AJCN. First published ahead of print May 6, 2009 as doi: 10.3945/ajcn.2009.27230G.

Methods of assessment of zinc status in humans: a systematic review[1–5]

Nicola M Lowe, Katalin Fekete, and Tamás Decsi

ABSTRACT

Background: Zinc is an essential micronutrient for human health and has numerous structural and biochemical roles. The search for a reliable, sensitive, and specific index of zinc status has been the subject of considerable research, which has resulted in the identification of a number of potentially useful biomarkers.

Objective: The objective was to assess the usefulness of biomarkers of zinc status in humans.

Design: The methods included a structured search strategy using Ovid MEDLINE, EMBASE (Ovid), and the Cochrane Library CENTRAL databases; formal inclusion and exclusion criteria; data extraction into an Access database; quality and validity assessment; and meta-analysis.

Results: Data on 32 potential biomarkers from 46 publications were analyzed. Plasma zinc concentration responded in a dose-dependent manner to dietary manipulation in adults, women, men, pregnant and lactating women, the elderly, and those at low and moderate baseline zinc status. Urinary zinc excretion responded to zinc status overall and in all subgroups for which there were sufficient data. Hair zinc concentration also responded, but there were insufficient studies for subgroup analysis. Platelet, polymorphonuclear cell, mononuclear cell, and erythrocyte zinc concentration and alkaline phosphatase activity did not appear to be effective biomarkers of zinc status.

Conclusions: This systematic review confirms that in healthy individuals, plasma, urinary, and hair zinc are reliable biomarkers of zinc status. Further high-quality studies using these biomarkers are required, particularly in infants, adolescents, and immigrant population groups for whom there are limited data. Studies are also required to fully assess a range of additional potential zinc biomarkers. *Am J Clin Nutr* 2009;89(suppl):1S–12S.

INTRODUCTION

Zinc is well established as an essential micronutrient for human health because it has numerous structural and biochemical functions at the cellular and subcellular level, which include enzyme function, DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation, and cell-mediated immunity. The ubiquitous nature of zinc in human biological systems indicates the widespread consequences and the complexity of inadequate dietary supply of zinc and zinc depletion.

Zinc is absorbed in the small intestine, primarily via transporter-mediated processes. Rich sources of dietary zinc include meat, fish, shellfish, nuts, seeds, legumes, and whole-grain cereals (1, 2). However, plant sources are considered to be less bioavailable because of the presence of phytic acid that binds to zinc-forming insoluble complexes, which thus inhibits zinc's absorption (1). The current recommendations for dietary zinc intake in adults range from 7 mg/d (UK Reference Nutrient Intake) to 11 mg/d (US Recommended Dietary Allowance) (2). This broad range reflects in part the variation in requirements due to differences in the bioavailability of zinc from different national diets and also the difficulties associated with estimating the requirements for optimal health, which depends on a reliable indicator of status (3).

Unlike other micronutrients such as iron, there is no storage form of zinc in the body that can be readily mobilized when intakes are inadequate, which emphasizes the need for a regular dietary supply (4). A highly effective homeostatic mechanism responds to alterations in zinc intake, upregulating absorption and conserving losses via the gastrointestinal tract and kidneys when intakes fall. By using isotope tracer techniques, it was predicted that when dietary zinc fell from 12.2 to 0.23 mg/d in a group of adult men, fractional zinc absorption could increase to virtually 100%, with urinary excretion falling from 0.36 to 0.006 mg/d and fecal excretion falling from 11.8 to 0.23 mg/d (4). When homeostatic mechanisms fail to ensure that requirements are met, clinical symptoms of zinc deficiency ensue. Severe deficiency is associated with stunted growth, immune dysfunction, and poor wound healing. These symptoms of severe zinc deficiency are most dramatically observed in acrodermatitis enteropathica, a congenital condition in which the

[1] From the Centre for Applied Sport and Exercise Sciences, School of Psychology, the University of Central Lancashire, Preston, United Kingdom (NML) and the Department of Pediatrics, the University of Pécs, Pécs, Hungary (KF and TD).

[2] Presented at the EURRECA workshop "Biomarkers of Micronutrient Status," held in Sveti Stefan, Montenegro, 9 June 2008.

[3] This manuscript does not necessarily reflect the views of the Commission of the European Communities and in no way anticipates their future policy in this area.

[4] Supported partially by the Commission of the European Communities, specific RTD Programme "Quality of Life and Management of Living Resources," within the 6th Framework Programme (contract no. FP6-036196-2 EURRECA: EURopean micronutrient RECommendations Aligned).

[5] Address correspondence to N Lowe, Centre for Applied Sport and Exercise Sciences, School of Psychology, University of Central Lancashire, Preston PR1 2HE, United Kingdom. E-mail: nmlowe@uclan.ac.uk.

doi: 10.3945/ajcn.2009.27230G.

infant is born with impaired gastrointestinal zinc transport, which limits the ability to absorb zinc (5). In European populations, severe primary zinc deficiency is extremely uncommon, but marginal deficiency is likely to be much more prevalent (6). The lack of a reliable, responsive, and specific indicator of zinc status means that the diagnosis of marginal zinc deficiency is difficult.

The aim of this systematic review was to assess the usefulness of the biomarkers of zinc status in healthy humans to determine which biomarkers appropriately reflect changes in zinc status in response to supplementation or depletion.

METHODS

Inclusion criteria

Both supplementation and depletion studies were included in our search. The forms of zinc used for supplementation included zinc sulfate, gluconate, methionine, and acetate, all of which have been shown to be readily absorbable (7). For the purposes of this review, a minimum duration of the supplementation or depletion intervention of 2 wk was deemed sufficient to elicit a biomarker response. This decision was based on a study of zinc depletion and repletion in men, where there was an increase in plasma zinc concentration of 260% within 1 wk of the reintroduction of zinc to the diet after a period of depletion with very little further increase in plasma zinc concentration after 2 wk (8).

Types of study

To be included, a study needed to meet all of the following criteria: *1*) be an intervention study in humans (including supplementation and/or depletion studies) without restriction in study design, which could include randomized controlled trials (RCTs), controlled clinical trials, and before-after studies (B/A); *2*) report the zinc status in humans at baseline and after supplementation or depletion; *3*) span a time period of $\geq$2 wk over which the change was measured; *4*) report the daily dose of the zinc supplement; *5*) use one of the following supplements: zinc sulfate; zinc acetate; zinc gluconate, or zinc methionine; and *6*) involve healthy participants who had not recently used mineral or vitamin supplements.

Search strategy

Electronic searches were performed with Ovid MEDLINE (www.ovid.com), EMBASE (Ovid; www.ovid.com), and the Cochrane Library CENTRAL (www.thecochranelibrary.com) database, which were searched from inception to October 2007 for intervention studies by using text terms with appropriate truncation and relevant indexing terms. The search was in the form: [zinc terms] and [intervention study terms] and [human studies]. The full Ovid MEDLINE search strategy can be found in Table S1 under ''Supplemental data'' in the online issue. The searches of the above-mentioned databases were also based on this strategy.

An Ovid MEDLINE search was conducted for reviews of the methods of assessing zinc status; 6 of these reviews (9–14) were collected in full text, and the reference lists were checked. Studies that appeared to be intervention studies but that had not been already assessed for inclusion were collected.

One expert, Rosalind Gibson, was asked if she could suggest additional intervention studies for the review. She suggested additional articles for assessment and these were then subjected to the same criteria listed above before they were accepted for inclusion.

Data extraction

The methodology of this review is based on the standard methodology developed for this set of reviews in this supplement (15) and is abbreviated below, mainly noting differences from the main methodology. Titles and abstracts were screened for inclusion by a single reviewer (KF). The full text of all articles collected was screened for inclusion by using an inclusion and exclusion form by 2 independent reviewers. Where the 2 reviewers disagreed, the study was discussed and a consensus decision was reached, or a third reviewer was asked to arbitrate.

Data for each included study were extracted onto an Access (Microsoft Corp, Redmond, WA) database file by a single reviewer (KF). In doubtful cases, studies were discussed with the review team before beginning full data extraction and, in some cases, study authors were contacted for clarification. When necessary, units of measurement were converted to a standard form to facilitate comparison across studies. Data extraction and synthesis for primary and secondary measures of interest were undertaken as discussed in the methodology article (15).

RESULTS

The flow diagram for this review is shown in **Figure 1**. A total of 1334 titles and abstracts were screened after electronic and bibliographic searches or were recommended by experts. Of these, 182 appeared potentially relevant and were collected as full-text articles to be assessed for inclusion, and 180 full-text articles were assessed (2 articles could not be traced); 48 studies were found to fulfill the inclusion criteria. One article had elements of both an RCT and a B/A in the study design (16) and one article contained a supplementation and a depletion study (17). These data were analyzed as 2 separate studies, giving a total of 48 studies from 46 publications: 24 described RCT studies and 24 described B/A studies. In some cases, studies were further subdivided into data sets, when, for example, the study cohort was assigned to groups receiving different amounts of supplementation.

Quality of included studies

The characteristics of the studies included in the analysis are presented in **Table 1** (supplementation studies) and **Table 2** (depletion studies). In terms of the distribution of the age of the population groups studied, 67% (32/48) of the studies were in healthy adults, and 19% (9/48) in elderly people. There were 5 studies in pregnant or lactating women (36, 38, 43, 48, 49), one study in postmenopausal women (58), and one study in children and adolescents (40). There were no studies in infants, and none that selected for immigrant or low-socioeconomic groups. As discussed in the methodology article (15), quality assessment was undertaken as part of the data extraction process. A summary of the reasons for dropping out in the intervention group, the methods of randomization, and compliance checking are summarized in **Table 3**. In the majority of studies,

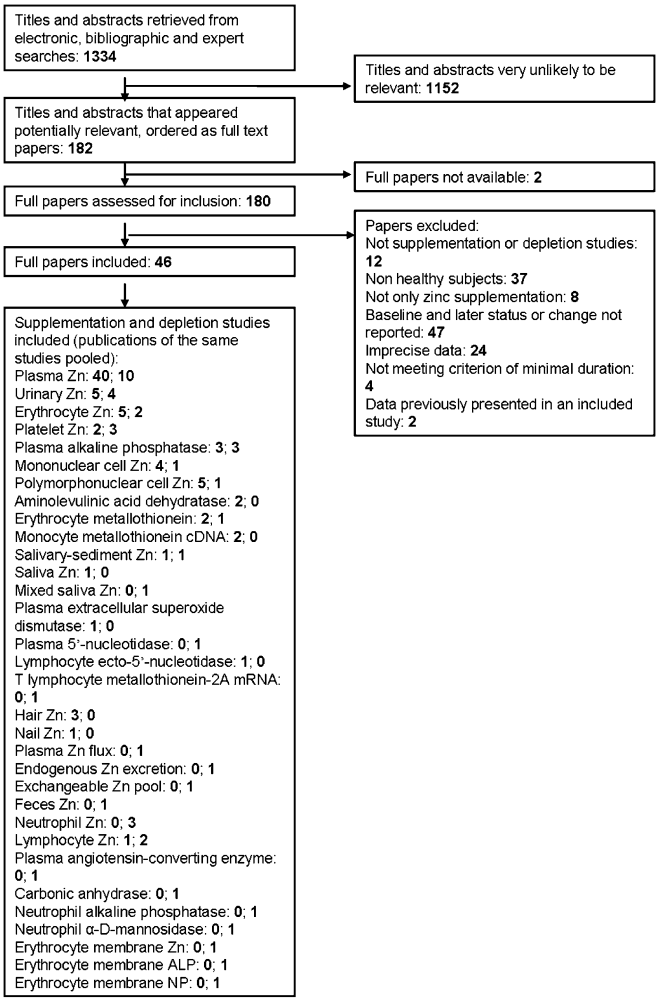

FIGURE 1. Flow diagram of systematic literature search on biomarkers of zinc status. Numbers in bold represent the number of publications identified. ALP, alkaline phosphatase; NP, neutral phosphatase.

the reasons for dropping out and the method and outcome of compliance testing were not reported. In studies that claimed to be randomized, only 2 of 26 described the methods used.

Biomarkers identified

In the 48 studies included in this review, a total of 32 potential zinc biomarkers, 17 biomarkers of zinc status in zinc supplementation trials, and 25 biomarkers in zinc depletion trials were identified. A summary of all the biomarkers identified, including the number of studies, participants, and the results of the primary analysis where relevant is presented in **Table 4**. A large proportion of the studies included in this review measured plasma or serum zinc concentration, and for simplicity, here the term ''plasma'' will be used to refer to both. The results of the secondary analysis of biomarkers for which there were sufficient data for subgroup analysis are described below.

Plasma zinc concentration

Plasma zinc concentration was the most frequently investigated marker of zinc status, with a total of 40 data sets from 35 supplementation studies, which involved 1375 participants and 10 data sets from 10 depletion studies involving 79 participants. Combining data from the depletion and supplementation studies, primary analysis revealed an overall significant ($P < 0.00001$) response of plasma zinc concentration to dietary zinc intake [weighted mean difference (WMD): 2.9 μmol/L; 95% CI: 2.2, 3.5; $I^2 = 94\%$] (*see* Figure S1 under ''Supplemental data'' in the online supplement), but with high levels of heterogeneity between studies. A summary of the subgroup analysis of the response of plasma zinc concentration to zinc supplementation and depletion is given in **Table 5**. The data included in this analysis were mostly collected from studies in adults and the elderly. The response of plasma zinc concentration to supplementation was significant in adults, the elderly, pregnant and lactating women, men, women, and mixed-sex groups, but there were insufficient data to draw firm conclusions on children and adolescents or on postmenopausal women.

Individuals with a low or moderate baseline status responded significantly to supplementation. Data from 2 studies suggest that individuals with high baseline status do not respond to supplementation, but further studies are required. Sulfate, gluconate, and acetate all elicited a significant response, although there were only 2 studies of acetate. Secondary analysis revealed that all levels of zinc supplementation resulted in a significant increase in plasma zinc concentration and that the WMD in plasma zinc concentration increased in a dose-dependent manner (**Figure 2**).

All 11 depletion studies included in this review were B/A studies (Table 2) with between 5 and 15 participants per arm, including 7 studies of males, 2 studies of females, and 1 mixed-sex group study. As with the supplementation studies, most trials were carried out with adults (8 studies, 59 participants), with one study in 5 postmenopausal women (58) and one study in 15 elderly people (53). All participants had moderate baseline plasma zinc concentrations. However, failure to meet the minimal criteria for the severe and marginal intake categories precluded a definitive conclusion regarding the efficacy of plasma zinc concentration as a biomarker of zinc depletion. The subgroup analysis revealed a significant fall in plasma zinc concentration in response to a marginally depleted diet (Figure 2). Overall, plasma zinc appears to be a good marker of zinc status in all subgroupings for which we have sufficient studies to judge.

Urinary zinc excretion

Data on the response of urinary zinc excretion to changes in dietary intake were extracted from 5 supplementation and 4 depletion studies, comprising 6 studies in adults (47 individuals from supplementation studies and 25 individuals from depletion studies), 2 studies in elderly people (both supplemented, with a total of 326 participants), and one depletion study in postmenopausal women (5 participants). However, the units used for supplementation studies (mmol/mol creatinine) differed from those in the depletion studies (mmol/d), so only the supplementation studies could be pooled. Primary analysis (highest dose arm and longest duration for each included study) of the supplementation studies revealed a significant effect of zinc intake on urinary zinc excretion (WMD: 0.31 mmol/mol creatinine; 95% CI: 0.20, 0.43) without important heterogeneity ($I^2 = 0\%$) and was supported by depletion studies (WMD: 3.89 μmol/d; 95% CI: 1.01, 6.76; $I^2 = 93\%$).

TABLE 1
Basic characteristics of the included supplementation studies[1]

Studies	Country(s); age; sex; no. included	Description of intervention; latest time point; no. in intervention; no. in control at latest time	Micronutrient type	Study design	Biomarkers reported
Abdulla and Svensson,[2] 1979 (16)	Sweden; 25 y; X; 12	135 mg Zn; 12 wk; 7; 5	Zinc sulfate	RCT p	Pl Zn; ALAD
Abdulla and Svensson,[3] 1979 (16)	Sweden; 25 y; X; 7	45 mg Zn; 12 wk; 7	Zinc sulfate	B/A	Pl Zn; ALAD
Abdulla and Suck, 1998 (18)	India and Pakistan; 40 y; X; 45	15 mg Zn; 30 mg Zn; 45 mg Zn; 6 wk; 15 + 15 + 15	Zinc gluconate	B/A	Pl Zn
Barrie et al, 1987 (19)	United States; students; X; 15	50 mg Zn; 4 + 4 wk; 15; 15	Zinc gluconate	RCT c	Pl Zn; E Zn; hair Zn
Black et al, 1988 (20)	United States; 19–29 y; M; 45	50 mg Zn; 75 mg Zn; 12 wk; 13 + 9; 9	Zinc gluconate	RCT p	Pl Zn; urinary Zn
Bogden et al, 1988 (21)	United States; 71 y; X; 103	15 mg Zn; 100 mg Zn; 12 wk; 32 + 32; 32	Zinc acetate	RCT p	Pl Zn; MNC Zn; Pl ALP; PMNC Zn; Plat Zn
Crouse et al, 1984 (22)	United States; 20–55 y; M; 44	28.7 mg Zn; 8 wk; 11 + 12; 10 + 11	Zinc sulfate	RCT p	Pl Zn
Demetree et al, 1980 (23)	United States; 27–34 y; M; 10	100 mg Zn; 3 wk; 5; 5	Zinc sulfate	RCT p	Pl Zn
Donangelo et al, 2002 (24)	United States; 20–28 y; F; 11	22 mg Zn; 6 wk; 11	Zinc gluconate	B/A	Pl Zn; urinary Zn
Duchateau et al, 1981 (25)	Belgium; 20–60 y; F; M; 83	150 mg Zn; 4 wk; 20 + 20 + 20 + 23	Zinc sulfate	B/A	Pl Zn
Field et al, 1987 (26)	United Kingdom; 71–93 y; F; 15	50 mg Zn; 100 mg Zn; 150 mg Zn; 4 wk; 5 + 5 + 5	Zinc sulfate	B/A	Pl Zn; MNC Zn; PMNC Zn;
Fischer et al, 1984 (27)	Canada; adults; M; 26	50 mg Zn; 6 wk; 13; 13	Zinc gluconate	RCT p	Pl Zn
Gatto and Samman, 1995 (28)	Australia; 24.3 ± 4.2 y; M; 10	50 mg Zn; 4 + 4 wk; 10; 10	Zinc sulfate	RCT c	Pl Zn
Grider et al, 1990 (29)	United States; 25–32 y; M; 6	50 mg Zn; 9 wk; 6	Zinc gluconate	B/A	E MT
Gupta et al, 1998 (30)	India; 50 ± 10.65 y; X; 20	150 mg Zn; 6 wk; 20	Zinc sulfate	B/A	Pl Zn
Hayee et al, 2005 (31)	Bangladesh; 51.62 ± 10.49 y; X; 20	150 mg Zn; 6 wk; 20	Zinc sulfate	B/A	Pl Zn
Heckmann 2005 (32)	Germany; 41–82 y; X; 50	20 mg Zn; 12 wk; 24; 26	Zinc gluconate	RCT p	Pl Zn; saliva Zn
Hininger-Favier et al, 2007 (33)	France, United Kingdom, and Italy; 55–85 y; X; 387	15 mg Zn; 30 mg Zn; 26 wk; 126 + 131; 130	Zinc gluconate	RCT p	Pl Zn; E Zn; urinary Zn; Pl ALP
Hodkinson et al, 2007 (34)	Northern Ireland; 55–70 y; X; 101	15 mg Zn; 30 mg Zn; 26 wk; 28 + 34; 31	Zinc gluconate	RCT p	Pl Zn; E Zn; urinary Zn
Hollingsworth et al, 1987 (35)	United States; 66–85 y; X; 8	100 mg Zn; 12 wk; 8	Zinc sulfate	B/A	Pl Zn; L ecto-5′-NT
Hunt et al, 1985 (36)	United States; 16 y; F; 138	20 mg Zn; 19 wk; 56; 47	Zinc sulfate	RCT p	Pl Zn
Medeiros et al, 1987 (37)	United States; 19–29 y; M; 31	50 mg Zn; 75 mg Zn; 12 wk; 13 + 9; 9	Zinc gluconate	RCT p	Pl Zn; urinary Zn; hair Zn
O'Brien et al, 2007 (38)	United States; 31 ± 4 y; F; 26	15 mg Zn; 26 wk; 16; 10	Zinc sulfate	RCT p	Pl Zn
Pachotikarn et al, 1985 (39)	United States; 18–29 y; M; 23	50 mg Zn; 6 wk; 23	Zinc gluconate	B/A.	Pl Zn
Palin et al, 1979 (40)	United States; 16.8 ± 5.1 y; X; 17	23 mg Zn; 8 wk; 7; 10	Zinc sulfate	RCT p	Pl Zn
Peretz et al, 1993 (41)	Belgium; 24–46 y; X; 9	45 mg Zn; 9 wk; 9	Zinc gluconate	B/A	Pl Zn; MNC Zn; PMNC Zn
Prasad et al, 1996 (17)	United States; 64 ± 9 y; M; 9	30 mg Zn; 26 wk; 5	Zinc gluconate	B/A	Pl Zn; L Zn; PMNC Zn
Samman and Roberts, 1987 (42)	Australia; 28 y; F; M; 47	150 mg Zn; 6 + 6 wk; 41; 41	Zinc sulfate	RCT c	Pl Zn
Shaaban et al, 2005 (43)	Egypt; pregnant women; F; N/A	10 mg Zn; 8 wk; 30; 30	Zinc sulfate	RCT p	Nail Zn; hair Zn
Stur et al, 1996 (44)	Austria; 71 y; X; 112	45 mg Zn; 104 wk; 38; 42	Zinc sulfate	RCT p	Pl Zn
Sullivan and Cousins, 1997 (45)	United States; 19–35 y; M; 20	50 mg Zn; 2 wk; 10; 10	Zinc gluconate	RCT p	Pl Zn; monocyte MT cDNA
Sullivan et al, 1998 (46)	United States; 19–35 y; M; 25	50 mg Zn; 2 wk; 11; 11	Zinc gluconate	RCT p	Pl Zn; monocyte MT cDNA; E MT
Swanson et al, 1988 (47)	Switzerland; 64–95 y; X; 34	30 mg Zn; 4 wk; 17; 17	Zinc acetate	RCT p	Pl Zn; MNC Zn; PMNC Zn; Plat Zn
Tamura et al, 1996 (48)	United States; 13–39 y; F; 135	25 mg Zn; 17 wk; 70; 65	Zinc sulfate	RCT p	Pl Zn; E Zn
Tamura et al, 2001 (49)	United States; pregnant women; F; 63	25 mg Zn; 20 wk; 31; 32	Zinc sulfate	RCT p	Pl Zn; E Zn; Pl ALP; Pl EC-SOD
Weismann et al, 1977 (50)	Denmark; 17–37 y; X; 39	135 mg Zn; 12 wk; 13; 12	Zinc sulfate	RCT p	Pl Zn
Yadrick et al, 1989 (51)	United States; 25–40 y; F; 9	50 mg Zn; 10 wk; 9	Zinc gluconate	B/A	Pl Zn; salivary-sediment Zn

[1] M, exclusively male group; F, exclusively female group; X, mixed group; RCT p, randomized controlled trial—parallel; RCT c, randomized controlled trial—crossover study; B/A, before-after study; N/A, not available; Pl, plasma; ALAD, amino levulinic acid dehydratase; E, erythrocytes; MNC, mononuclear cells; Pl ALP, plasma alkaline phosphatase; PMNC, polymorphonuclear cells; Plat, platelet; E MT, erythrocyte metallothionein; L ecto-5′-NT, ecto-5′-nucleotidase; L, lymphocyte; monocyte MT cDNA, monocyte metallothionein cDNA; Pl EC-SOD, plasma extracellular superoxide dismutase.

[2] Study 1.

[3] Study 2.

TABLE 2
Basic characteristics of the included depletion studies[1]

Studies	Country; age range; sex; no. included	Description of intervention; latest time point; no. in intervention; no. in control at latest time	Study design	Biomarkers reported
Allan et al, 2000 (52)	United States; 27–47 y; M; 7	4.6 mg Zn; 10 wk; 7	B/A	Pl Zn; TL MT-2A mRNA
Bales et al, 1994 (53)	United States; 59–78 y; X; 15	3.97 ± 0.21 mg Zn; 2 wk; 15	B/A	Pl Zn; Pl ALP; Pl 5′NT
Freeland-Graves et al, 1981 (54)	United States; 23–44 y; F; 12	3.2 mg Zn; 3 wk; 12	B/A	Pl Zn; Mixed saliva Zn; salivary-sediment Zn
Lowe et al, 2004 (55)	United States; 20–35 y; M; 5	0.23 mg Zn; 6 wk; 5	B/A	Pl Zn; Pl ALP; EZE; Pl Zn flux; urinary Zn; EZP
Lukaski et al, 1984 (56)	United States; 23–57 y; M; 5	3.6 mg Zn; 17 wk; 5	B/A	Pl Zn
Mahajan et al, 1992 (57)	United States; 21–30 y; M; 8	3.2–5.6 mg Zn; 24 wk; 8	B/A	Pl Zn; Plat Zn; L Zn; Neutr Zn
Milne et al, 1987 (58)	United States; 50–63 y; F; 5	2.6 mg Zn; 17 wk; 5	B/A	Pl Zn; Plat Zn; MNC Zn; E Zn; Neutr Zn; CA; urinary Zn; feces Zn; Pl ALP; Pl ACE
Pinna et al, 2002 (59)	United States; 27–47 y; M; 8	4.6 mg Zn; 10 wk; 8	B/A	Pl Zn
Prasad et al, 1996 (17)	United States; 27 y; M; 4	4.9 mg Zn; 20 wk; 4	B/A	L Zn; PMNC Zn
Ruz et al, 1992 (60)	Canada; 25.3 ± 3.3 y; M; 15	4 mg Zn; 7 wk; 14	B/A	Pl Zn; urinary Zn; Neutr Zn; Neutr ALP; Neutr αDM; Plat Zn; EM Zn; EM ALP; EM NP
Thomas et al, 1992 (61)	United States; 22–35 y; M; 5	3.2 mg Zn; 6 wk, 5	B/A	Pl Zn; E Zn; E MT; urinary Zn

[1] M, exclusively male group; F, exclusively female group; X, mixed group; B/A, before-after study; Pl, plasma; TL MT-2A mRNA, T lymphocyte metallothionein-2A mRNA; Pl ALP, plasma alkaline phosphatase; Pl 5′NT, plasma 5′-nucleotidase; EZE, endogenous zinc excretion; EZP, exchangeable zinc pool; Plat, platelet; L, lymphocytes; Neutr, neutrophils; MNC, mononuclear cells; E, erythrocytes; CA, carbonic anhydrase; Pl ACE, plasma angiotensin-converting enzyme; PMNC, polymorphonuclear cells; Neutr ALP, neutrophil alkaline phosphatase; Neutr αDM, neutrophil α-D-mannosidase; EM, erythrocyte membrane; EM ALP, erythrocyte membrane alkaline phosphatase; EM NP, erythrocyte membrane neutral phosphatase; E MT, erythrocyte metallothionein.

All of the supplementation trials that measured urinary zinc concentration used zinc gluconate, with 3 data sets (326 participants) in the 15–25 mg/d range, 4 data sets (370 participants) in the 26–50 mg/d range, and 2 data sets (36 participants) in the 51–100 mg/d range. A statistically significant increase in urinary zinc excretion was seen in response to all 3 dose ranges, but only the 15–25 and the 26–50 mg/d subgroups included enough studies to declare the marker useful (**Figure 3**). The data do suggest a dose response (*see* Table S2 under ''Supplemental data'' in the online issue).

Significant responses were recorded in studies of adults, the elderly, males, mixed-sex groups, and females and in those with a low or moderate zinc status at baseline; however, there were enough studies ($\geq$3) and participants ($\geq$50) to declare urinary zinc a useful marker of zinc status only in those with moderate zinc status at baseline (*see* Table S2 under ''Supplemental data'' in the online issue).

Erythrocyte zinc concentration

A total of 5 supplementation and 2 depletion studies reported values for erythrocyte zinc concentration. Neither primary analysis (Figure S2 under ''Supplemental data'' in the online issue) nor any individual study suggested a response of this biomarker to changes in zinc intake (for more subgroup analysis details, *see* Table S3 under ''Supplemental data'' in the online issue).

Mononuclear cell zinc concentration

Five studies, including 95 participants, assessed the effect of the change in zinc intake on mononuclear cell zinc concentration. Pooling these 5 studies suggested that this is not a useful biomarker of zinc status (WMD: -0.05 μmol/10^{10} cells; 95% CI: -0.21, 0.11; $I^2 = 38\%$).

Polymorphonuclear cell zinc concentration

Five supplementation trials and one depletion study that measured polymorphonuclear cells (PMNCs) as a biomarker of zinc status were identified. Population groups represented in the studies included adults (2 studies) and elderly persons (4 studies). Individual studies were variable, which suggests both significantly positive and negative effects of increased zinc status on PMNC zinc concentration. Neither primary (Figure S3 under ''Supplemental data'' in the online issue) nor secondary analyses (Table S4 under ''Supplemental data'' in the online issue) revealed any significant response of this biomarker to changes in zinc intake. Our data suggest that PMNC zinc concentration is not a useful marker of zinc status.

Platelet zinc concentration

Five studies that measured platelet zinc concentration—2 RCT supplementation studies and 3 depletion B/A studies—were identified. Of the supplementation studies, there were data for each of the following intake ranges: 15–25 mg/d (21), 26–50 mg/d (47), and 51–100 mg/d (21). Of the depletion studies, there was one in the moderate range of depletion (58) and 2 in the marginal range (57, 60). The primary analysis, combining data from supplementation and depletion studies, did not reveal a significant response to changes in dietary zinc intake (WMD: 0.09 nmol/10^9 cells; 95% CI: -1.12, 1.30; $I^2 = 76\%$; Figure S4 under ''Supplemental data'' in the online supplement).

Hair zinc concentration

Data were analyzed from 3 RCT supplementation studies, which included a total of 93 adult participants with either low or moderate baseline status and intakes in the ranges of 15–25, 26–50, and 51–100 mg/d. Primary analysis revealed that hair zinc

TABLE 3
Validity of included studies[1]

Studies	Randomized? Method of randomization	Reasons for dropouts by intervention group	Method for checking; results of compliance check
Abdulla and Svensson,[2] 1979 (16)	Randomized; N/A	No information on dropouts	N/A; N/A
Abdulla and Svensson,[3] 1979 (16)	Nonrandomized	No information on dropouts	N/A; N/A
Abdulla and Suck, 1998 (18)	Randomized; N/A	No information on dropouts	N/A; N/A
Allan et al, 2000 (52)	Nonrandomized	No exclusion	N/A; N/A
Bales et al, 1994 (53)	Nonrandomized	No information on dropouts	N/A; N/A
Barrie et al, 1987 (19)	Randomized; N/A	No exclusion	N/A; N/A
Black et al, 1988 (20)	Randomized; N/A	Lack of compliance, illness [8][4]	N/A; N/A
Bogden et al, 1988 (21)	Randomized; random number tables	No information on dropouts	Count of returned capsules; 87%
Crouse et al, 1984 (22)	Randomized; N/A	No exclusion	Daily records; >95%
Demetree et al, 1980 (23)	Randomized; N/A	No exclusion	N/A; N/A
Donangelo et al, 2002 (24)	Nonrandomized	No exclusion	N/A; N/A
Duchateau et al, 1981 (25)	Nonrandomized	No exclusion	N/A; N/A
Field et al, 1987 (26)	Randomized; N/A	No exclusion	N/A; N/A
Fischer et al, 1984 (27)	Randomized; N/A	No information on dropouts	N/A; N/A
Freeland-Graves et al, 1981 (54)	Nonrandomized	No exclusion	N/A; N/A
Gatto and Samman, 1995 (28)	Randomized; N/A	No exclusion	Count of returned capsules; 98%
Grider et al, 1990 (29)	Nonrandomized	No information on dropouts	N/A; N/A
Gupta et al, 1998 (30)	Nonrandomized	No exclusion	N/A; N/A
Hayee et al, 2005 (31)	Nonrandomized	No exclusion	N/A; N/A
Heckmann 2005 (32)	Randomized; software program	No exclusion	N/A; N/A
Hininger-Favier et al, 2007 (33)	Randomized; N/A	No information on dropouts	Count of returned capsules; ≥98%
Hodkinson et al, 2007 (34)	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A
Hollingsworth et al, 1987 (35)	Nonrandomized	No exclusion	N/A; N/A
Hunt et al, 1985 (36)	Randomized; N/A	Lack of compliance [14]	N/A; N/A
Lowe et al, 2004 (55)	Not randomized	Reasons not reported, lack of compliance [7]	N/A; N/A
Lukaski et al, 1984 (56)	Nonrandomized	No exclusion	N/A; N/A
Mahajan et al, 1992 (57)	Nonrandomized	No exclusion	N/A; N/A
Medeiros et al, 1987 (37)	Randomized; N/A	Lack of compliance, illness [8]	N/A; N/A
Milne et al, 1987 (58)	Nonrandomized	No exclusion	Strict control; N/A
O'Brien et al, 2007 (38)	Randomized; N/A	No exclusion	Count of returned capsules; >90%
Pachotikarn et al, 1985 (39)	Nonrandomized	Lack of compliance, mononucleosis [2]	N/A; N/A
Palin et al, 1979 (40)	Randomized; N/A	No exclusion	N/A; N/A
Peretz et al, 1993 (41)	Nonrandomized	No exclusion	N/A; N/A
Pinna et al, 2002 (59)	Nonrandomized	No exclusion	N/A; N/A
Prasad et al, 1996 (17)[5]	Nonrandomized	No exclusion	N/A; N/A
Prasad et al, 1996 (17)[6]	Nonrandomized	Reasons not reported [4]	N/A; N/A
Ruz et al, 1992 (60)	Nonrandomized	No exclusion	Strict control; satisfactory
Samman and Roberts, 1987 (42)	Randomized; N/A	Side effects [6]	N/A; N/A
Shaaban et al, 2005 (43)	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A
Stur et al, 1996 (44)	Randomized; N/A	Side effects, personal reasons [18]	N/A; ≥80%
Sullivan and Cousins, 1997 (45)	Randomized; N/A	No exclusion	By consumption in the presence of a dietitian
Sullivan et al, 1998 (46)	Randomized; N/A	No information on dropouts	N/A; N/A
Swanson et al, 1988 (47)	Randomized; N/A	No exclusion	Count of returned capsules; excellent
Tamura et al, 1996 (48)	Randomized; N/A	No information on dropouts	Count of returned capsules; 78%
Tamura et al, 2001 (49)	Randomized; N/A	No exclusion	N/A; N/A
Thomas et al, 1992 (61)	Nonrandomized	No exclusion	N/A; N/A
Weismann et al, 1977 (50)	Randomized; N/A	Side effects, lack of compliance [7]	Count of returned capsules; N/A
Yadrick et al, 1989 (51)	Nonrandomized	No information on dropouts	N/A; N/A

[1] N/A, not available.
[2] Study 1.
[3] Study 2.
[4] No. of subjects in brackets (all such values).
[5] Depletion study.
[6] Supplementation study.

TABLE 4
Primary analyses (the greatest duration and the greatest supplementation dose) for each of the identified biomarkers for supplementation with zinc and zinc depletion[1]

Biomarker	No. of studies (no. of included participants)	Pooled effect size, WMD (95% CI)	Measure of heterogeneity, I^2	Appears effective as a biomarker?
			%	
Plasma/serum Zn (μmol/L)	50 (1454)	2.88 (2.24, 3.51)	93.6	Yes
Urinary Zn (mmol/mol creatinine) supplementation	5 (373)	0.31 (0.20, 0.43)	0	Yes
Urinary Zn (μmol/d) depletion	4 (30)	3.89 (1.01, 6.76)	92.9	Unclear
Erythrocyte Zn (μmol/L)	7 (537)	2.20 (−4.58, 8.98)	0	No
Mononuclear cell Zn (μmol/10^{10} cells)	5 (95)	−0.05 (−0.21, 0.11)	37.7	No
Polymorphonuclear cell Zn (μmol/10^{10} cells)	6 (101)	0.05 (−0.13, 0.22)	83.3	No
Platelet Zn (nmol/10^9 cells)	5 (105)	0.09 (−1.12, 1.30)	76.0	No
Hair Zn (ppm)	3 (93)	13.24 (11.91, 14.56)	0	Yes
Plasma alkaline phosphatase (IU/L)	6 (410)	4.14 (−2.38, 10.65)	56.6	No
Aminolevulinic acid dehydratase (IU/L RBC)	2 (19)	7.88 (−7.90, 23.66)	89.4	Unclear
Erythrocyte metallothionein (μg MT/g protein) supplementation	2 (25)	121.82 (−22.65, 266.29)	90.7	Unclear
Erythrocyte metallothionein (nmol/g protein) depletion	1 (5)	0.30 (−0.43, 1.03)	N/A	Unclear
Monocyte metallothionein cDNA (pg cDNA/ng RNA)	2 (40)	1.02 (0.48, 1.56)	0	Unclear
Saliva Zn (mg/dL)	1 (50)	2.82 (−2.67; 8.31)	N/A	Unclear
Salivary-sediment Zn (μmol/g dry wt)	2 (14)	0.27 (−0.07, 0.60)	N/A	Unclear
Plasma extracellular superoxide dismutase (IU/mL)	1 (52)	0.50 (−1.46, 2.46)	N/A	Unclear
Lymphocyte Zn (μmol/10^{10} cells)	3 (18)	−0.36 (−1.61, 0.90)	99.7	Unclear
Lymphocyte ecto-5′-nucleotidase (nmol · h^{-1} · 10^{-6} cells)	1 (6)	−0.60 (−3.91, 2.71)	N/A	Unclear
Nail Zn (ppm)	1 (60)	24.10 (4.69, 43.51)	N/A	Unclear
Plasma angiotensin-converting enzyme (IU/L)	1 (5)	−19.40 (−38.34, −0.46)	N/A	Unclear
Neutrophil Zn (μg/10^{10} cells)	3 (26)	7.44 (−15.71, 30.58)	95.0	Unclear
T lymphocyte metallothionein −2A mRNA (fg MT-2A mRNA/pg β-actin mRNA)	1 (7)	6.60 (−1.77, 14.97)	N/A	Unclear
Plasma 5′-nucleotidase (Shinowara units)	1 (15)	1.75 (0.54, 2.96)	N/A	Unclear
Mixed-saliva Zn (μmol/L)	1 (7)	−0.73 (−2.49, 1.03)	N/A	Unclear
Endogenous Zn excretion (μmol/d)	1 (5)	36.70 (33.96, 39.44)	N/A	Unclear
Plasma Zn flux (mmol/d)	1 (5)	3.74 (2.42, 5.06)	N/A	Unclear
Exchangeable Zn pool (mmol)	1 (5)	0.92 (0.27, 1.57)	N/A	Unclear
Carbonic anhydrase (IU/g Hgb)	1 (5)	−0.10 (−0.89, 0.69)	N/A	Unclear
Feces Zn (μmol/d)	1 (5)	60.39 (57.00, 63.78)	N/A	Unclear
Neutrophil α-D-mannosidase (nmol product · h^{-1} · mg $protein^{-1}$)	1 (15)	−5.30 (−58.75, 48.15)	N/A	Unclear
Neutrophil alkaline phosphatase (nmol product · h^{-1} · mg $protein^{-1}$)	1 (15)	−122.80 (−294.85, 49.25)	N/A	Unclear
Erythrocyte membrane Zn (μmol/g protein)	1 (15)	0.05 (−0.11, 0.21)	N/A	Unclear
Erythrocyte membrane alkaline phosphatase (nmol product· min^{-1} · mg $protein^{-1}$)	1 (15)	0.15 (−0.04, 0.34)	N/A	Unclear
Erythrocyte membrane NP (nmol product · min^{-1} · mg $protein^{-1}$)	1 (15)	0.00 (−0.15, 0.15)	N/A	Unclear

[1] To claim that a biomarker was effective (ie, reflected change in status) within a review, there had to be the following: *1*) statistical significance within a forest plot (95% CI did not include 0 or $P < 0.05$), *2*) ≥3 trials contributing data, and *3*) ≥50 participants contributing data in the intervention arm, control arm, or both. To claim that a biomarker was ineffective, there had to be the following: *1*) a lack of statistical significance within a forest plot (95% CI included 0 or $P \geq 0.05$); *2*) ≥3 trials contributing data; *3*) ≥50 participants contributing data in the intervention arm, control arm, or both; and *4*) roughly similar study results (acceptable heterogeneity levels so that I^2 <50%). RBC, red blood cell; N/A, no available data.

concentration was significantly elevated after supplementation (WMD: 13.24 ppm; 95% CI: 11.91, 14.56; $I^2 = 0\%$) (**Figure 4**). Insufficient data precluded subgroup analyses.

Plasma alkaline phosphatase activity

Six studies investigating the response of plasma alkaline phosphatase activity to changes in zinc intake were included: 3 supplementation (RCTs) and 3 depletion (B/A) studies. Overall, the primary analysis (combining the supplementation and depletion trials) revealed no significant effect of zinc intakes on plasma alkaline phosphatase activity (WMD: 4.14 IU/L; 95% CI: −2.38, 10.66; $I^2 = 56.6\%$; Figure S5 under "Supplemental data" in the online issue), which suggests that this is not a useful zinc biomarker. Subgrouping also did not reveal any groups in which this was a clearly useful biomarker of zinc status (Table S5 under "Supplemental data" in the online issue).

Other potential markers

We found at least one study each to assess the effects of zinc supplementation or depletion on the following potential zinc biomarkers: 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 angiotensin-converting enzyme, neutrophil zinc, T lymphocyte metallothionein −2A mRNA, plasma 5′-nucleotidase, endogenous zinc excretion, plasma zinc flux, exchangeable zinc pool, carbonic anhydrase, fecal zinc,

TABLE 5
Subgroup analysis of the results of the systematic review of data on changes in plasma or serum zinc after supplementation with zinc and zinc depletion[1]

Analysis	Mean effect, WMD (95% CI)	Study design: RCT supplementation	Study design: Before/after supplementation	Study design: Before/after depletion	Heterogeneity I^2	Biomarker useful?
	μmol/L	*no. of studies included (no. of participants within these studies)*			%	
All studies (primary outcome)	2.88 (2.24, 3.51)	25 (1157)	15 (218)	10 (79)	93.6	Yes
Children and adolescents	0.22 (−4.34, 4.78)	1 (17)	N/A	N/A	N/A	Unclear
Pregnancy and lactation	0.37 (0.32, 0.43)	4 (325)	N/A	N/A	0	Yes
Adults	3.50 (2.47, 4.54)	15 (311)	12 (197)	8 (59)	91.5	Yes
Postmenopausal women	−1.00 (−2.72, 0.72)	N/A	N/A	1 (5)	N/A	Unclear
Elderly	2.78 (1.28, 4.28)	5 (504)	3 (21)	1 (15)	89.7	Yes
Males	3.23 (2.13, 4.33)	10 (189)	3 (51)	7 (53)	83.8	Yes
Mixed	2.98 (1.47, 4.49)	10 (623)	6 (79)	1 (15)	95.3	Yes
Females	1.76 (0.98, 2.53)	5 (345)	6 (88)	2 (11)	84.2	Yes
Low status at baseline	0.43 (0.07, 0.79)	5 (324)	1 (11)	N/A	48.6	Yes
Moderate status at baseline	3.24 (2.36, 4.11)	18 (789)	14 (207)	10 (79)	91.2	Yes
High status at baseline	2.66 (−1.01, 6.32)	2 (44)	N/A	N/A	0	Unclear
Micronutrient type (zinc sulfate)	3.55 (2.36, 4.74)	14 (564)	9 (143)	X	94.7	Yes
Micronutrient type (zinc gluconate)	2.47 (1.63, 3.32)	9 (495)	6 (75)	X	78.3	Yes
Micronutrient type (zinc acetate)	3.95 (2.96, 4.95)	2 (98)	N/A	X	0	Unclear
Dose						
1 (<1 mg Zn/d)	8.70 (7.05, 10.35)	X	X	1 (5)	N/A	Unclear
2 (1–2.9 mg Zn/d)	−1.00 (−2.72, 0.72)	X	X	1 (5)	N/A	Unclear
3 (3–5 mg Zn/d)	1.59 (0.28, 2.89)	X	X	8 (69)	89.1	Yes
4 (15–25 mg Zn/d)	0.7 (0.38, 1.03)	9 (771)	2 (26)	X	49.3	Yes
5 (26–50 mg Zn/d)	2.61 (1.88, 3.34)	13 (621)	8 (91)	X	76.3	Yes
6 (51–100 mg Zn/d)	4.21 (3.15, 5.26)	4 (110)	2 (13)	X	0	Yes
7 (101–150 mg Zn/d)	4.94 (2.18, 7.70)	4 (119)	7 (128)	X	91.4	Yes

[1] To claim that a biomarker was effective (reflected change in status) within a review, 3 conditions needed to be met: *1*) statistical significance within a forest plot (95% CI did not include 0 or $P < 0.05$), *2*) ≥3 trials contributing data, and *3*) ≥50 participants contributing data in the intervention arm, control arm, or both. To claim that a biomarker was ineffective, 4 conditions had to be met: *1*) lack of statistical significance within a forest plot (95% CI included 0 or $P \geq 0.05$); *2*) ≥3 trials contributing data; *3*) ≥50 participants contributing data in the intervention arm, control arm, or both; and *4*) roughly similar study results (heterogeneity levels were acceptable so that $I^2 < 50\%$). X indicates that this design category is not applicable to the subgroup analysis. RCT, randomized controlled trial; WMD, weighted mean difference; N/A, no available data.

neutrophil α-D-mannosidase, neutrophil alkaline phosphatase, erythrocyte membrane zinc, erythrocyte membrane alkaline phosphatase, and erythrocyte membrane NP. However, there were not enough eligible studies of these markers to allow us to decide whether they were effective markers of zinc status.

DISCUSSION

Data were extracted and analyzed on 32 potential biomarkers from 46 publications. Plasma zinc concentration responded to dietary manipulation in adults, women, men, pregnant and lactating women, the elderly, and those at low and moderate baseline zinc status and in both depletion and supplementation studies. Urinary zinc excretion also appeared to respond to change in zinc status for all groups for which we had data, but with fewer studies there were fewer subgroupings with enough studies to make a clear decision about urinary zinc response. Hair zinc concentration also responded to zinc supplementation, but there were insufficient studies to assess in which subgroups these may be effective markers. For platelet, PMNC, mononuclear cell, and erythrocyte zinc concentration and alkaline phosphatase activity, there were sufficient data to judge them as likely to be ineffective as biomarkers of zinc status.

More high-quality studies are required to assess the effects of most potential zinc biomarkers and in a variety of populations. Ideally, these would be highly controlled studies or RCTs, with clearly presented methodology indicating that they are at low risk of bias. Studies need to describe their randomization methodology, clearly describe the numbers of dropouts or exclusions and the reasons for their cessation of inclusion, check compliance, and report the results of such checks.

The majority of studies identified and included in this review were zinc supplementation studies that covered a broad range of zinc intake levels, which ranged from intakes that could be achieved through diet alone to pharmacologic doses at values of >10 times the European and US dietary recommendations. This abundant data set enabled subgroup analysis according to life stage, sex, baseline status, and dose for some of the biomarkers. Fewer zinc depletion studies were identified, and participant numbers were low compared with the supplementation trials because of the practical and ethical difficulties of conducting depletion trials. The depletion studies investigated a broad range of potential biomarkers. There were some notable gaps in the availability of data from certain population groups; in particular, there was a complete absence of data regarding infants and immigrant population groups and a paucity of studies of zinc status in adolescents.

The search for a reliable indicator for zinc has been problematic because the effective regulation of zinc homeostasis buffers the functional response to dietary deficiency and excess. The total amount of zinc present in the human body ranges from

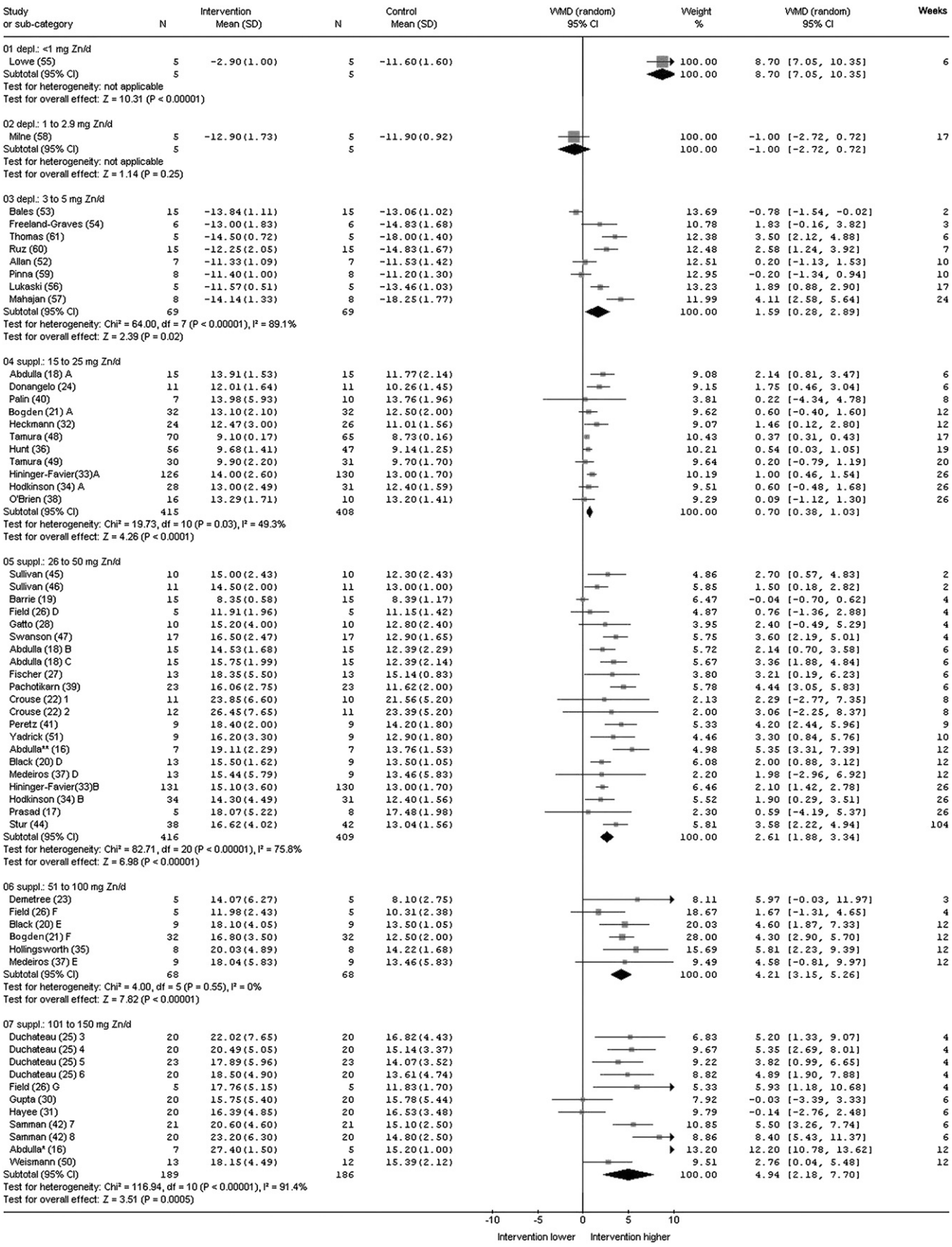

FIGURE 2. Secondary analysis of the response of plasma zinc concentration (μmol/L) to zinc supplementation and zinc depletion with subgrouping by dose (mg/d). *Study 1; **study 2; A, 15-mg Zn/d group; B, 30-mg Zn/d group; C, 45-mg Zn/d group; D, 50-mg Zn/d group; E, 75-mg Zn/d group; F, 100-mg Zn/d group; G, 150-mg Zn/d group; 1, endurance-trained male group; 2, sedentary male group; 3, male group (20–40 y); 4, female group (20–40 y); 5, female group (20–40 y + oral contraceptive); 6, female group (40–50 y); 7, male group; 8, female group. WMD, weighted mean difference.

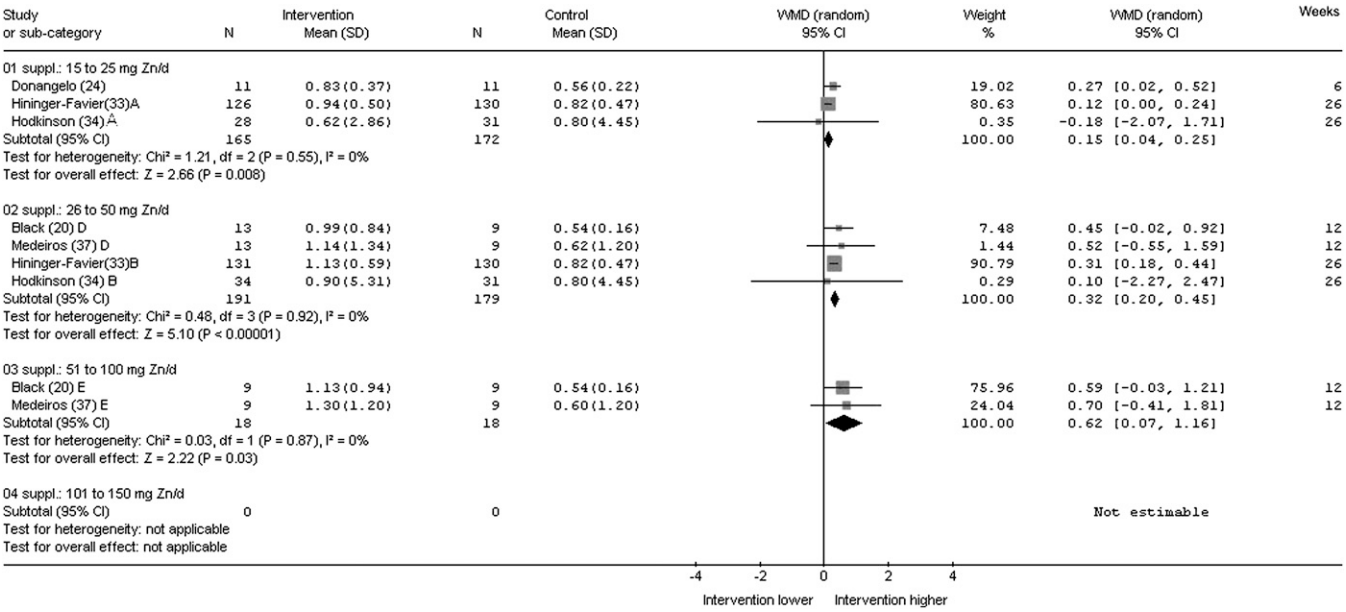

FIGURE 3. Secondary analysis of the response of urinary zinc excretion to zinc supplementation (mmol/mol creatinine) with subgrouping by dose (mg/d). A, 15-mg Zn/d group; B, 30-mg Zn/d group; D, 50-mg Zn/d group; E, 75-mg Zn/d group. WMD, weighted mean difference.

1.5 to 2.5 mg, most of which is found intracellularly within skeletal muscle tissue (57%), bone (29%), and other tissues, including skin and organs (62). 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 (≈10%) represents the ''functional pool'' (3, 63, 64), which is composed of zinc, located within the liver and other tissues, that exchanges rapidly with the plasma. When this functional pool is depleted, zinc deficiency ensues (3).

Plasma zinc, which represents <0.2% of total body zinc content, was the most frequently measured biomarker of zinc status, thus enabling the most comprehensive analysis of this biomarker across the subgroups. The result of the primary analysis of data from depletion and supplementation studies yielded conclusive evidence that plasma zinc concentration reflects zinc intake. However, although plasma zinc concentration responds to altered intake over short periods, the homeostatic mechanisms that act to maintain plasma zinc concentration within the physiologic range (namely, adaptive changes in efficiency of absorption and levels of endogenous excretion) may prevent high plasma concentrations from being sustained over a prolonged period.

All studies included in the analysis were undertaken in apparently healthy individuals. It is well established that plasma zinc concentration can fall in response to factors unrelated to zinc status or dietary zinc intake, including infection, inflammation, stress, or trauma. Conversely, tissue catabolism during starvation can release zinc into the circulation, causing a transient increase in circulating zinc levels. Furthermore, postprandial falls in plasma zinc concentration of ≤22% have been reported (65, 66). Clearly, the interpretation of plasma zinc concentration requires knowledge of all of these possible confounders.

The reliability of plasma zinc concentration as a marker of zinc status is also dependent on the proper collection and storage of the sample, because adventitious zinc can easily be added to samples by environmental exposure and inappropriate handling of samples. Care must be taken to avoid contamination from the

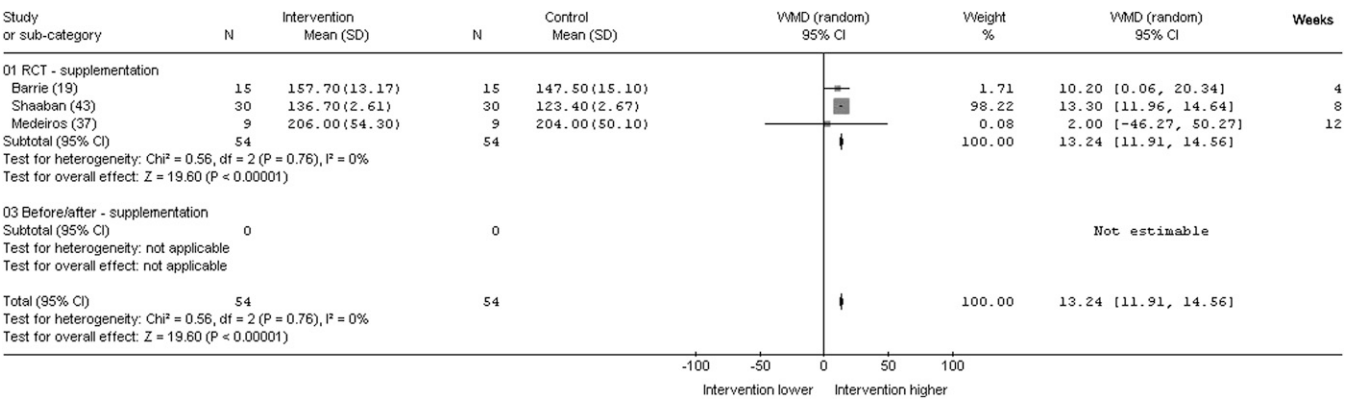

FIGURE 4. Primary analysis of the response of hair zinc (ppm) to zinc supplementation. WMD, weighted mean difference; RCT, randomized controlled trial.

collection or storage vessel, hemolysis of the sample when zinc is released from the red blood cells into the plasma. The time between taking the sample and the separation of the plasma from the red blood cells can also be crucial. Guidance for the collection of plasma for zinc determination has been prepared by the International Zinc Nutrition Collaborative Group (67).

Of all the other biomarkers evaluated in this systematic review, both 24-h urinary zinc excretion and hair zinc appear to respond to zinc supplementation, but the effect of zinc depletion is inconclusive due to insufficient data. On the basis of the results of this review, erythrocytes, PMNCs, mononuclear cells, platelet zinc, and plasma alkaline phosphatase did not appear to be useful biomarkers of zinc status.

Measurement of zinc status in vulnerable groups, such as immigrant populations in Europe whose ability to absorb zinc may be compromised by high phytate concentrations, are required. In addition, there is a notable absence of data on zinc status in infants. Although zinc depletion in children across Europe is rare, situations in which general malnutrition is present with concurrent zinc deficiency may provide opportunities to monitor the response of biomarkers to supplementation.

Further research is needed to evaluate potentially useful biomarkers, including enzymes and other zinc-binding proteins that were measured in only 1 or 2 studies and for which conclusions were unable to be drawn. Kinetic parameters measured using stable isotope techniques, including the exchangeable zinc pool, fractional zinc absorption, and endogenous zinc excretion, also have potential value in the search for biomarkers. Although stable isotope studies are expensive, technically demanding, and unsuitable for large population studies, they may be useful tools for evaluating more accessible potential biomarkers and to develop new biomarkers. At the present time, on the basis of the results of this review, plasma zinc concentration is the only biomarker of status that can be used to measure zinc status in individuals with either a low or a high supply of dietary zinc, but with many limitations and constraints. Urinary zinc excretion (24 h) and hair zinc can provide useful information on zinc status in zinc-supplemented individuals, but whether these reflect zinc status in depleted individuals is not certain. It is clear that there is an urgent need to develop new biomarkers of zinc status. (Other articles in this supplement to the Journal include references 15 and 68–74.)

We acknowledge the expert advice and support from Lee Hooper and Rosalind Gibson in the preparation of this article.

The authors' responsibilities were as follows—KF and NML: assessed the studies for inclusion, extracted data, and assessed validity; KF: conducted meta-analyses and tabulated data; NML: wrote the first draft of the manuscript; and TD: contributed to writing the manuscript and approved the final version. None of the authors had a conflict of interest.

REFERENCES

1. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 2003;78(suppl):633S–9S.
2. Geissler C, Powers H. Human nutrition. 11th ed. London, United Kingdom: Elsevier Churchill Livingstone, 2005.
3. King JC. Assessment of zinc status. J Nutr 1990;120(suppl):1474–9.
4. King JC, Shames DM, Lowe NM, et al. Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. Am J Clin Nutr 2001; 74:116–24.
5. Atherton DJ, Muller DP, Aggett PJ, Harries JT. A defect in zinc uptake by jejunal biopsies in acrodermatitis enteropathica. Clin Sci 1979;56: 505–7.
6. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. Br J Nutr 2008;99(suppl): S14–23.
7. Allen LH. Zinc and micronutrient supplements for children. Am J Clin Nutr 1998;68(suppl):495S–8S.
8. Baer MT, King JC. Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am J Clin Nutr 1984;39: 556–70.
9. Wood RJ. Assessment of marginal zinc status in humans. J Nutr 2000; 130(suppl):1350S–4S.
10. Brown KH. Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. Am J Clin Nutr 1998;68(suppl):425S–9S.
11. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2002; 75:1062–71.
12. Hotz C, Lowe NM, Araya M, Brown KH. Assessment of the trace element status of individuals and populations: the example of zinc and copper. J Nutr 2003;133(suppl):1563S–8S.
13. Hambidge M. Biomarkers of trace mineral intake and status. J Nutr 2003;133(suppl):948S–55S.
14. Thompson RP. Assessment of zinc status. Proc Nutr Soc 1991;50:19–28.
15. Hooper L, Ashton K, Harvey LJ, Decsi T, Fairweather-Tait SJ. Assessing potential biomarkers of micronutrient status by using a systematic review methodology: methods. Am J Clin Nutr 2009; 89(suppl):1953S–9S.
16. Abdulla M, Svensson S. Effect of oral zinc intake on delta-aminolaevulinic acid dehydratase in red blood cells. Scand J Clin Lab Invest 1979;39: 31–6.
17. Prasad AS, Mantzoros CS, Beck FW, Hess JW, Brewer GJ. Zinc status and serum testosterone levels of healthy adults. Nutrition 1996;12: 344–8.
18. Abdulla M, Suck C. Blood levels of copper, iron, zinc, and lead in adults in India and Pakistan and the effect of oral zinc supplementation for six weeks. Biol Trace Elem Res 1998;61:323–31.
19. Barrie SA, Wright JV, Pizzorno JE, Kutter E, Barron PC. Comparative absorption of zinc picolinate, zinc citrate and zinc gluconate in humans. Agents Actions 1987;21:223–8.
20. Black MR, Medeiros DM, Brunett E, Welke R. Zinc supplements and serum lipids in young adult white males. Am J Clin Nutr 1988;47:970–5.
21. Bogden JD, Oleske JM, Lavenhar MA, et al. Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. Am J Clin Nutr 1988;48:655–63.
22. Crouse SF, Hooper PL, Atterbom HA, Papenfuss RL. Zinc ingestion and lipoprotein values in sedentary and endurance-trained men. JAMA 1984; 252:785–7.
23. Demetree JW, Safer LF, Artis WM. The effect of zinc on the sebum secretion rate. Acta Derm Venereol 1980;60:166–9.
24. Donangelo CM, Woodhouse LR, King SM, Viteri FE, King JC. Supplemental zinc lowers measures of iron status in young women with low iron reserves. J Nutr 2002;132:1860–4.
25. Duchateau J, Delespesse G, Vereecke P. Influence of oral zinc supplementation on the lymphocyte response to mitogens of normal subjects. Am J Clin Nutr 1981;34:88–93.
26. Field HP, Whitley AJ, Srinivasan TR, Walker BE, Kelleher J. Plasma and leucocyte zinc concentrations and their response to zinc supplementation in an elderly population. Int J Vitam Nutr Res 1987;57:311–7.
27. Fischer PW, Giroux A, L'Abbe MR. Effect of zinc supplementation on copper status in adult man. Am J Clin Nutr 1984;40:743–6.
28. Gatto LM, Samman S. The effect of zinc supplementation on plasma lipids and low-density lipoprotein oxidation in males. Free Radic Biol Med 1995;19:517–21.
29. 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.
30. Gupta R, Garg VK, Mathur DK, Goyal RK. Oral zinc therapy in diabetic neuropathy. J Assoc Physicians India 1998;46:939–42.
31. Hayee MA, Mohammad QD, Haque A. Diabetic neuropathy and zinc therapy. Bangladesh Med Res Counc Bull 2005;31:62–7.
32. Heckmann SM, Hujoel P, Habiger S, et al. Zinc gluconate in the treatment of dysgeusia: a randomized clinical trial. J Dent Res 2005;84:35–8.
33. Hininger-Favier I, Andriollo-Sanchez M, Arnaud J, et al. Age- and sex-dependent effects of long-term zinc supplementation on essential

trace element status and lipid metabolism in European subjects: the Zenith Study. Br J Nutr 2007;97:569–78.
34. Hodkinson CF, Kelly M, Alexander HD, et al. Effect of zinc supplementation on the immune status of healthy older individuals aged 55-70 years: the ZENITH Study. J Gerontol A Biol Sci Med Sci 2007;62: 598–608.
35. Hollingsworth JW, Otte RG, Boss GR, Fryberger MF, Strause LG, Saltman P. Immunodeficiency and lymphocyte ecto-5′-nucleotidase activity in the elderly: a comparision of the effect of a trace mineral supplement (1USRDA) with high zinc (6.7 X USRDA). Nutr Res 1987; 7:801–11.
36. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. Am J Clin Nutr 1985;42:815–28.
37. Medeiros DM, Mazhar A, Brunett EW. Failure of oral zinc supplementation to alter hair zinc levels among healthy human males. Nutr Res 1987;7:1109–15.
38. O'Brien CE, Krebs NF, Westcott JL, Dong F. Relationships among plasma zinc, plasma prolactin, milk transfer, and milk zinc in lactating women. J Hum Lact 2007;23:179–83.
39. Pachotikarn C, Medeiros DM, Windham F. Effect of oral zinc supplementation upon plasma lipids, blood pressure, and other variables in young adult white males. Nutr Rep Int 1985;32:373–82.
40. Palin D, Underwood BA, Denning CR. The effect of oral zinc supplementation on plasma levels of vitamin A and retinol-binding protein in cystic fibrosis. Am J Clin Nutr 1979;32:1253–9.
41. Peretz A, Neve J, Jeghers O, Pelen F. Zinc distribution in blood components, inflammatory status, and clinical indexes of disease activity during zinc supplementation in inflammatory rheumatic diseases. Am J Clin Nutr 1993;57:690–4.
42. Samman S, Roberts DC. The effect of zinc supplements on plasma zinc and copper levels and the reported symptoms in healthy volunteers. Med J Aust 1987;146:246–9.
43. Shaaban SY, El-Hodhod MA, Nassar MF, Hegazy AE, El-Arab SE, Shaheen FM. Zinc status of lactating Egyptian mothers and their infants: effect of maternal zinc supplementation. Nutr Res 2005;25:45–53.
44. Stur M, Tittl M, Reitner A, Meisinger V. Oral zinc and the second eye in age-related macular degeneration. Invest Ophthalmol Vis Sci 1996;37: 1225–35.
45. Sullivan VK, Cousins RJ. Competitive reverse transcriptase-polymerase chain reaction shows that dietary zinc supplementation in humans increases monocyte metallothionein mRNA levels. J Nutr 1997;127: 694–8.
46. Sullivan VK, Burnett FR, Cousins RJ. Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. J Nutr 1998;128:707–13.
47. Swanson CA, Mansourian R, Dirren H, Rapin CH. Zinc status of healthy elderly adults: response to supplementation. Am J Clin Nutr 1988;48: 343–9.
48. Tamura T, Goldenberg RL, Johnston KE, Freeberg LE, DuBard MB, Thomas EA. In vitro zinc stimulation of angiotensin-converting enzyme activities in human plasma. J Nutr Biochem 1996;7:55–9.
49. Tamura T, Olin KL, Goldenberg RL, Johnston KE, Dubard MB, Keen CL. Plasma extracellular superoxide dismutase activity in healthy pregnant women is not influenced by zinc supplementation. Biol Trace Elem Res 2001;80:107–13.
50. Weismann K, Wadskov S, Sondergaard J. Oral zinc sulphate therapy for acne vulgaris. Acta Derm Venereol 1977;57:357–60.
51. 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.
52. Allan AK, Hawksworth GM, Woodhouse LR, Sutherland B, King JC, Beattie JH. Lymphocyte metallothionein mRNA responds to marginal zinc intake in human volunteers. Br J Nutr 2000;84:747–56.
53. Bales CW, DiSilvestro RA, Currie KL, et al. Marginal zinc deficiency in older adults: responsiveness of zinc status indicators. J Am Coll Nutr 1994;13:455–62.
54. Freeland-Graves JH, Hendrickson PJ, Ebangit ML, Snowden JY. Salivary zinc as an index of zinc status in women fed a low-zinc diet. Am J Clin Nutr 1981;34:312–21.
55. Lowe NM, Woodhouse LR, Sutherland B, et al. Kinetic parameters and plasma zinc concentration correlate well with net loss and gain of zinc from men. J Nutr 2004;134:2178–81.
56. 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.
57. Mahajan SK, Prasad AS, Brewer GJ, Zwas S, Lee DJ. Effect of changes in dietary zinc intake on taste acuity and dark adaptation in normal human subjects. J Trace Elem Exp Med 1992;5:33–45.
58. 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.
59. Pinna K, Kelley DS, Taylor PC, King JC. Immune functions are maintained in healthy men with low zinc intake. J Nutr 2002;132:2033–6.
60. Ruz M, Cavan KR, Bettger WJ, Gibson RS. Erythrocytes, erythrocyte membranes, neutrophils and platelets as biopsy materials for the assessment of zinc status in humans. Br J Nutr 1992;68:515–27.
61. Thomas EA, Bailey LB, Kauwell GA, Lee DY, Cousins RJ. Erythrocyte metallothionein response to dietary zinc in humans. J Nutr 1992;122: 2408–14.
62. Jackson MJ. Physiology of zinc: general aspects. In: Mills CF, ed. Zinc in human biology. London, United Kingdom: Springer-Verlag, 1989:1–14.
63. Foster DM, Aamodt RL, Henkin RI, Berman M. Zinc metabolism in humans: a kinetic model. Am J Physiol 1979;237:R340–9.
64. Miller LV, Hambidge KM, Naake VL, Hong Z, Westcott JL, Fennessey PV. Size of the zinc pools that exchange rapidly with plasma zinc in humans: alternative techniques for measuring and relation to dietary zinc intake. J Nutr 1994;124:268–76.
65. Hambidge KM, Goodall MJ, Stall C, Pritts J. Post-prandial and daily changes in plasma zinc. J Trace Elem Electrolytes Health Dis 1989;3: 55–7.
66. Lowe NM, Woodhouse LR, King JC. A comparison of the short-term kinetics of zinc metabolism in women during fasting and following a breakfast meal. Br J Nutr 1998;80:363–70.
67. IZiNCG (International Zinc Nutrition Collaborative Group). Assessing population zinc status with serum zinc concentration. IZiNCG Technical Brief No. 02. 2007. Available from: http://www.izincg.org (cited 7 July 2008).
68. Hoey L, McNulty H, Strain JJ. Studies of biomarker responses to intervention with riboflavin: a systematic review. Am J Clin Nutr 2009; 89(suppl):1960S–80S.
69. Hoey L, Strain JJ, McNulty H. Studies of biomarker responses to intervention with vitamin B-12: a systematic review of randomized controlled trials. Am J Clin Nutr 2009;89(suppl):1981S–96S.
70. Seamans KM, Cashman KD. Existing and potentially novel functional markers of vitamin D status: a systematic review. Am J Clin Nutr 2009; 89(suppl):1997S–2008S.
71. Harvey LJ, Ashton K, Hooper L, Casgrain A, Fairweather-Tait SJ. Methods of assessment of copper status in humans: a systematic review. Am J Clin Nutr 2009;89(suppl):2009S–24S.
72. Ashton K, Hooper L, Harvey LJ, Hurst R, Casgrain A, Fairweather-Tait SJ. Methods of assessment of selenium status in humans: a systematic review. Am J Clin Nutr 2009;89(suppl):2025S–39S.
73. Ristic-Medic D, Piskackova Z, Hooper L, et al. Methods of assessment of iodine status in humans: a systematic review. Am J Clin Nutr 2009; 89(suppl):2052S–69S.
74. Fekete K, Marosvölgyi T, Jakobik V, Decsi T. Methods of assessment of n−3 long-chain polyunsaturated fatty acid status in humans: a systematic review. Am J Clin Nutr 2009;89(suppl):2070S–84S.