Effect of zinc supplementation on serum zinc concentration and T cell proliferation in nursing home elderly: A randomized, double-blind, placebo-controlled trial1

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Effect of zinc supplementation on serum zinc concentration and T cell proliferation in nursing home elderly: a randomized, double-blind, placebo-controlled trial[1]

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ABSTRACT

Background: Zinc is essential for the regulation of immune response. T cell function declines with age. Zinc supplementation has the potential to improve the serum zinc concentrations and immunity of nursing home elderly with a low serum zinc concentration.

Objective: We aimed to determine the effect of supplementation with 30 mg Zn/d for 3 mo on serum zinc concentrations of zinc-deficient nursing home elderly.

Design: This was a randomized, double-blind, placebo-controlled study. Of 53 nursing home elderly (aged ≥65 y) who met eligibility criteria, 58% had a low serum zinc concentration (serum zinc <70 μg/dL); these 31 were randomly assigned to zinc (30 mg Zn/d) (n = 16) or placebo (5 mg Zn/d) (n = 15) groups. The primary outcome measure was change in serum zinc concentrations between baseline and month 3. We also explored the effects of supplementation on immune response.

Results: Baseline characteristics were similar in the 2 groups. The difference in the mean change in serum zinc was significantly higher, by 16%, in the zinc group than in the placebo group (P = 0.007) when baseline zinc concentrations were controlled for. In addition, controlling for baseline C-reactive protein, copper, or albumin did not change the results. However, supplementation of participants with ≤60 μg serum Zn/dL failed to increase their serum zinc to ≥70 μg/dL. Zinc supplementation also significantly increased anti-CD3/CD28 and phytohemagglutinin-stimulated T cell proliferation, and the number of peripheral T cells (P < 0.05). When proliferation was expressed per number of T cells, the significant differences between groups were lost, suggesting that the zinc-induced enhancement of T cell proliferation was mainly due to an increase in the number of T cells.

Conclusions: Zinc supplementation at 30 mg/d for 3 mo is effective in increasing serum zinc concentrations in nursing home elderly; however, not all zinc-deficient elderly reached adequate concentrations. The increase in serum zinc concentration was associated with the enhancement of T cell function mainly because of an increase in the number of T cells. *Am J Clin Nutr* 2016;103:942–51.

Keywords: T cell proliferation, nursing home elderly, serum zinc concentration, zinc gluconate, zinc supplementation

INTRODUCTION

The elderly have been described as having low zinc status or decreased zinc intake (1–3). Previously, we showed that a high proportion of nursing home elderly (~30%) had low serum zinc concentrations at baseline and after 1 y of follow-up (4). In that study, we observed that elderly patients with low serum zinc concentrations (<70 μg/dL) had a significantly higher incidence and longer duration of pneumonia, as well as all-cause mortality, than did those with adequate serum zinc concentrations (≥70 μg/dL) (4). Zinc deficiency has been reported to impair immunity (5, 6) and increase susceptibility to infectious diseases, a major cause of morbidity and mortality in the elderly (5, 7–9). Zinc has also been shown to play an important role in the regulation of the immune response, particularly that of T cell–mediated function (10, 11). Zinc deficiency is associated with changes in T cell–mediated function similar to those observed with aging (10, 12, 13). The burden of both aging and zinc deficiency on the immune system may further predispose frail elderly populations to higher infectious disease risk. Zinc supplementation has been shown to improve T cell–mediated function (1, 6, 14–18) and to reduce infections in the elderly (19). Zinc supplementation may therefore play an important role in increasing serum zinc concentrations, improving immunity, and preventing infectious diseases such as pneumonia in the elderly (1, 5, 7).

To our knowledge, studies on zinc supplementation in elderly nursing home adults with zinc deficiency, and thus the population that is most in need of supplementation, are scarce. The potential for reduction in infections and other chronic diseases is high if zinc supplementation is found to be effective in increasing serum

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zinc concentrations in this easily targeted population. In this study we aimed to show that it is feasible to identify and supplement zinc-deficient elderly patients in nursing homes in the United States with zinc. In addition, we hypothesized that zinc supplementation of 30 mg/d over a period of 3 mo would significantly increase serum zinc concentrations in this population. Exploratory analyses were also conducted to determine the impact of zinc supplementation on T cell function, specifically lymphocyte proliferation, in this population. These findings may pave the way for future longer-term research on simple low-cost zinc supplementation interventions to prevent infections such as pneumonia in the high-risk population of nursing home elderly (20–22).

METHODS

Study design and intervention

A randomized, double-blind, placebo-controlled zinc supplementation trial in nursing home elderly with a low serum zinc concentration was conducted between July 2009 and January 2011. For 3 mo, those in the placebo group received daily a capsule containing ~50% Dietary Reference Intakes (DRIs)[8] of most essential vitamins (e.g., vitamins A, C, D, E, and B complex) and minerals (copper, iron, and selenium), including zinc (5 mg/d) in the form of zinc gluconate, whereas those in the zinc group received daily a similar capsule, but with 30 mg Zn also in the form of zinc gluconate. The mixture of micronutrients was provided to all subjects to ensure Adequate Intake of micronutrients, given that they were asked to stop their multivitamin supplement. Fifty percent DRIs were selected because few subjects meeting our eligibility criteria would have a dietary intake of <50% of the DRIs for micronutrients (23). The source of the capsules was Tishcon Corporation (Westbury, NY). Before study initiation, the zinc and placebo multimicronutrient supplements were assayed in triplicate to confirm the zinc concentration in the study capsules. The mean ± SD (% CV) zinc concentrations for the zinc and placebo capsules were 30.2 ± 1.5 mg (5%) and 4.7 ± 0.4 mg (7%), respectively. Personnel from the Human Nutrition Research Center on Aging's Nutritional Evaluation Laboratory, blinded to the content of the supplements, performed the quality control measurements. The capsules were packaged by one of the participating nursing homes in blister packs. The nursing home unit dose dispensing system was used to deliver the capsules. The clinical nursing home staff administered the capsules during routine medication rounds in the mornings. Adherence to the study protocol was verified by review of nursing home medication records.

Recruitment and enrollment

Participants were recruited from 3 nursing homes in Boston, Massachusetts, i.e., the Hebrew Rehabilitation Center for the Elderly, the Bostonian Nursing Home, and the Harborlights Nursing Home. The Tufts University New England Medical Center and the Hebrew Senior Life institutional review boards approved the protocol. The administrator, director of nursing, medical director, consulting pharmacist, and/or infection control director of each institution approved and supported the study. Staff at each nursing home initially identified potential candidates. Residents were included based on the following criteria: ≥65 y old; >6 mo life expectancy, in the judgment of their physician; willingness to be randomly assigned to one of the treatment groups; ability to swallow pills; not currently on antibiotics; willingness to replace their nutrient supplement with our study supplement. Those consuming supplements of calcium, vitamin D, and iron were not excluded (and were allowed to continue consuming these supplements throughout the study). Residents were excluded based on the following criteria: anticipated transfer or discharge within 3 mo of enrollment; bed- or room-bound continuously for the previous 3 mo; presence of lung neoplastic diseases or other active neoplastic diseases requiring chemotherapy and/or use of immunosuppressive drugs (including >10 mg prednisone/d); nasogastric or other tube feeding; long-term intravenous or urethral catheters (30 d); presence of tracheostomy or chronically ventilator-dependent; consuming supplements containing more than the DRI concentration of nutrients known to affect the immune response, i.e., vitamins E, C, or B-6; selenium; zinc; or β-carotene, and unwilling to stop; chronic prophylactic antibiotic treatment; protein energy malnutrition defined as albumin <3.0 g/dL; and BMI (in kg/m^2) <18. Written, signed, informed institutional review board approved consent was obtained from each eligible participant or his or her proxy before enrollment in the study. After informed consent, eligible participants were screened for serum zinc concentrations. For this study, we used a serum zinc concentration cutoff of <70 μg/dL to indicate low serum zinc concentrations (24).

Sample size calculation and randomization

Our previous study in nursing home elderly (25) suggested that the treatment group would have a 29 μg/dL increase in mean serum zinc over the placebo group, with a within-group SD of 18 μg/dL. A sample size of 15/group would give a 99% chance of declaring the difference between the 2 groups in mean increase of serum zinc concentrations to be statistically significant at the 0.05 concentration of significance.

Eligible participants were randomly assigned to either the placebo or the zinc group at a 1:1 ratio. Enrollment ended after recruitment of 31 participants. A randomization scheme (a sequentially numbered list of 3 blocks of 12 subjects) known only to the study statistician was created (26). Treatment was assigned by the study statistician, who had no contact with subjects and had no role in data collection. Placebo and zinc capsule packets were labeled with the subjects' names by the statistician who held the randomization code (all other investigators and the study nurse were blinded to this information).

Data collection

Information regarding subject characteristics, diseases, infections, use of supplements and medications, and vaccination history was obtained from medical records at baseline. Medical records and use of supplements were also reviewed, and data

[8] Abbreviations used: CBC, complete blood count; ccpm, corrected count per minute; CRP, C-reactive protein; DRI, Dietary Reference Intake; LDH, lactate dehydrogenase.

were collected at months 1, 2, and 3. Fasting blood was collected at baseline and at study completion.

Outcomes

The primary outcome of this intervention study was change in serum zinc concentration between baseline and month 3. In addition, the impact of zinc supplementation on ex vivo measures of cell-mediated immunity, specifically, lymphocyte proliferation with the use of anti-CD3/CD28 antibodies, and phytohemagglutinin, as well as other measures of interest, such as C-reactive protein (CRP), metallothionein, lactate dehydrogenase (LDH), copper, and albumin, were also explored between baseline and month 3.

Laboratory assays

Serum samples were collected with the use of trace metal–free tubes. Serum zinc and copper were measured by direct-current plasma emission spectroscopy (Beckman SpectraSpan VI Direct Current Plasma Emission Spectrophotometer; Beckman Instruments) according to the technical and application manual, and according to Smith (27) and Dawson (28) at the Nutrition Evaluation Laboratory, Human Nutrition Research Center on Aging, Tufts University. Measurement of serum zinc has been recommended by the WHO, UNICEF, International Atomic Energy Agency, and International Zinc Nutrition Consultative Group as the best available population-concentration biomarker of zinc deficiency (24, 29). Fasting blood measures included clinical chemistries, complete blood count (CBC), and other measures such as copper, globulin, and albumin as previously described (25). CRP (30) and LDH (31) concentrations were also measured. Serum metallothionein concentrations were measured with the use of a sandwich enzyme immunoassay procedure [Human Metallothionein (MT) ELISA kit, BlueGene Biotech].

Lymphocyte subpopulations

Percentages of lymphocyte subpopulations were determined from whole blood with flow cytometry. Both reagents and protocols from BD Biosciences were used. The following subpopulations were assessed with the use of monoclonal antibodies: T cells (CD3), helper T cells (CD4), cytotoxic T cells (CD8), B cells (CD19), natural killer cells, and natural killer T cells (natural killer cells and CD3). White blood cells were stained, fixed, and analyzed on a FACS-Calibur flow cytometer (BD Biosciences), and acquired data were analyzed with the use of FlowJo software (version 10.0.6; Tree Star). Isotype controls for each antibody class and fluorochrome were used as negative controls.

Lymphocyte proliferation

To assess the impact of zinc on T cell–mediated function, the ability of T cells to proliferate in response to anti–T cell receptor or a mitogen was determined before and after zinc supplementation. This measure has been shown consistently to decline with aging and be affected by zinc deficiency and supplementation (10–13). Twenty percent fresh whole blood diluted in cell culture media was stimulated with anti-CD3/CD28 antibody or phytohemagglutinin. The culture media was Roswell Park Memorial Institute 1640, supplemented with HEPES (25 mmol/L), glutamine (2 mmol/L), and penicillin (100 kU/L)/streptomycin (100 mg/L) (Gibco Invitrogen). Blood was cultured in 96-well round-bottom cell culture plates (Nunc) with phytohemagglutinin (Difco Laboratories) at 25 μg/mL. Phytohemagglutinin from different sources and different batches have different stimulation potencies; the concentration 25 μg/mL was determined to be optimal after a broad range of concentrations was tested according to standard procedure in our laboratory. For antigenic stimulation, plates were precoated with 10 μg/mL anti-CD3 antibody (BD Biosciences), incubated at 37°C, 5% CO_2 for 90 min, and then washed twice with media. Blood was then added with anti-CD28 antibody (BD Biosciences) at 2 μg/mL. We used whole-blood culture to determine lymphocyte proliferation rather than peripheral blood mononuclear cells, because it is a better representative of in vivo conditions and the data produced by the 2 techniques have been shown to correlate strongly with each other (32). All samples were cultured in triplicate and incubated at 37°C, 5% CO_2 for 68 h and then 0.5 μcurie [^{3}H]-thymidine/well was added for an additional 4 h as previously described (33). Cells were harvested onto glass fiber mats with the use of a Perkin Elmer cell harvester (model no. C961961), and cell proliferation was quantified as the amount of [^{3}H]-thymidine incorporation into DNA determined by liquid scintillation counting in a Perkin Elmer counter (model no. 2450–0060). T cell proliferation is expressed as corrected count per minute (ccpm), i.e., stimulated count per minute − unstimulated count per minute. Because phytohemagglutinin and anti-CD3/CD28 only stimulate T cell proliferation, we normalized the proliferation data with the number of T cells per microliter whole blood and computed the ccpm/T cells per microliter whole blood. All materials were acid-washed with hydrochloride (5 mol/L) for trace metal removal. Reagents were purchased from the same lot unless limited by expiration dates. Mitogens were portioned into small concentrated amounts and stored at −70°C.

Statistical analysis

The percentage distribution of demographic, clinical, and medical characteristics of participants in the zinc group compared with the placebo group at baseline was computed. Blood concentrations of zinc, copper, CRP, and metallothionein, as well as other laboratory results, including a CBC with differential, blood lymphocyte profile, and lymphocyte proliferation, were compared between groups at baseline with the use of Student's *t* test for independent samples, and within groups between the 2 time points with the use of Student's *t* test for paired samples. Use of supplements and medications known and/or suspected to influence serum zinc concentrations during the study was compared between groups with the use of Fisher's exact test. Serum zinc concentrations at baseline and at month 3 were also compared between those with and without various baseline medical conditions with the use of Student's *t* test for independent samples. ANCOVA was performed to assess the change in serum zinc concentration between baseline and month 3 as the outcome measure, treatment (where zinc group = 1 and placebo group = 0) as a study factor, and baseline serum zinc concentration as a covariate. This model assumes that subjects with the same baseline serum zinc concentration should experience the

same expected change except for the effect of treatment. Further analyses were done, additionally controlling for baseline albumin, copper, and CRP, each in a separate model because of our small sample size. Analyses of impact of zinc supplementation on measures of lymphocyte proliferation and other measures were also conducted with the use of our main ANCOVA model, i.e., controlling for baseline concentrations. Correlations between change in serum zinc concentration and change in lymphocyte proliferation were conducted with the use of a Pearson correlation. The difference in percentage change in outcomes measures between groups was computed as follows: [(outcome measure for zinc group/baseline measure for zinc group) × 100] − [(outcome measure for placebo group/baseline measure for placebo group) × 100]. Two-sided observed significance concentrations (P values) < 0.05 were considered to be statistically significant. Analyses were performed with the use of SAS for Windows, version 9.2 and IBM SPSS Statistics, version 21.

RESULTS

Study population

A total of 442 medical charts were screened from the 3 participating nursing homes; 53 nursing home elderly were eligible and therefore were screened for low serum zinc concentrations (<70 μg/dL) (**Figure 1**). Of these, 31 (58%) had low screening serum zinc concentrations. Participants were randomly assigned to either the placebo group (n = 16) or the zinc-supplemented group (n = 15) and were scheduled to start the study as soon as possible after the screening blood draw. However, because of end-of-year public holidays and other factors such as the hesitation of family members to have participants participate in the study after determination of eligibility, a few participants did not start the study until several weeks later. Baseline blood samples were drawn immediately before the start of placebo or zinc supplementation. Baseline and month 3 blood samples were analyzed for serum zinc concentrations at the study end. We found that, in some participants who completed the study, baseline zinc concentrations fell below their screening levels, whereas in others, concentrations were above their screening levels. Consequently, few subjects in both groups had serum zinc concentrations higher than 70 μg/dL at baseline. Six participants did not complete the study for various reasons, including refusal to take study capsules and advice from their physicians; one participant in the zinc group experienced nausea on 2 consecutive days after ingestion of the zinc capsule at the beginning of the study. A total of 25 participants completed the study, with 13 and 12 receiving the placebo and zinc capsules, respectively, over a period of 3 mo (Figure 1).

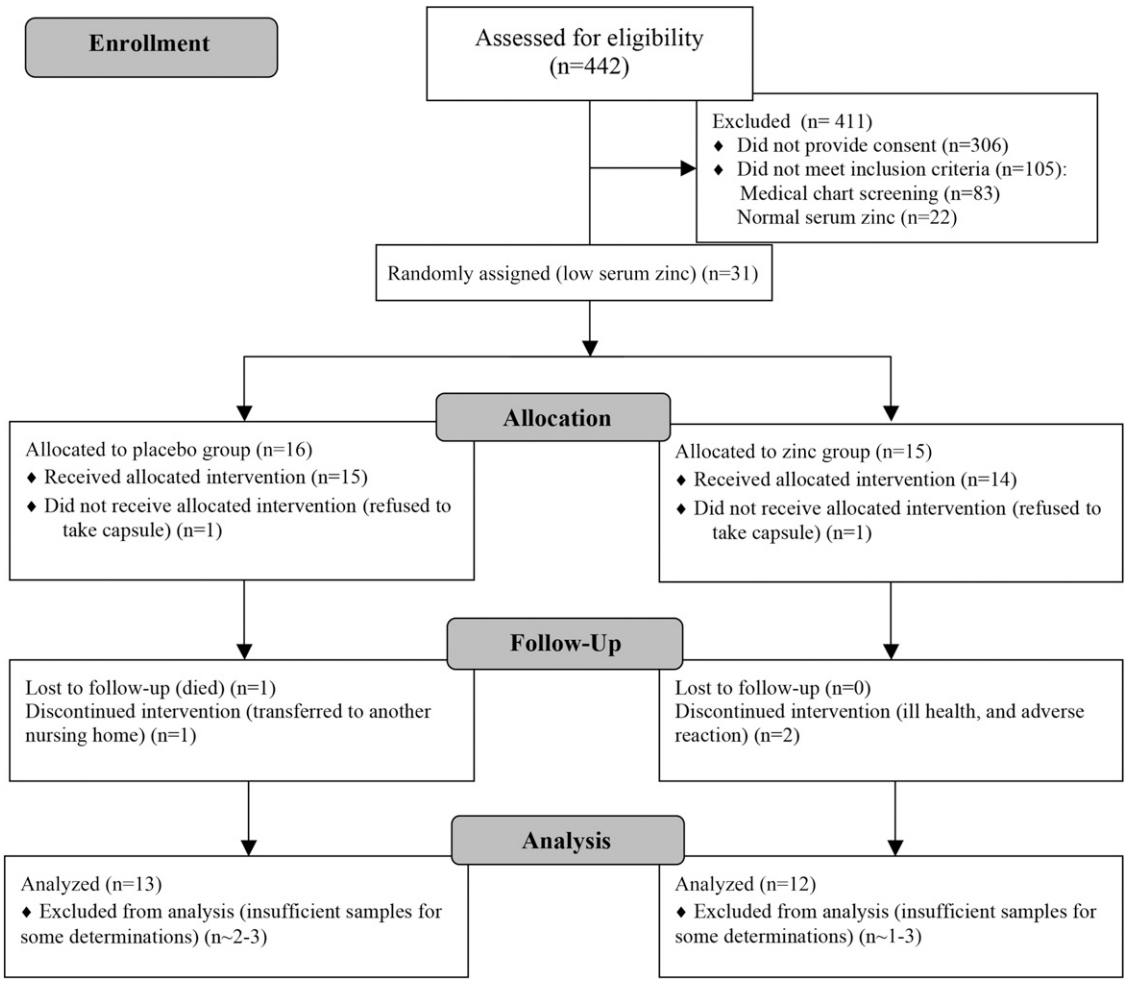

FIGURE 1 Study profile.

Characteristics of study participants

The baseline demographic characteristics of participants in both the placebo and zinc groups were similar for all variables (**Table 1**). The baseline distribution of participants who were diagnosed with diseases such as congestive heart failure, hypertension, diabetes, Alzheimer disease, dementia, asthma, emphysema/chronic obstructive pulmonary disease, and cancer were not different between the 2 groups (data not shown). However, significantly more participants in the placebo group were diagnosed with depression (77% compared with 33%, respectively) ($P = 0.025$). There were no significant differences between the placebo and zinc groups in the use of allowed supplements (calcium carbonate, vitamin D, and ferrous sulfate) (62%, 46%, and 15% in the placebo group, and 58%, 58%, and 17% in the zinc group, respectively; the mean percentage use of these supplements was thus 44% compared with 41%, respectively). Use of medications (e.g., sodium polystyrene sulfonate and ciprofloxacin) in the placebo and zinc groups (75% compared with 46%, respectively) known or suspected to influence serum zinc concentrations was also not different at any time during the study. Furthermore, despite the numerical difference in percentage of participants who had used these medications, because the use of the drugs did not change during the study and because the baseline zinc concentrations were not different between the 2 groups, they are unlikely to have had an impact on serum zinc concentrations that would have resulted in a significant between-group difference.

Serum zinc, lymphocyte proliferation, and other immune and biochemical measures

Serum zinc, copper, metallothionein, CRP, lymphocyte profile (percentage T cells, helper T cells, cytotoxic T cells, B cells, natural killer cells, and natural killer T cells), lymphocyte proliferation, CBC differentials, and other measures were not found to be significantly different at baseline between those in the placebo and zinc groups (**Tables 2–4**). Outcome measure results other than serum zinc are secondary analyses.

With the use of ANCOVA and controlling for baseline serum zinc concentrations, the difference in the mean change in serum zinc was significantly higher by 16% in the zinc group than in the placebo group ($\beta \pm$ SE: 10.7 $\pm$ 3.6 μg/dL, $P = 0.007$) (Table 2). In addition, controlling for baseline CRP, copper, and albumin, each in separate models because of the small sample size, did not change this finding ($P = 0.01$, 0.004, and 0.01, respectively). Despite the mean increase, 42% of those who completed the study in the zinc group did not reach adequate concentrations ($\geq$70 μg/dL) at month 3; these participants were highly zinc deficient at baseline with serum zinc concentrations $\leq$60 μg/dL (mean $\pm$ SD baseline serum zinc: 53 $\pm$ 6 μg/dL; range: 44–60 μg/dL).

With the use of similar ANCOVA models, differences in the mean changes in lymphocyte proliferation induced by anti-CD3 (10 μg/mL)/CD28 (2 μg/mL) [$\beta \pm$ SE: 9.7 $\pm$ 3.7 ($\times$ 1000 ccpm)] and phytohemagglutinin (25 μg/μL) [$\beta \pm$ SE: 12.4 $\pm$ 5.8 ($\times$ 1000 ccpm)] were statistically significantly higher by 47% ($P = 0.02$) and 45% ($P = 0.05$) in the zinc group than in the placebo group, respectively (Table 4). Furthermore, we observed a significant increase in the number of T cells in the zinc group after 3 mo of supplementation. The increase in the T cell number was significantly higher in the zinc group than in the control group ($P = 0.03$). Thus, we normalized the whole-blood lymphocyte proliferation results with the numbers of blood T cells for each subject and found that the difference in lymphocyte proliferation no longer existed (Table 4). These data suggest that the observed enhancement in T cell proliferation is mainly due to a zinc supplementation–induced increase in the number of blood T cells, and not to the ability of each cell to proliferate. Accordingly, changes in serum zinc concentration were found to be correlated with changes in both lymphocyte proliferation induced by anti-CD3 (10 μg/mL)/CD28 (2 μg/mL) ($r = 0.52$, $P = 0.02$) (**Figure 2**) and the number of blood T cells ($r = 0.39$, $P = 0.06$) (**Figure 3**).

Metallothionein concentrations did not differ at baseline between groups, and the difference in mean change in metallothionein after supplementation also was not significant. Of note, although serum LDH concentrations did not differ at baseline, the difference in mean change in LDH was higher by 22% in the zinc group than in the placebo group after supplementation ($P = 0.03$) (Table 2). The results remained significant after controlling further for baseline CRP, copper, and albumin, each in separate models.

TABLE 1
Baseline characteristics of study participants[1]

Characteristics	Placebo	Zinc
Age, y	84.4 $\pm$ 8.8	87.0 $\pm$ 5.0
Weight, kg	72.9 $\pm$ 14.2	66.1 $\pm$ 13.4
Height, m	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1
BMI, kg/m^2	27.8 $\pm$ 5.4	26.7 $\pm$ 7.2
Female	75.0 (12)	73.3 (11)
Caucasian	87.5 (14)	86.7 (13)
Self-consented	25.0 (4)	60.0 (9)
NSAID[2] use	31.3 (5)	53.3 (8)
Did not smoke	87.5 (14)	100 (15)
Did not consume alcohol	100.0 (15)*	93.3 (14)
No aspiration risk	76.9 (10)†	86.7 (13)
Received flu vaccine within 1 y	93.3 (14)*	92.3 (12)†
Received pneumococcal vaccine within 5 y	88.9 (8)‡	77.8 (7)‡

[1]Values are means $\pm$ SDs or % (n). $n = 16$ for placebo and $n = 15$ for zinc, except *$n = 15$, $^\dagger n = 13$, and $^\ddagger n = 9$.
[2]NSAID, nonsteroidal anti-inflammatory drug.

DISCUSSION

The key finding from this study is that it is feasible to increase serum zinc concentrations in nursing home residents with a low serum zinc concentration through supplementation with zinc. Serum zinc has been cited as the best available biomarker of zinc deficiency in a population (24, 29). The difference in the mean change in serum zinc concentration was significantly higher in zinc-deficient elderly supplemented with 30 mg Zn/d than in those supplemented with 5 mg Zn/d over a 3-mo period. As observed in this study, the plasma zinc concentration has been shown by Feillet-Coudray et al. (34) to increase in healthy late-middle-aged men with normal zinc concentrations given a similar dose of 30 mg Zn/d and over a longer period (6 mo). The same study reported a significant increase in plasma zinc concentrations after supplementation with a lower dose of 15 mg/d over a 6-mo period (34). Although some studies of zinc supplementation

TABLE 2
Effect of zinc supplementation on serum zinc and other biochemical measures in elderly nursing home residents with low serum zinc concentration[1]

	Placebo				Zinc				Difference in outcome measures between zinc and placebo treatment groups[2]			
	Baseline		Month 3		Baseline		Month 3					
Measures	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	β ± SE	%Δ	*P*
Serum zinc, μg/dL	16	66.3 ± 10.0	13	65.4 ± 8.8	14	63.9 ± 9.7	12	73.2 ± 14.6	24	10.7 ± 3.6	16	0.007
Serum copper, μg/dL	14	104.7 ± 19.2	13	109.5 ± 16.9	12	111.3 ± 16.6	12	113.4 ± 17.6	24	0.3 ± 6.0	−3	0.96
Serum globulin, g/dL	13	2.8 ± 0.5	13	2.8 ± 0.5	12	2.7 ± 0.5	12	2.8 ± 0.4	24	0.3 ± 6.0	4	0.49
Serum albumin, g/dL	16	3.6 ± 0.4	13	3.6 ± 0.3	14	3.8 ± 0.5	11	3.5 ± 0.4	23	−0.09 ± 0.1	−8	0.35
LDH, IU/L	13	142.3 ± 40.9	13	128.5 ± 21.3	12	147.7 ± 26.2	12	165.9 ± 52.5	22	35.8 ± 15.6	22	0.03
Metallothionein, ng/mL	10	0.4 ± 0.4	9	0.3 ± 0.3	12	0.4 ± 0.3	12	0.3 ± 0.3	16	0.17 ± 0.1	0	0.15
Serum CRP,[3] mg/L	12	7.7 ± 9.1	10	7.2 ± 6.1	12	8.9 ± 11.9	11	10.4 ± 14.1	19	1.85 ± 4.4	23	0.88

[1] *t* test for independent samples between zinc and placebo groups at baseline is not significant for all variables. CRP, C-reactive protein; LDH, lactate dehydrogenase.
[2] "Outcome measures" refers to the change in concentrations of measures of interest between baseline and month 3. β [difference in the mean outcome measures between treatment groups (zinc group = 1 and placebo group = 0)] ± SE and *P* value obtained with the use of ANCOVA with outcome measures of interest, controlling for their corresponding baseline concentrations. %Δ obtained as follows: [(outcome measure for zinc group/baseline measure for zinc group) × 100] − [(outcome measure for placebo group/baseline measure for placebo group) × 100].
[3] *P* values for this variable were obtained with data analyses done in the natural logarithmic scale to normalize distribution; however, unlogged data are presented in the table.

in both independently living and institutionalized elderly patients have reported increased circulating zinc concentrations (14, 18, 35–37), findings have not been consistent, however, because of different study designs; the zinc status of study populations; and the type, dose, and duration of zinc supplements used (reviewed in 36).

In the present study, supplementation of zinc-deficient elderly patients with a small amount of zinc (5 mg/d) (as provided in our placebo group) was not effective in maintaining or increasing mean serum zinc concentrations. The latter is also supported by findings from our previous study in 617 nursing home elderly in which supplementation with 7 mg Zn/d over a period of 1 y failed to increase serum zinc concentrations. In that study, ~30% of subjects were found to be deficient in zinc at baseline and at follow-up (4). Thus, our data suggest that a higher dose of zinc per day is needed to prevent a decrease in or maintain serum zinc concentrations in the elderly, especially in zinc-deficient elderly.

In this study, participants with serum zinc ≤60 μg/dL failed to attain adequate concentrations of serum zinc (≥70 μg/dL), despite supplementation with 30 mg Zn/d for 3 mo. Despite the small sample size, the mean difference in serum zinc concentrations between those with baseline serum zinc ≤60 μg/dL (n = 5) was significantly lower by 8% than in those with baseline serum zinc >60 μg/dL (n = 7) (with the use of our main ANCOVA model; β ± SE: −22.6 ± 10.1 μg/dL, P = 0.05). No differences were observed in the distribution of participants diagnosed with congestive heart failure, hypertension, diabetes, Alzheimer disease, dementia, asthma, emphysema/chronic obstructive pulmonary disease, cancer, depression (including

TABLE 3
Effect of zinc supplementation on complete blood count differentials in elderly nursing home residents with low serum zinc concentration[1]

	Placebo				Zinc				Difference in outcome measures between zinc and placebo treatment groups[2]			
	Baseline		Month 3		Baseline		Month 3					
Measures	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	β ± SE	%Δ	*P*
Red blood cells, $\times 10^6/\mu$L	13	4.1 ± 0.6	13	4.0 ± 0.6	12	4.0 ± 0.5	12	3.9 ± 0.4	24	−0.13 ± 0.09	0%	0.16
Hemoglobin, g/dL	13	12.2 ± 1.4	13	12.2 ± 1.6	12	12.1 ± 1.2	12	11.7 ± 1.0	24	−0.42 ± 0.25	−3%	0.11
Platelets, $\times 10^3/\mu$L	13	252.5 ± 79.5	13	267.5 ± 85.7	12	240.9 ± 75.4	12	256.8 ± 78.8	24	−1.82 ± 23.39	1%	0.94
White blood cells, $\times 10^3/\mu$L	13	6.4 ± 2.3	13	6.8 ± 2.1	12	5.9 ± 1.5	12	6.4 ± 1.5	24	−0.12 ± 0.45	2%	0.78
Lymphocytes, %	13	40.9 ± 16.7	13	34.5 ± 10.6	12	31.8 ± 9.4	12	32.6 ± 7.8	23	1.29 ± 3.35	18%	0.71
T cells in lymphocyte, %	13	65.0 ± 9.8	13	64.7 ± 9.3	12	61.7 ± 19.0	12	66.7 ± 15.7	24	4.28 ± 3.0	8.6%	0.17
T cells/μL whole blood	13	1653 ± 908	13	1517 ± 777	12	1160 ± 757	12	1400 ± 750	24	297.9 ± 124.0	29%	0.03
Eosinophils, %	13	3.8 ± 1.8	13	3.8 ± 2.0	12	3.2 ± 0.9	11	3.5 ± 1.4	23	0.18 ± 0.38	9%	0.65
Basophils, %	13	0.9 ± 0.8	13	0.8 ± 0.4	12	0.6 ± 0.5	11	0.7 ± 0.8	24	−0.13 ± 0.26	28%	0.63
Neutrophils, %	13	48.8 ± 15.5	13	53.8 ± 9.3	12	57.7 ± 9.7	11	56.4 ± 8.2	23	−0.19 ± 3.21	−12%	0.95
Monocytes, %	13	6.3 ± 2.1	13	7.0 ± 1.2	12	6.8 ± 1.2	11	6.8 ± 1.2	23	−0.33 ± 0.46	−11%	0.48

[1] *t* test for independent samples between zinc and placebo groups at baseline is not significant for all variables; paired *t* tests within a group are not significantly different except for the number of white blood cells (P = 0.04) and T cells per microliter whole blood in the zinc group (P = 0.013).
[2] "Outcome measures" refers to the change in values of measures of interest between baseline and month 3. β [difference in the mean outcomes measures between treatment groups (zinc group = 1 and placebo group = 0)] ± SE and *P* value obtained with the use of ANCOVA with outcome measures of interest, controlling for their corresponding baseline values. %Δ obtained as follows: [(outcome measure for zinc group/baseline measure for zinc group) × 100] − [(outcome measure for placebo group/baseline measure for placebo group) × 100].

TABLE 4
Effect of zinc supplementation on lymphocyte proliferation measures in elderly nursing home residents with low serum zinc concentration[1]

	Placebo				Zinc				Difference in outcome measures between zinc and placebo treatment groups[2]			
	Baseline		Month 3		Baseline		Month 3					
Lymphoproliferation and measures	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	β ± SE	%Δ	*P*
Anti-CD3/CD28, 10 μg/mL/2 μg/mL												
ccpm (× 1000)[3]	10	28.1 ± 14.8	10	21.7 ± 11.8	11	18.8 ± 14.6	11	23.3 ± 17.6	20	9.7 ± 3.7	47	0.02
ccpm/T[4]	10	21 ± 18	10	17 ± 11	11	19 ± 15	11	18 ± 13	20	2.78 ± 2.58	14	0.29
Phytohemagglutinin, 25 μg/mL												
ccpm (× 1000)	11	31.2 ± 13.7	11	24.0 ± 10.3	9	26.8 ± 17.6	9	32.5 ± 25.1	19	12.4 ± 5.8	45	0.05
ccpm/T	11	21 ± 8	11	17 ± 8	9	27 ± 21	9	26 ± 19	19	3.85 ± 3.97	15	0.35

[1] *t* test for independent samples between zinc and placebo groups at baseline is not significant for all variables. ccpm, corrected count per minute; cpm, count per minute.

[2] "Outcome measures" refers to change in concentrations of measures of interest between baseline and month 3. β [difference in the mean outcome measures between treatment groups (zinc group = 1 and placebo group = 0)] ± SE and *P* value obtained with the use of ANCOVA with outcome measures of interest, controlling for their corresponding baseline concentrations. %Δ obtained as follows: [(outcome measures for zinc group/baseline measure for zinc group) × 100] − [(outcome measures for placebo group/baseline measure for placebo group) × 100].

[3] ccpm = (stimulated cpm) − (unstimulated cpm). Unstimulated cpm values were as follows—placebo (baseline: 89 ± 62 and month 3: 71 ± 15); zinc (baseline: 93 ± 81 and month 3: 76 ± 20).

[4] ccpm/T (ccpm normalized to number of T cells/μL whole blood) = ccpm · T cells^{-1} · μL whole blood^{-1}.

manic depression and anxiety), and use of allowed supplements (calcium carbonate, vitamin D, and ferrous sulfate) and medications known or suspected to influence serum zinc concentrations between these 2 groups at baseline and at month 3.

Failure to respond to zinc supplementation by participants with baseline zinc ≤60 μg/dL or these highly zinc-deficient participants may be due to various factors. Adjustments to increased zinc intake after habitually low intake in highly zinc-deficient participants may take months (38, 39), possibly longer than the duration of this study, to establish a new state of equilibrium. Furthermore, participants who failed to reach adequate concentrations of serum zinc despite supplementation may have

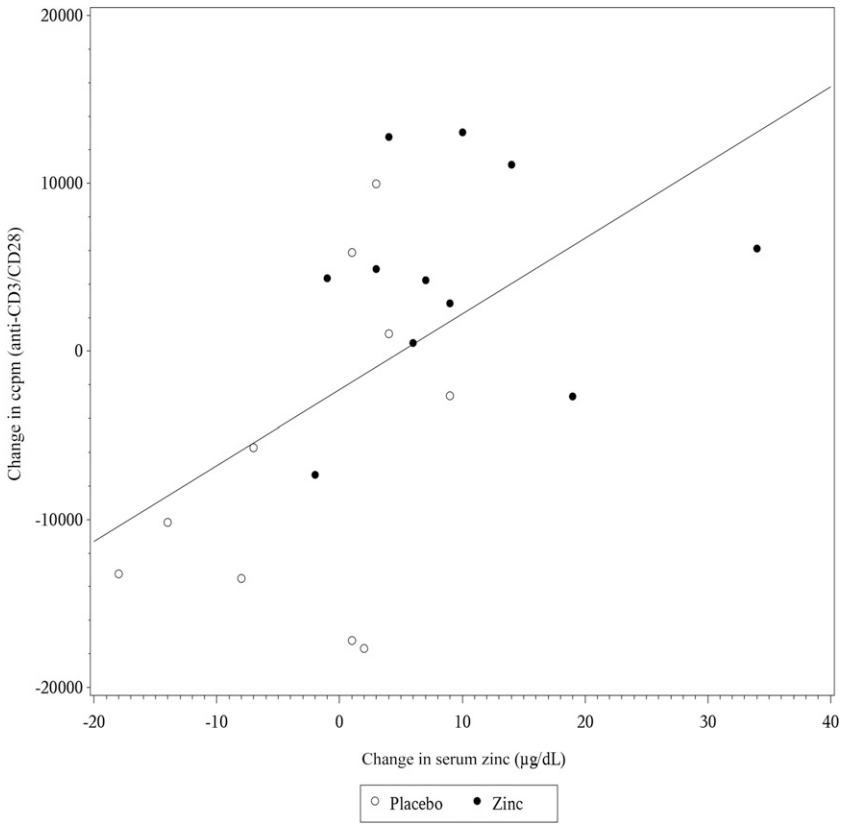

FIGURE 2 Correlation between changes in T cell proliferation in response to anti-CD3/CD28 and those in serum zinc concentrations between baseline and month 3. $n = 21$; $r = 0.52$ and $P = 0.02$ obtained with the use of a Pearson correlation. ccpm, corrected count per minute.

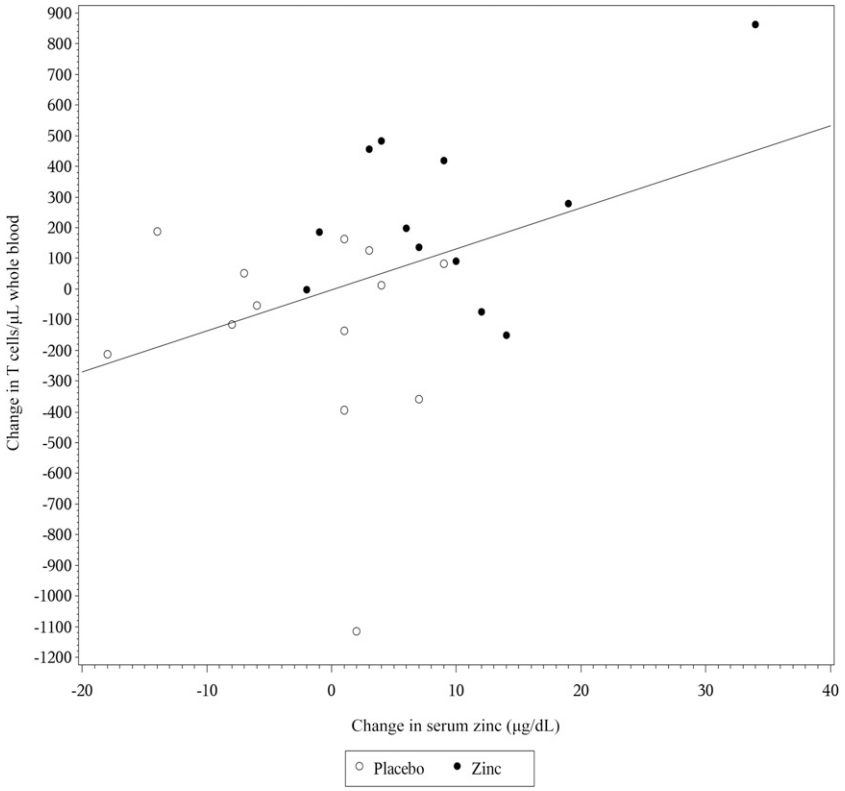

FIGURE 3 Correlation between changes in number of T cells per microliter whole blood and those in serum zinc concentrations between baseline and month 3. $n = 25$; $r = 0.39$ and $P = 0.06$ obtained with the use of a Pearson correlation.

a poorer ability to synthesize metallothionein (40), a zinc-binding protein shown to decline in the elderly (41, 42); metallothionein helps maintain zinc homeostasis in the gut and other tissues (43, 44). In the present study, however, we did not find any difference in the baseline and month 3 serum metallothionein concentrations between those with baseline serum zinc concentrations ≤60 μg/dL and >60 μg/dL. Genetics may also influence a participant's response to zinc supplementation (45, 46). The composition of elderly gut microbiota may also be a contributing factor; zinc absorption competition within the gut between the enterocytes and the gut microbiota has been shown to exist, limiting available zinc (47).

Potential side effects of high-dose zinc supplementation include manifestations of overt toxicity symptoms, including vomiting, epigastric pain, lethargy, and fatigue, as well as induced copper deficiency, which is associated with anemia, neutropenia, and impaired immune function (48–50). Our dose of 30 mg Zn/d was well-tolerated by all participants in the zinc group, except for one who experienced the nausea mentioned above. Given that low dietary intake is a major contributor to low serum zinc concentrations (24), especially in the elderly (1, 34, 51, 52), the total zinc intake in this study from food and supplement likely did not exceed the recommended upper limit of zinc intake (40 mg/d). This is confirmed by the fact that we did not observe any adverse effects from zinc toxicity in those who completed the study. It is important to monitor closely an individual's reaction to the dose given, especially in the first few days of administration, so appropriate measures can be taken, if needed, should any adverse reaction be observed. In this study, serum copper concentrations did not change after zinc supplementation (Table 2). This may result be due to the tolerable moderate zinc dose provided and/or the 0.8 mg Cu/d provided in the capsules given to the participants.

Zinc deficiency, even a mild zinc deficiency, adversely affects clinical, biochemical, and immunologic functions (12, 53, 54). This is because zinc has diverse biological functions in enzymatic catalysis, redox regulation, cellular signal transduction, neurons, and the immune system; >300 catalytically active zinc metallothioneins and >2000 zinc-dependent transcription factors have been recognized thus far (55, 56). In this study, LDH, a zinc-dependent enzyme (57), was observed to increase in the zinc group compared with the placebo group (Table 2), but remained within the normal range (58). The activity of LDH has been shown by Yousef et al. (59) to decrease in serum in a dose-dependent manner with zinc deficiency. The authors concluded that dietary zinc deficiency exerts alterations in the serum enzymatic activity (59).

Adequate serum zinc concentration is essential for proper function of the immune system, particularly T cell–mediated function. Zinc deficiency has been shown to be associated with impaired T cell–mediated function. Aging is also associated with impaired T cell–mediated function; thus, improving serum zinc concentrations through supplementation has the potential to enhance T cell–mediated function and resistance to infection in this at-risk population. In this study, we found that zinc supplementation significantly increased T cell proliferation, as well as the number of peripheral T cells. When T cell proliferation was normalized for the number of blood T cells in each subject, a statistically significant difference between the 2 groups was no longer observed, suggesting that zinc-induced enhancement of T

cell proliferation in this population mainly was due to an increase in the number of T cells. Although the underlying mechanism behind the zinc-induced increase in T cell numbers was not determined in this study, based on previous investigations reviewed by Fraker et al. (10), we speculate that this effect of zinc might be due to an increase in the production of T cells and/or a decrease in the loss of T cell precursors via apoptosis. This is further supported by the observation that changes in serum zinc concentration were positively correlated with changes in both lymphocyte proliferation and the number of T cells per microliter of blood (Figures 2 and 3). Furthermore, given that our previous study showed a significant correlation between serum zinc concentration and incidence of pneumonia in nursing home elderly, and the importance of T cells in susceptibility to infection, our data suggest that improving serum zinc concentrations in elderly patients who are zinc deficient might be helpful in reducing their risk of pneumonia and other infections. Others have reported an improvement in immune response and a lower incidence of infection in the elderly (14, 15, 19). Zinc supplementation has also been shown to reduce both the risk and duration of pneumonia in children (60, 61).

This study confirms the feasibility of identifying and supplementing zinc-deficient elderly with a low-cost intervention of 30 mg Zn/d in nursing homes, and that this dose of zinc is safe in zinc-deficient elderly, because no side effects were observed in supplemented individuals who completed the 3-mo study. In addition, it demonstrated that the supplementation of zinc-deficient nursing home elderly with 30 mg Zn/d for 3 mo in the form of gluconate successfully increased serum zinc concentrations to adequate concentrations of >70 μg/dL in those with baseline zinc concentrations >60 μg/dL, but it suggests that those with zinc concentrations ≤ 60 μg/dL may need a larger dose or longer duration of supplementation. Furthermore, exploratory analyses suggest that zinc supplementation improves T cell–mediated function by increasing the number of functional T cells in the periphery. As mentioned in the results section above, we do not expect potential biases from the use of supplements and medications in the placebo and the zinc groups. Also, we do not expect potential biases from differences in the distribution of participants who self-consented, given that the data presented are not based on any recall information and we did not observe a significant difference at baseline between the 2 groups in the outcomes reported. However, there is a potential for bias in the estimate of treatment effect because of the loss of 52–71% of the 31 randomly assigned participants who were included in these analyses, depending on outcome. Future more definitive studies of a larger size and longer duration and with additional doses of zinc are needed to determine the impact of baseline serum zinc concentrations and genetic and gut microbiota on the optimal dose of zinc needed to maintain adequate zinc concentrations, improve the immune response, and decrease the incidence and duration of infectious diseases, such as pneumonia, in zinc-deficient nursing home elderly. Findings from such studies may have a significant impact on improving health span and quality of life in the elderly as well as reducing the economic costs associated with their care.

The authors' responsibilities were as follows—SNM: had full access to all of the data in the study and was responsible for the integrity of the data and the accuracy of the data analysis; JBB, DHH, RK, GB, GED, PFJ, RS, and SNM: were responsible for the study concept and design; JBB, MCD, DHH, RK, GB, RS, EK, and SNM: were responsible for data acquisition; JBB, MCD, and GED: were responsible for statistical analyses; JBB, DHH, DW, GED, and SNM: were responsible for the interpretation of study findings; JBB: drafted the manuscript; and all authors: reviewed the draft manuscript and provided feedback for and final approval of the final version. None of the authors reported a conflict of interest related to the study.

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