 4/24 abnormal ECG, ↓ESOD, ↑fructose, ↓pl Leu- & Met-enkephalins, ↑pl beta-endorphins, ↑cholesterol | [132–134] |
| 7 | M | 108–120 | 0.89 ± 0.09 | 19 | 21.3 | | 4/7 ↓ESOD, 2/7 ↓plCu,, 1/7 ↓ENZCp, 2/7 ↓oral glucose tolerance test | [135,136] |
| 1 | M | 105 | 0.79 | 19 | 24 | | Abnormal ECG, ↓ESOD, ↓plCu, ↑cholesterol | [137] |
| 11 | M | 42 | 0.79 | 14.7? | 18.6 | Food formula, vitamins | Nil | [138] |
| 8 | F | 42 and 42 with vit C | 0.63–0.76/ energy | 8.5 + diet | ~25 | Food formula, 50 mg Fe | ↓ENZCp, ↓MNC CCO, ↑stress blood pressure | [139,140] |
| 10 | M | 36 | 0.6–0.7 | | | | ↓ENZCp, 6/10 ↓MNC CCO, 4/10 ↑glutathione | [141] |
| 6 | M | 49 | 0.6 | | | High fructose | 3/6 abnormal ECG, ↓plCu, ↓ESOD, ↑glutathione, ↑fructose | [142,143] |
| 12 | F | 105 | 0.64/2500 kcal | 12/2500 kcal | 18.8 | Food formula | 3/12 abnormal ECG; ↓Plt CCO, ↓ESOD, ↓PltCu, ↓EGPX, ↑clotting factors V&VIII, no anemia or leukopenia | [144] |
| 11 | M | 42 | 0.38 | 14.7 | 38.7 | Food formula, vitamins | ↓plCu, ↓ENZCp | [145] |
| 13 | F | 90 | 1.0/2000 kcal | 53 | 53 | | ↓ short term general recall, ↓plCu, ↓ENZCp, ↓Plt CCO | [146] |
| 1 | F | 365 or more | | 200–400 | | | ↓plCu, ↓ENZCp, ↑plZn, ↓Hgb, ↓leukocytes, myelopathy similar to ''combined systems disease'' of vitamin $B_{12}$ deficiency | [147] |

[a] *Abbreviations*: ECG, electrocardiogram; ESOD, erythrocyte superoxide dismutase; plCu, plasma copper; ENZCp, enzymatic activity of ceruloplasmin; MNC (Plt) CCO, monocyte (platelet) cytochrome c oxidase; EGPX, erythrocyte glutathione peroxidase; plZn, plasma zinc; Hgb, hemoglobin.

research on the zinc/copper interaction and its clinical significance. In dietary and supplemental intakes, zinc and copper should be proportionate [148].

## Requirements

A 70 kg adult contains about 2–3 g zinc. The amount of zinc needed daily is relatively small, about 2–3 mg in adults, i.e. only 1/1000 of the total is renewed daily, in agreement with a biological half-life of zinc of about 280 days [149].

Factorial calculations suggest healthy adults have an absolute need for 2–3 mg zinc per day to compensate for the relatively small loss of zinc in urine, stool, and sweat [37]. In the previous recommended dietary allowance (RDA) [150], this approach and results of balance studies led to recommendations for zinc requirements that were higher than the current RDA in the USA. The current RDA of the Food and Nutrition Board [151] was derived using different methodology and assumptions. Recommendations for men are 11 mg, and for women are 8 mg. These values are thought to be adequate for 97–98% of the US population. Noteworthy, the remaining 2–3% corresponds to 5–7.5 million Americans that could be at risk [152]. Identification of this sub-population is an important health issue. The Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, and Schweizerische Vereinigung für Ernährung (DACH) recommend 10 and 7 mg, respectively [153].

In addition to gender, recommendations are stratified for age and conditions of greater metabolic need such as pregnancy and lactation. Lower values are given for younger individuals. For vegetarians, the requirement is at least 50% higher because zinc in not readily available from a vegetarian diet [154]. Zinc requirements for pregnant and nursing women are also higher. An increase of the daily intake by 4 and 3 mg, respectively, is recommended. However, recommendations do not specifically consider effects of diets rich in inhibitors of zinc absorption on requirements of healthy people. Effects of disease on requirements are not within the purview of the RDA. Resolution of these issues is an important task for the future.

## Approaches to establish requirements

Several approaches have been used to derive human requirements for zinc. A traditional but demanding method involves measurement of metabolic balance. The method involves feeding constant diets of similar foods, which provide several levels of zinc intake, to a group of subjects that agree to consume all of the diet and to collect all excreta. This is best achieved in a highly controlled environment such as is possible at some clinical research centers. Total input and output are determined precisely and the input necessary for equilibrium is determined by regression analysis of the data. Because the method is fraught with the potential for errors, reliable balance data are difficult to obtain. Thus the method is not used except at a few research centers with high technical expertise. Another drawback of the method is that it is highly expensive both in time and money. Thus few reports include a sufficient number of subjects to lend high confidence to the findings.

An alternative to the balance approach is estimation of requirements by the factorial method, which estimates dietary requirements based on likely losses and anabolic needs (Table 4). Table 4 also gives calculated amounts required if the percentage of bioavailable zinc is 20% or 30%, and if the coefficient of variation (CV) of the absolute requirement is 15%. The intent of such an estimate would be to suggest a recommended daily intake that meets the needs of nearly all adults. Because the actual CV of the requirement is unknown, selection of the number is a critical issue in determination of an RDA, which by convention is two standard deviations above the estimated requirement.

Most recently, the difficulties associated with measurement of chemical balance and factorial estimates prompted the use of radioisotopes and stable isotopes of zinc to determine the amount of zinc needed to replace losses. The method determines the fractional retention (net retention) of orally administered isotopic zinc tracers. Again, discussion of the methodology is beyond the scope of this article. Table 5 shows zinc absorption data obtained mostly by methods using the radioisotope $^{65}$Zn. When stable isotopes ($^{67}$Zn, $^{68}$Zn, and $^{70}$Zn) are used, the amount of absorbed zinc is determined by mass-spectroscopic measurement of the urinary excretion of an orally administered zinc tracer relative to excretion of an intravenously administered, different zinc isotope tracer [164–166]. The 2002 US Zinc Recommended Dietary Allowance (RDA) issued by the National Research Council [155] was derived by this approach and is given together with estimates by committees in other countries (Table 6). With the exception of the recommendation by the World Health Organization (WHO) [155], the recommendations do not include adjustments for bioavailability. In addition, it is unclear which CV for the absolute requirement should be used. This is important because the lower the CV, the lower the calculated RDA.

## Reference dose (RfD)

The US Environmental Protection Agency [168] used data from Yadrick et al. [169] on the effects of zinc on copper and iron absorption as the basis for a

**Table 4.** Comparison of factorial estimates of adult zinc requirements [37]

| Source available | Group | Absolute need[a] (mg/d) | Need if 20% available[b] | 1.3[c] × 20% available | Need if 30% available | 1.3 × 30% available |
|---|---|---|---|---|---|---|
| WHO[d] [19] | Adults | 2.2 | 11.0 | 14.3 | 7.3 | 9.5 |
| King[e][156] | Adults | 2.5 | 12.5 | 16.3 | 8.3 | 10.8 |
| UK [157] | Men | 2.2 | 11.0 | 14.3 | 7.3 | 9.5 |
| | Women | 1.7 | 8.3 | 10.8 | 5.5 | 7.2 |
| Canada [158] | Men | 2.1 | 10.5 | 13.7 | 7.0 | 9.1 |
| | Women | 1.8 | 9.0 | 11.7 | 6.0 | 7.8 |

[a]Absolute (minimal) need: factorially calculated "absolute" requirement.
[b]Available: amount of zinc (mg/d) 'bioavailable', i.e., absorbed and utilized by the body each day.
[c]The factor 1.3 refers to the amount of zinc bioavailable plus two 15% coefficients of variation ($2 \times 15 = 30\%$) of the amount bioavailable.
[d]The WHO estimate was based on the assumption that the zinc concentration of fat-free tissue is 30 μg/g, i.e. equivalent to 2.0 and 1.2 g of total zinc in soft tissue of a man or a woman, respectively. Bone zinc was not included in the calculation because it is relatively sequestered from the exchangeable zinc pool. The zinc content of sweat was based on a surface loss of 1 mg/L. The urinary excretion of zinc was based on reported normal levels.
[e]Based on more recent data than the WHO estimate.

**Table 5.** Fractional zinc absorption from total diets as measured by isotope techniques [159]

| Subjects | Isotope | Diets | Zinc content, μmol (mg) | Phytate:zinc, molar ratio | Zinc absorption, % ($x \pm$SD) |
|---|---|---|---|---|---|
| Young adults [160] | Radio | High fiber | 163 (10.7) | 7 | $27 \pm 6$ |
| Young women [161] | Stable | Habitual | 124 (8.1) | 10 | $34 \pm 9$ |
| Women (20–42 yr) [162] | Radio | Lactoovo-vegetarian | 139 (9.1) | 14 | 26 pooled SD = 5 |
| | | Omnivorous | 169 (11.1) | 5 | 33 |
| Women (20–42 yr) [162] | Radio | "Low meat" | 102 (6.7) | — | 30 pooled SD = 4.6 |
| Postmenopausal women [163] | Radio | "High meat" | 198 (13.0) | — | 28 pooled SD = 4.6 |

"lowest-observed adverse-effect level, LOAEL." RfDs of 1.66 and 0.83 mg/kg/d were calculated for bioavailability of 15% and 30% [170]. For zinc supplements that might be 95% absorbed, an RfD of 0.25 mg/kg/d was calculated. This value corresponds to 17.5 mg of zinc for a 70 kg man and 15 mg for a 60 kg woman, and obviously is in conflict to zinc supplements that contain up to 50 mg zinc or even more. An RfD of 0.33 mg/kg/d is meant to protect individuals, but not to predict toxicity. Thus the RfD is similar to or below the 1989 RDA for zinc [150], and below the provisional zinc requirement suggested by the WHO at 15% bioavailability [171]. Taken together, these data seem to indicate that there is no AROI for 95% of the population, i.e. a group that is homogeneous in terms of age, sex, and other characteristics believed to affect requirement, but not demographically or culturally. The issue may be further compounded by variability in sensitivity among individuals to both deficiency and toxicity.

Perhaps estimation of the LOAEL and upper limit (UL) for zinc should relate to the Zn:Cu ratio. Relationships between diet zinc, copper and protein (Table 3) suggest a zinc LOAEL of 13.7 mg when diet copper is 0.83 mg (Zn:Cu ratio = 16.5, per weight; 16.1, per molar ratio). The UL would be determined by the intake of copper. For example at an intake of 1.5 mg copper and 13.7 mg zinc (Zn:Cu ratio = 9.1), the zinc intake would be safe and adequate. However, if a zinc supplement of 10 mg of highly bioavailable zinc were added to the intake to increase the total zinc intake to 23.7 mg (Zn:Cu ratio = 15.8), experiments in humans suggest the resulting intake might be unsafe [132]. Clearly, inclusion of bioavailability data would improve the estimate of safety.

## Evaluation of risk vs. benefit to human health

Adverse effects on health can arise from either zinc deficiency or conditioned deficiency of copper secondary to excess zinc. Diet is the major factor determining zinc deficiency while supplements are the major factor determining toxicity.

The recommendations issued by various committees are guidelines, not precisely defined limits. Supplementation with quantities of zinc above the suggested upper

**Table 6.** Dietary reference values for zinc (mg/d)

| Age | United Kingdom [160] | | | USA RDA[a] [151] | WHO DRI [155] | European DRI [167] |
|---|---|---|---|---|---|---|
| | LNRI | EAR | RNI | | | |
| *Infants* | | | | | | |
| 0–3 months | 2.6 | 3.3 | 4.0 | 2.0 | | |
| 4–6 months | 2.6 | 3.3 | 4.0 | 3.0 | | |
| 7–12 months | 3.0 | 3.8 | 5.0 | 3.0 | 5.6 | 4.0 |
| 1–3 yr | 3.0 | 3.8 | 5.0 | 3.0 | 5.5 | 4.0 |
| 4–6 yr | 4.0 | 5.0 | 6.5 | 5.0 | 6.5 | 6.0 |
| 7–10 yr | 4.0 | 5.4 | 7.0 | 8.0 | 7.5 | 7.0 |
| *Males* | | | | | | |
| 11–14 yr | 5.3 | 7.0 | 9.0 | 8.0 | 12.1 | 9.0 |
| 15–18 yr | 5.5 | 7.3 | 9.5 | 11.0 | 13.1 | 9.5 |
| 19–50 + yr | 5.5 | 7.3 | 9.5 | 11.0 | 9.4 | 9.5 |
| *Females* | | | | | | |
| 11–14 yr | 5.3 | 7.0 | 9.0 | 8.0 | 10.3 | 9.0 |
| 15–18 yr | 4.0 | 5.5 | 7.0 | 9.0 | 10.2 | 7.0 |
| 19–50 + yr | 4.0 | 5.5 | 7.0 | 8.0 | 6.5 | 7.1 |
| Pregnancy | | | | 11.0 | 7.3–13.3 | |
| *Lactation* | | | | | | |
| 0–4 months | | | | 12.0 | 12.7 | +5.0 |
| 4+ months | | | | 12.0 | 11.7 | +5.0 |

DRI, dietary reference intake; EAR, estimated average requirement; LNRI, lower reference nutrient intake; RDA, recommended dietary allowance; RNI, recommended nutrient intake.

[a]The age groups for the RDA do not coincide exactly to the groups of the other standards.

limit can result in copper deficiency, especially if the form of zinc in the supplement is readily bioavailable. However, the threshold for this effect is unknown. The concern is for both the effects of copper deficiency and the potential for long-term damage. The literature provides many examples of copper deficiency related to excess supplemental zinc, affecting many tissues and functions. An example is a report of adolescents who had been treated for years with over-the-counter zinc supplements for acne. They displayed anemia and leucopenia [172,173]. Supplements of 80 mg/d are immunosuppressive and inhibit allogenic reactions [174,175]. In the "Health Professionals Follow-up Study" men consuming $\geqslant$100 mg/d zinc had a 2.9-fold higher risk for metastatic prostate cancer [176]. A supplement of 53 mg/d zinc impaired copper status and behavior [146]. Because of this negative impact, the safety of zinc supplements should be carefully considered. Long-term supplementation with pharmacological amounts of highly bioavailable forms of zinc should probably not be done without close medical supervision. In addition, supplementation with physiological amounts of zinc should certainly not exceed the RDA in healthy people, and for safety's sake, should probably not exceed 50% of the RDA. It appears clear that safe intakes of bioavailable zinc are related to the intake of copper. Disproportionately high dietary and/or supplemental intake of readily bioavailable zinc increases the risk of copper deficiency. The prevalence is unknown. For practical purposes and until research indicates otherwise, zinc intakes should probably not exceed 20 mg zinc in adults, and at that level, copper intake must be in sufficient amounts that intakes of highly bioavailable zinc and copper do not exceed a ratio of 10–12.

In order to find a balance in recommendations for maximum public health, critical effects of deficiency and toxicity should have similar clinical significance [177]. In terms of severity, there are lethal effects, obvious clinical effects, and subclinical or hidden effects. For example, for zinc, there seems to be no carcinogenic effect at high zinc intake. However, the observation of zinc deficiency as a risk factor for cancer and other diseases should be carefully weighed against the adverse effects of high zinc intake.

## Zinc supplementation in disease

### Zinc therapy – acrodermatitis enteropathica and Wilson's disease

Pharmacological doses of zinc are given for the treatment of acrodermatitis enteropathica to ascertain

that the patients obtain enough zinc and Wilson's disease to avoid the accumulation of copper in tissues. Patients with copper overload from Wilson's disease benefit from treatment with 50 mg zinc acetate three times daily or more [178]. Treatment with zinc was highly efficacious for up to 10 yr [179]. Morbidity in untreated Wilson's disease includes cirrhosis of the liver, neuromotor dysfunctions, and psychosis. Death ensues if untreated.

Our knowledge about adequacy of zinc in populations, subclinical zinc deficiency, and indications for supplementation with zinc is fragmented. Worldwide, zinc supplementation is an enormously important intervention against mortality from diarrhea, pneumonia, and perhaps malaria [180,181]. Without unambiguous data on zinc uptake and a method to determine zinc status, it is inappropriate to make general statements about the utility of zinc supplementation in disease. Yet ascertaining adequate zinc intake is a most important health issue. However, one must caution that the considerable potential for zinc therapy in some diseases is offset by a lack of knowledge of how supplemental zinc may adversely affect the progression of yet other diseases.

### Diabetes as a candidate disease for zinc therapy

Diabetes is accompanied by zincuria [182]. For diabetics with an increased risk for zinc deficiency more clinical data would be very important as zinc has an insulinomimetic effect and protects against oxidative damage that is associated with the disease. Moreover, it needs to be determined whether or not zinc has a protective function in prevention of the disease in humans. In this regard it is of interest that zinc lowers high blood glucose in genetically obese mice [183,184]. Supplementation with 30 mg/d zinc over 6 months reduced the burden of oxidative stress – monitored by plasma thiobarbituric acid-reactive substances – in human adults with type-2 diabetes by 15% without apparent effects on copper metabolism [185]. Also, zinc protects against oxidative stress in diabetic retinopathy [186]. The relation between zinc and redox metabolism [187,188] and risk of zinc deficiency and oxidative stress in diabetics suggest efficacy of a combined therapy with antioxidants and zinc. Zinc alone may not suffice because zinc deficiency seldom occurs as an isolated phenomenon. It may be necessary to decrease the oxidative stress in diabetics and adjust the redox state of the cell to its normal reductive state so that zinc can bind where and when it is needed. Better diagnostics of zinc and redox status are important to determine the efficacy of optimal and individual doses and to use the full therapeutic potential without running the risk of complications when over-dosing.

## Conclusions

The significance of iron deficiency is undisputed. For zinc deficiency, there is a similar, but largely untapped potential for improving public health. Considering the myriad of functions of zinc, the benefits of assuring adequate zinc nutriture is likely to be at least as great as assurance of iron adequacy. Based on the critical mass of knowledge on zinc in the basic sciences, numerous opportunities exist for translational research in inter- and multidisciplinary settings such as nutrition and toxicology. The following summarizes this article:

1. Requirements are more readily established than upper safe limits. Recommendations are higher than actual requirements and lower than upper safe limits as they both suffer from uncertainties in bioavailability of zinc and CV due to variable sensitivities of individuals in populations. Subpopulations at risk for zinc deficiency or excess need to be identified with special attention to children and the elderly and individuals with different metabolic demands.
2. Recommendations have no practical relevance for public health without data on the actual zinc uptake in populations and relationship to health. Food availability, composition, and preferences can change. Therefore uptakes need to be re-evaluated from time to time.
3. Recommendations are made for healthy individuals. There is an enormous potential for reducing the burden from morbidity with targeted zinc supplementation in both developed and developing countries. Zinc supplementation is most likely to be efficacious if other potentially limiting micronutrients are administered simultaneously [189].
4. Considerations for supplementation will differ for developed and developing countries as different risks vs. benefits apply. In developing countries, remarkable results can be expected: zinc supplementation might reduce child death globally by 63%. Zinc deficiency is one of the ten most important factors contributing to the burden of disease in developing countries with high mortality [190]. Hence, zinc supplementation is one of the major preventive public health strategies. It might be enhanced by interventions such as food fortification and diversification [190].
5. RfDs are meant to protect the public, not necessarily to indicate acute toxicity. The main known mechanism for adverse health effects of high zinc intakes is that they cause a conditioned copper deficiency. There is virtually no knowledge on genetic susceptibility of populations to excess zinc.
6. RDAs and RfDs for zinc are almost identical, leaving almost no gap for supplementation. Therefore the window for pharmacological intervention requires

further studies and definitions. Indications for supplementation may override concerns about side effects, in particular since known side effects can be managed by copper and iron supplementation. For zinc supplementation, a balanced and informed approach is therefore necessary.

7. There is no single reliable clinical test for all the biological functions of zinc. Thus there is not even a "baseline" regarding the definition of an adequate zinc status for all zinc-dependent functions, some of which seem to be improved by additional intakes.
8. What seems to be at issue is not the supplementation for the purpose of eliminating zinc deficiency per se, but the supplementation above RDAs in view of claims of pharmacological effects. With regard to readily available zinc supplements and planned intervention trials in humans, this topic clearly needs additional attention in experimental research.
9. The role of zinc in diseases deserves much more attention. Zinc deficiency is a cause of disease and determines the progression of disease.

## References


[1] Fraker PJ, King LE. Reprogamming the immune system during zinc deficiency. Annu Rev Nutr 2004;24:277–98.

[2] Wellinghausen N, Kirchner H, Rink L. The immunobiology of zinc. Immunol Today 1997;18:519–21.

[3] Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993;73:79–118.

[4] Berg JM, Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. Science 1996;271: 1081–5.

[5] Apgar J. Effect of zinc deficiency on maintenance of pregnancy in the rat. J Nutr 1970;100:470–6.

[6] Hurley LS, Shrader RE. Abnormal development of preimplantation rat eggs after three days of maternal dietary zinc deficiency. Nature 1975;254:427–9.

[7] Hurley LS, Swenerton H. Congenital malformations resulting from zinc deficiency in rats. Proc Soc Exp Biol Med 1966;123:692–6.

[8] Grahn BH, Paterson PG, Gottschall-Pass KT, Zhang Z. Zinc and the eye. J Am Coll Nutr 2001;20:106–8.

[9] Russell RM, Cox ME, Solomons NW. Zinc and the special senses. Ann Intern Med 1983;99:227–39.

[10] Lokken PM, Halas ES, Sandstead HH. Influence of zinc deficiency on behavior. Proc Soc Exp Biol Med 1973;144:680–2.

[11] Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr 1995;125:2263S–71S.

[12] Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. I. Impaired acquisition of granule cells. Brain Res 1983;271:217–26.

[13] Dvergsten CL, Johnson LK, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. III. Impaired dendritic differentiation of basket and stellate cells. Brain Res 1984;318:21–6.

[14] Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. II. Impaired maturation of Purkinje cells. Brain Res 1984;318:11–20.

[15] Dvergsten CL. Retarded synaptogenesis and differentiation of cerebellar neurons in zinc-deficient rats. In: Fredericksen C, Howell G, Kasarkis E, editors. The neurobiology of zinc, part B: deficiency, toxicity, and pathology. New York: Alan R Liss; 1984. p. 17–31.

[16] Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. Nat Rev Neurosci 2005;6: 449–62.

[17] Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. Am J Hum Genet 2002; 71:66–73.

[18] Kury S, Dreno B, Bezieau S, Giraudet S, Kharfi M, Kamoun R, et al. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. Nat Genet 2002;31:239–40.

[19] World Health Organization. E. C., Environmental Health Criteria 221: Zinc, Geneva: World Health Organization, 2001. p. 360.

[20] Alaimo K, McDowell MA, Briefel RR, Bischof AM, Caughman CR, Loria CM, et al. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, phase 1, 1988–91. Adv Data, 1994. p. 1–28.

[21] Cotton PA, Subar AF, Friday JE, Cook A. Dietary sources of nutrients among US adults, 1994 to 1996. J Am Diet Assoc 2004;104:921–30.

[22] Ma J, Betts NM. Zinc and copper intakes and their major food sources for older adults in the 1994–96 continuing survey of food intakes by individuals (CSFII). J Nutr 2000;130:2838–43.

[23] Arsenault JE, Brown KH. Zinc intake of US preschool children exceeds new dietary reference intakes. Am J Clin Nutr 2003;78:1011–7.

[24] Henderson L, Irving K, Gregory J, Bates C, Prentice A, Perks J, et al. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Vitamin and mineral intake and urinary analytes. 1st ed. Norwich: Her Majesty's Stationery Office; 2003.

[25] Thane CW, Bates CJ, Prentice A. Zinc and vitamin A intake and status in a national sample of British young people aged 4–18 y. Eur J Clin Nutr 2004;58: 363–75.

[26] Ervin RB, Kennedy-Stephenson J. Mineral intakes of elderly adult supplement and non-supplement users in the third national health and nutrition examination survey. J Nutr 2002;132:3422–7.

[27] Eichenberger-Gilmore JM, Hong L, Broffitt B, Levy SM. Longitudinal patterns of vitamin and mineral supplement use in young white children. J Am Diet Assoc 2005;105:763–72.

[28] Sichert-Hellert W, Kersting M. Vitamin and mineral supplements use in German children and adolescents

between 1986 and 2003: results of the DONALD Study. Ann Nutr Metab 2004;48:414–9.
[29] Anon. Home and garden bulletin no. 72, Nutritive value of foods. Washington, DC: USDA; 2002.
[30] Gibson R. Zinc nutrition in developing countries. Nutr Res Rev 1994;7:151–73.
[31] Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 1994;102(Suppl 2):5–46.
[32] Yokoi K, Alcock NW, Sandstead HH. Iron and zinc nutriture of premenopausal women: associations of diet with serum ferritin and plasma zinc disappearance and of serum ferritin with plasma zinc and plasma zinc disappearance. J Lab Clin Med 1994;124:852–61.
[33] Yokoi K, Egger NG, Ramanujam VM, Alcock NW, Dayal HH, Penland JG, et al. Association between plasma zinc concentration and zinc kinetic parameters in premenopausal women. Am J Physiol 2003;285: E1010–20.
[34] Yokoi K, Egger N, Ramanujam VMS, Alcock N, Dayal H, Penland J, et al. Associations between iron and zinc nutriture in premenopausal women. FASEB J 2003; 17 (415).
[35] Sandstead HH, Henriksen LK, Greger JL, Prasad AS, Good RA. Zinc nutriture in the elderly in relation to taste acuity, immune response, and wound healing. Am J Clin Nutr 1982;36:1046–59.
[36] Milne DB, Canfield WK, Mahalko JR, Sandstead HH. Effect of dietary zinc on whole body surface loss of zinc: impact on estimation of zinc retention by balance method. Am J Clin Nutr 1983;38:181–6.
[37] Sandstead HH, Smith Jr JC. Deliberations and evaluations of approaches, endpoints and paradigms for determining zinc dietary recommendations. J Nutr 1996;126:2410S–8S.
[38] Oberleas D, Muhrer ME, O'Dell BL. Dietary metal-complexing agents and zinc availability in the rat. J Nutr 1996;90:56–62.
[39] Ferguson EL, Gibson RS, Thompson LU, Ounpuu S. Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. Am J Clin Nutr 1989;50:1450–6.
[40] Gibson RS, Ferguson EL. Assessment of dietary zinc in a population. Am J Clin Nutr 1998;68:430S–4S.
[41] Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J Nutr 1986;116:927–35.
[42] Milne DB, Canfield WK, Mahalko JR, Sandstead HH. Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. Am J Clin Nutr 1984;39: 535–9.
[43] Simmer K, Iles CA, James C, Thompson RP. Are iron-folate supplements harmful? Am J Clin Nutr 1987;45: 122–5.
[44] Gallaher DD, Johnson PE, Hunt JR, Lykken GI, Marchello MJ. Bioavailability in humans of zinc from beef: intrinsic vs. extrinsic labels. Am J Clin Nutr 1988;48:350–4.
[45] Prasad AS, Miale Jr A, Farid Z, Sandstead HH, Schulert AR, Darby WJ. Biochemical studies on dwarfism, hypogonadism, and anemia. Arch Intern Med 1963;111: 407–28.
[46] Sandstead HH, Prasad AS, Schulert AR, Farid Z, Miale Jr A, Bassilly S, et al. Human zinc deficiency, endocrine manifestations and response to treatment. Am J Clin Nutr 1967;20:422–42.
[47] Brown K, Wuehler S, Peerson J. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bull 2001; 22:113–25.
[48] McClain CJ. Zinc metabolism in malabsorption syndromes. J Am Coll Nutr 1985;4:49–64.
[49] Solomons NW, Rosenberg IH. Zinc and inflammatory bowel disease. Am J Clin Nutr 1981;34:1447–8.
[50] Solomons NW, Rosenberg IH, Sandstead HH, Vo-Khactu KP. Zinc deficiency in Crohn's disease. Digestion 1977;16:87–95.
[51] McClain C, Soutor C, Zieve L. Zinc deficiency: a complication of Crohn's disease. Gastroenterology 1980;78:272–9.
[52] Prasad AS, Ortega J, Brewer GJ, Oberleas D, Schoomaker EB. Trace elements in sickle cell disease. JAMA 1976;235:2396–8.
[53] Prasad AS. Malnutrition in sickle cell disease patients. Am J Clin Nutr 1997;66:423–4.
[54] Prasad AS, Diwany M, Gabr M, Sandstead HH, Mokhtar N, Hefny AE. Biochemical studies in thalassemia. Ann Intern Med 1965;62:87–96.
[55] Farid Z, Patwardhan VN, Darby WJ. Parasitism and anemia. Am J Clin Nutr 1969;22:498–503.
[56] Lindeman RD. Trace minerals and the kidney: an overview. J Am Coll Nutr 1989;8:285–91.
[57] Vallee BL, Wacker WEC, Bartholomay AF, Robin ED. Zinc metabolism in hepatic dysfunction. NE J Med 1956;255:403–8.
[58] Vallee BL, Wacker WEC, Bartholomay AF, Hoch FL. Zinc metabolism in hepatic dysfunction. Ann Intern Med 1959;50:1077–91.
[59] Sullivan JF, Lankford HG. Urinary excretion of zinc in alcoholism and postalcoholic cirrhosis. Am J Clin Nutr 1962;10:153–7.
[60] McClain CJ, Antonow DR, Cohen DA, Shedlofsky SI. Zinc metabolism in alcoholic liver disease. Alcohol Clin Exp Res 1986;10:582–9.
[61] Sullivan JF, Lankford HG. Zinc metabolism and chronic alcoholism. Am J Clin Nutr 1965;17:57–63.
[62] McClain CJ, Su LC. Zinc deficiency in the alcoholic: a review. Alcohol Clin Exp Res 1983;7:5–10.
[63] McClain CJ, McClain ML, Boosalis MG, Hennig B. Zinc and the stress response. Scand J Work Environ Health 1993;19(Suppl 1):132–3.
[64] Powanda MC. Host metabolic alterations during inflammatory stress as related to nutritional status. Am J Vet Res 1980;41:1905–11.
[65] Goldblum SE, Cohen DA, Jay M, McClain CJ. Interleukin 1-induced depression of iron and zinc: role of granulocytes and lactoferrin. Am J Physiol 1987;252:E27–32.

[66] Cuthbertson DP, Fell GS, Smith CM, Tilstone WJ. Metabolism after injury. I. Effects of severity, nutrition, and environmental temperature on protein potassium, zinc, and creatine. Br J Surg 1972;59:926–31.
[67] Golden MH, Harland PS, Golden BE, Jackson AA. Zinc and immunocompetence in protein-energy malnutrition. Lancet 1978;1:1226–8.
[68] Golden MH, Jackson AA, Golden BE. Effect of zinc on thymus of recently malnourished children. Lancet 1977; 2:1057–9.
[69] Prasad AS. Zinc and immunity. Mol Cell Biochem 1998;188:63–9.
[70] Neldner KH, Hambidge KM, Walravens PA. Acrodermatitis enteropathica. Int J Dermatol 1978;17:380–7.
[71] Weismann K, Hoyer H. Zinc deficiency dermatoses. Etiology, clinical aspects and treatment. Hautarzt 1982; 33:405–10.
[72] Arakawa T, Tamura T, Igarashi Y, Suzuki H, Sandstead HH. Zinc deficiency in two infants during total parenteral alimentation for diarrhea. Am J Clin Nutr 1976;29:197–204.
[73] Baer MT, King JC, Tamura T, Margen S. Acne in zinc deficiency. Arch Dermatol 1978;114:1093.
[74] Sandstead H, Carter J, Darby W. Clinical diagnosis of nutritional disease. Nutr Today 1969;4:20–6.
[75] Henzel JH, DeWeese MS, Lichti EL. Zinc concentrations within healing wounds. Significance of postoperative zincuria on availability and requirements during tissue repair. Arch Surg 1970;100:349–57.
[76] Husain SL, Fell GS, Scott R, Trethewie ER. Zinc and healing. Lancet 1970;2:1361–2.
[77] Larson DL, Maxwell R, Abston S, Dobrkovsky M. Zinc deficiency in burned children. Plast Reconstr Surg 1970;46:13–21.
[78] Sandstead HH, Shepard GH. The effect of zinc deficiency on the tensile strength of healing surgical incisions in the integument of the rat. Proc Soc Exp Biol Med 1968;128:687–9.
[79] Sandstead HH, Lanier Jr VC, Shephard GH, Gillespie DD. Zinc and wound healing. Effects of zinc deficiency and zinc supplementation. Am J Clin Nutr 1970;23: 514–9.
[80] Terril-Robb LA, Clemons DJ, Besch-Williford C, O'Brien DP, O'Dell BL. Morphophysiologic characterization of peripheral neuropathy in zinc-deficient guinea pigs. Proc Soc Exp Biol Med 1996;213:50–8.
[81] Henkin RI, Patten BM, Re PK, Bronzert DA. A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. Arch Neurol 1975;32:745–51.
[82] Sandstead HH. Zinc is essential for brain development and function. J Trace Elem Exp Med 2003;16:165–73.
[83] Ronaghy HA, Halsted JA. Zinc deficiency occurring in females. Report of two cases. Am J Clin Nutr 1975;28: 831–6.
[84] Abbasi A, Prasad A, Rabbani P, DuMouchelle E. Experimental zinc deficiency in man. Effect on testicular function. J Lab Clin Med 1980;96:544–50.
[85] Abbasi AA, Prasad AS, Ortega J, Congco E, Oberleas D. Gonadal function abnormalities in sickle cell anemia. Studies in adult male patients. Ann Intern Med 1976; 85:601–5.
[86] Prasad A, Abbasi A, Rabbani P, DuMouchelle E. Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects. Am J Hematol 1981;10:119–27.
[87] Meadows NJ, Ruse W, Smith MF, Day J, Keeling PW, Scopes JW, et al. Zinc and small babies. Lancet 1981;2: 1135–7.
[88] Goldenberg RL, Tamura T, Neggers Y, Copper RL, Johnston KE, DuBard MB, et al. The effect of zinc supplementation on pregnancy outcome. JAMA 1995; 274:463–8.
[89] Keen CL, Hurley LS. Zinc and reproduction: effects of deficiency on foetal and postnatal development. In: Mills CF, editor. Zinc in human biology. London: Springer; 1989. p. 183–220.
[90] Jameson S. Effects of zinc deficiency in human reproduction. Acta Med Scand Suppl 1976;593:1–89.
[91] Simmer K, Iles CA, Slavin B, Keeling PW, Thompson RP. Maternal nutrition and intrauterine growth retardation. Hum Nutr Clin Nutr 1987;41:193–7.
[92] Cavdar AO, Bahceci M, Akar N, Dincer FN, Erten J. Maternal hair zinc concentration in neural tube defects in Turkey. Biol Trace Elem Res 1991;30:81–5.
[93] Scholl TO, Hediger ML, Schall JI, Fischer RL, Khoo CS. Low zinc intake during pregnancy: its association with preterm and very preterm delivery. Am J Epidemiol 1993;137:1115–24.
[94] Walker CF, Black RE. Zinc and the risk for infectious disease. Annu Rev Nutr 2004;24:255–75.
[95] Sandstead HH, Penland JG, Alcock NW, Dayal HH, Chen XC, Li JS, et al. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. Am J Clin Nutr 1998;68:470S–5S.
[96] Penland JG. Behavioral data and methodology issues in studies of zinc nutrition in humans. J Nutr 2000;130: 361S–4S.
[97] Prasad AS, Oberleas D, Halsted JA. Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic subjects. J Lab Clin Med 1965;66:508–16.
[98] Hackley BM, Smith JC, Halsted JA. A simplified method for plasma zinc determination by atomic absorption spectrophotometry. Clin Chem 1968;14:1–5.
[99] Halsted JA, Smith Jr JC. Plasma-zinc in health and disease. Lancet 1970;1:322–4.
[100] Pilch SM, Senti FR. Analysis of zinc data from the second National Health and Nutrition Examination Survey (NHANES II). J Nutr 1985;115:1393–7.
[101] Prasad AS, Rabbani P, Abbasi A, Bowersox E, Fox MRS. Experimental zinc deficiency in humans. Ann Intern Med 1978;89:483–90.
[102] Lukaski HC, Bolonchuk WW, Klevay LM, Milne DB, Sandstead HH. Changes in plasma zinc content after exercise in men fed a low-zinc diet. Am J Physiol 1984; 247:E88–93.
[103] Milne DB, Canfield WK, Gallagher SK, Hunt JR, Klevay LM. Ethanol metabolism in postmenopausal

women fed a diet marginal in zinc. Am J Clin Nutr 1987;46:688–93.
[104] Penland JG. Cognitive performance effects of low zinc intakes in healthy adult men. FASEB J 1991;5:A938.
[105] Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M. Zinc deficiency in elderly patients. Nutrition 1993;9:218–24.
[106] Beck FW, Kaplan J, Fine N, Handschu W, Prasad AS. Decreased expression of CD73 (ecto-5'-nucleotidase) in the CD8+ subset is associated with zinc deficiency in human patients. J Lab Clin Med 1997;130:147–56.
[107] Meftah S, Prasad AS, Lee DY, Brewer GJ. Ecto 5' nucleotidase (5'NT) as a sensitive indicator of human zinc deficiency. J Lab Clin Med 1991;118:309–16.
[108] Bales CW, DiSilvestro RA, Currie KL, Plaisted CS, Joung H, Galanos AN, et al. Marginal zinc deficiency in older adults: responsiveness of zinc status indicators. J Am Coll Nutr 1994;13:455–62.
[109] Andree KB, Kim J, Kirschke CP, Gree JP, Paik HY, Joung H, et al. Investigation of lymphocyte gene expression for use as biomarkers for zinc status in humans. J Nutr 2004;134:1716–23.
[110] Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol 1997;272:E1002–7.
[111] Dardenne M, Savino W, Wade S, Kaiserlian D, Lemonnier D, Bach JF. In vivo and in vitro studies of thymulin in marginally zinc-deficient mice. Eur J Immunol 1984;14:454–8.
[112] Brummerstedt E. Animal model of human disease. Acrodermatitis enteropathica, zinc malabsorption. Am J Pathol 1977;87:725–8.
[113] Bach JF, Dardenne M. Thymulin, a zinc-dependent hormone. Med Oncol Tumor Pharmacother 1989;6:25–9.
[114] Prasad AS, Meftah S, Abdallah J, Kaplan J, Brewer GJ, Bach JF, et al. Serum thymulin in human zinc deficiency. J Clin Invest 1988;82:1202–10.
[115] Grider A, Bailey LB, Cousins RJ. Erythrocyte metallothionein as an index of zinc status in humans. Proc Natl Acad Sci USA 1990;87:1259–62.
[116] Domingo JL, Llobet JM, Paternain JL, Corbella J. Acute zinc intoxication: comparison of the antidotal efficacy of several chelating agents. Vet Hum Toxicol 1988;30:224–8.
[117] Karlsson N, Fangmark I, Haggqvist I, Karlsson B, Rittfeldt L, Marchner H. Mutagenicity testing of condensates of smoke from titanium dioxide/hexachloroethane and zinc/hexachloroethane pyrotechnic mixtures. Mutat Res 1991;260:39–46.
[118] Brown MA, Thom JV, Orth GL, Cova P, Juarez J. Food poisoning involving zinc contamination. Arch Environ Health 1964;34:657–60.
[119] Chow BF, Sherwin R, Hsueh AM, Blackwell BN, Blackwell RQ. Growth and development of rats in relation to maternal diet: A review. Nutr Dieta 1969;11:45–56.
[120] Godfrey KM, Barker DJP. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71:1344S–52S.
[121] Beach RS, Gershwin ME, Hurley LS. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science 1982;218:469–71.
[122] Vruwink K, Gershwin ME, Keen CL. Effects of gestational zinc deficiency in mice on growth and immune function. J Nutr Immunol 1993;2:25–41.
[123] Wellinghausen N. Immunobiology and gestational zinc deficiency. Br J Nutr 2001;85:S81–6.
[124] Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 1998;12:949–57.
[125] Jirtle RL, Waterland RA. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293–300.
[126] Barney GH, Orgebin-Crist MC, Macapinalac MP. Genesis of esophageal parakeratosis and histologic changes in the testes of the zinc-deficient rat and their reversal by zinc repletion. J Nutr 1968;95:526–34.
[127] Newberne PM, Broitman S, Schrager TF. Esophageal carcinogenesis in the rat: zinc deficiency, DNA methylation and alkyltransferase activity. Pathobiology 1997;65:253–63.
[128] Newberne PM, Schrager TF, Broitman S. Esophageal carcinogenesis in the rat: zinc deficiency and alcohol effects on tumor induction. Pathobiology 1997;65:39–45.
[129] Fong LY, Magee PN. Dietary zinc deficiency enhances esophageal cell proliferation and N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumor incidence in C57BL/6 mouse. Cancer Lett 1999;143:63–9.
[130] Fong LYY, Zhang L, Jiang Y, Farber JL. Dietary zinc modulation of Cox-2 expression and lingual and esophageal carcinogenesis in rats. J Natl Cancer Inst 2005;97:40–50.
[131] Johnson PE, Stuart MA, Hunt JR, Mullen L, Starks TL. 65-Copper absorption by women fed intrinsically and extrinsically labeled goose meat, goose liver, peanut butter and sunflower butter. J Nutr 1988;118:1522–8.
[132] Reiser S, Smith Jr JC, Mertz W, Holbrook JT, Scholfield DJ, Powell AS, et al. Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am J Clin Nutr 1985;42:242–51.
[133] Bhathena SJ, Recant L, Voyles NR, Timmers KI, Reiser S, Smith Jr JC, et al. Decreased plasma enkephalins in copper deficiency in man. Am J Clin Nutr 1986;43:42–6.
[134] Reiser S, Powell A, Yang C, Canary J. Effect of copper intake on blood cholesterol and its lipoprotein distribution in men. Nutr Rep Int 1987;36:641–9.
[135] Milne DB, Johnson P, Klevay L, Sandstead H. Effect of copper intake on balance, absorption, and status indices of copper in men. Nutr Res 1990;10:975–86.
[136] Klevay LM, Canfield WK, Gallagher SK. Decreased glucose tolerance in two men during experimental copper depletion. Nutr Rep Int 1986;33:371–82.
[137] Klevay LM, Inman L, Johnson LK, Lawler M, Mahalko JR, Milne DB, et al. Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism 1984;33:1112–8.

[138] Turnlund JR, Keen CL, Smith RG. Copper status and urinary and salivary copper in young men at three levels of dietary copper. Am J Clin Nutr 1990;51: 658–64.
[139] Milne D, Klevay L, Hunt J. Effects of ascorbic acid supplements and a diet marginal in copper on indices of copper nutriture in women. Nutr Res 1988;8:865–73.
[140] Lukaski HC, Klevay LM, Milne DB. Effects of dietary copper on human autonomic cardiovascular function. Eur J Appl Physiol Occup Physiol 1988;58:74–80.
[141] Nielsen F, Milne D, Mullen L, Gallagher S. Dietary sulfur amino acids, and generic make-up or interindividual variations affect the response of men to copper depletion. J Trace Elem Exp Med 1990;3:281–96.
[142] Nielsen F, Milne D. High dietary fructose affects plasma cholesterol concentrations and signs of short term copper deprivation in men. Proc North Dakota Acad Sci 1992;46:73.
[143] Milne D, Nielsen F. Effect of high dietary fructose on copper homeostasis and status indicators in men during copper deprivation. In: Anke M, Meissner D, Mills C, editors. Trace elements in man and animals-TEMA 8. Gersdorf, Germany: Verlag Nedia Touristik; 1993. p. 370–3.
[144] Milne DB, Nielsen FH. Effects of a diet low in copper on copper-status indicators in postmenopausal women. Am J Clin Nutr 1996;63:358–64.
[145] Turnlund JR, Scott KC, Peiffer GL, Jang AM, Keyes WR, Keen CL, et al. Copper status of young men consuming a low-copper diet. Am J Clin Nutr 1997; 65:72–8.
[146] Penland J, Milne D, Davis C. Moderately high zinc intake impairs verbal memory of healthy postmenopausal women on a low copper diet. In: Roussel A, Anderson R, Favier A, editors. Trace elements in man and animals, vol. 10. Evian, France: Kluwer Academic/ Plenum; 1999. p. 1025–30.
[147] Rowin J, Lewis SL. Copper deficiency myeloneuropathy and pancytopenia secondary to overuse of zinc supplementation. J Neurol Neurosurg Psychiatry 2005;76: 750–1.
[148] Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am J Clin Nutr 1995;61:621S–4S.
[149] Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol 1986;251:R398–408.
[150] Food and Nutrition Board. E. C. Zinc. In: Board FaN, editor. Recommended dietary allowances. 10 ed. Washington, DC: National Academy Press; 1989. p. 205–13.
[151] Food and Nutrition Board and Institute of Medicine. In: Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, editor. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press; 2001. p. 12.1–.47.
[152] Hambidge M. The new diatary reference intakes and zinc deficiency: is there a dichotomy? Nutr Today 2001; 36:278–80.
[153] Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung. Referenzwerte für die Naehrstoffzufuhr. 1. Auflage, Frankfurt/Main: Umschau Brauns GmbH, 2000.
[154] Gibson RS. Content and bioavailability of trace elements in vegetarian diets. Am J Clin Nutr 1994;59: 1223S–32S.
[155] World Health Organization, E. C. Zinc. In: Committee, editor. Trace Elements in Human Nutrition and Health. Geneva: World Health Organization; 1996. p. 72–104.
[156] King J, Turnlund J. Human zinc requirements. In: Mills C, editor. Zinc in human biology. London: Springer; 1989. p. 335–50.
[157] British Nutrition Foundation. E. C. Dietary reference values for food energy and nutrients for the United Kingdom. London: Her Majesty's Stationery Office; 1991.
[158] Ministry of Health and Welfare. E. C. Recommended nutrient intakes for Canadians. Ministry of Health and Welfare. Ottawa, Canada: Canadian Government Publishing Center Supply Services; 1990.
[159] Food and Agricultural Organization. E. C. Zinc. In: Human vitamin and mineral requirements. Rome, Italy: Food and Nutrition Division, FAO; 2001. p. 257–70.
[160] Petterson DS, Sandström B, Cederblad A. Absorption of zinc from lupin (Lupinus angustifolius)-based foods. Br J Nutr 1994;72:865–71.
[161] Knudsen E, Jensen M, Solgaard P, Sorensen SS, Sandström B. Zinc absorption estimated by fecal monitoring of zinc stable isotopes validated by comparison with whole-body retention of zinc radioisotopes in humans. J Nutr 1995;125:1274–82.
[162] Hunt JR, Matthys LA, Johnson LK. Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk. Am J Clin Nutr 1998;67:421–30.
[163] Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am J Clin Nutr 1995;62:621–32.
[164] Krebs NE, Hambidge KM. Zinc metabolism and homeostasis: the application of tracer techniques to human zinc physiology. Biometals 2001;14:397–412.
[165] Ruz M, Codoceo J, Rebolledo A, Vasquez M, Krebs N, Sian L, et al. The use of zinc stable isotopes in the study of iron-zinc interactions in Chilean women. Food Nutr Bull 2002;23:209–12.
[166] Tran CD, Miller LV, Krebs NF, Lei S, Hambidge KM. Zinc absorption as a function of the dose of zinc sulfate in aqueous solution. Am J Clin Nutr 2004;80: 1570–3.
[167] European Community, S. C. f. F. Nutrient and energy intakes for the European community. In: Directorate-General Industrial Affairs, editor. Report of the Scientific Committee for Food (series 31). Luxembourg: Office des publications officielles des Communautés Européenes; 1993.

[168] Dourson M. Methods for establishing oral reference doses. In: Mertz W, Abernathy C, Olin S, editors. Risk assessment of essential elements. Washington, DC: ILSI Press; 1994. p. 51–61.
[169] Yadrick MK, Kenney MA, Winterfeldt EA. Iron, copper, and zinc status: response to supplementation with zinc or zinc and iron in adult females. Am J Clin Nutr 1989;49:145–50.
[170] Sandstead HH. Zinc requirements, the recommended dietary allowance and the reference dose. Scand J Work Environ Health 1993;19(Suppl 1):128–31.
[171] World Health Organization. E. C. Zinc. In: Trace elements in human nutrition. 532 ed. Geneva: World Health Organization; 1973. p. 9–16.
[172] Porea TJ, Belmont JW, Mahoney DH. Zinc-induced anemia and neutropenia in an adolescent. J Pediatr 2000;136:688–90.
[173] Salzman MB, Smith EM, Koo C. Excessive oral zinc supplementation. J Pediatr Hematol Oncol 2002;24: 582–4.
[174] Chandra RK. Excessive intake of zinc impairs immune responses. JAMA 1984;252:1443–6.
[175] Faber C, Gabriel P, Ibs KH, Rink L. Zinc in pharmacological doses suppresses allogeneic reaction without affecting the antigenic response. Bone Marrow Transplant 2004;33:1241–6.
[176] Leitzmann MF, Stampfer MJ, Wu K, Colditz GA, Willett WC, Giovannucci EL. Zinc supplement use and risk of prostate cancer. J Natl Cancer Inst 2003;95: 1004–7.
[177] Nordberg G, Sandström B, Becking G, Goyer RA. Essentiality and toxicity of trace elements: Principles and methods for assessment of risk from human exposure to essential trace elements. J Trace Elem Exp Med 2000; 13:141–53.
[178] Brewer GJ. Zinc acetate for the treatment of Wilson's disease. Expert Opin Pharmacother 2001;2:1473–7.
[179] Brewer GJ, Dick RD, Johnson VD, Brunberg JA, Kluin KJ, Fink JK. Treatment of Wilson's disease with zinc: XV long-term follow-up studies. J Lab Clin Med 1998;132:264–78.
[180] Fischer Walker C, Black RE. Zinc and the risk for infectious disease. Annu Rev Nutr 2004;24:255–75.
[181] Black R. Micronutrient deficiency: an underlying cause of morbidity and mortality. Bull World Health Org 2003;81:79–89.
[182] Cunningham JJ, Fu AZ, Mearkle PL, Brown RG. Hyperzincuria in individuals with insulin-dependent diabetes mellitus – concurrent zinc zinc status and the effect of high-dose zinc supplementation. Metab: Clin Exp 1994;43:1558–62.
[183] Chen MD, Liou SJ, Lin PY, Yang VC, Alexander PS, Lin WH. Effects of zinc supplementation on the plasma glucose level and insulin activity in genetically obese (ob/ob) mice. Biol Trace Elem Res 1998;61:303–11.
[184] Simon SF, Taylor CG. Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med 2001;226:43–51.
[185] Roussel A- M, Kerkeni A, Zouari N, Mahjoub S, Matheau J- M, Anderson RA. Antioxidant effects of zinc supplementation in Tunisians with type 2 diabetes mellitus. J Am Coll Nutr 2003;22:316–21.
[186] Faure P, Benhamou PY, Perard A, Halimi S, Roussel AM. Lipid peroxidation in insulin dependant diabetic patients with early retinal degenerative lesions: effect of an oral zinc supplementation. Eur J Clin Nutr 1995; 49:282–8.
[187] Maret W. The function of zinc metallothionein: a link between cellular zinc and redox state. J Nutr 2000;130: 1455S–8S.
[188] Maret W. Cellular zinc and redox states converge in the metallothionein/thionein pair. J Nutr 2003;133: 1460S–2S.
[189] Sandstead HH. Improving study design. Am J Clin Nutr 1999;70:110–1.
[190] Shrimpton R, Gross R, Darnton-Hill I, Young M. Zinc deficiency: what are the most appropriate interventions? Br Med J 2005;330:347–9.