Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress[1–3]

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ABSTRACT

Background: Zinc deficiency, cell-mediated immune dysfunction, susceptibility to infections, and increased oxidative stress have been observed in elderly subjects (ie, those >55 y old). Zinc is an effective antiinflammatory and antioxidant agent.

Objectives: The primary objective was to determine the effect of zinc on the incidence of total infections in healthy elderly subjects. The secondary objective was to determine the effect of zinc on cytokines and oxidative stress markers.

Design: A randomized, double-blind, placebo-controlled trial of zinc supplementation was conducted in elderly subjects. Fifty healthy subjects of both sexes aged 55–87 y and inclusive of all ethnic groups were recruited for this study from a senior center. The zinc-supplemented group received zinc gluconate (45 mg elemental Zn/d) orally for 12 mo. Incidence of infections during the supplementation period was documented. The generation of inflammatory cytokines, T helper 1 and T helper 2 cytokines, and oxidative stress markers and the plasma concentrations of zinc were measured at baseline and after supplementation.

Results: Compared with a group of younger adults, at baseline the older subjects had significantly lower plasma zinc, higher ex vivo generation of inflammatory cytokines and interleukin 10, and higher plasma oxidative stress markers and endothelial cell adhesion molecules. The incidence of infections and ex vivo generation of tumor necrosis factor α and plasma oxidative stress markers were significantly lower in the zinc-supplemented than in the placebo group. Plasma zinc and phytohemagglutin-induced interleukin 2 mRNA in isolated mononuclear cells were significantly higher in the zinc-supplemented than in the placebo group.

Conclusions: After zinc supplementation, the incidence of infections was significantly lower, plasma zinc was significantly higher, and generation of tumor necrosis factor α and oxidative stress markers was significantly lower in the zinc-supplemented than in the placebo group. *Am J Clin Nutr* 2007;85:837–44.

KEY WORDS Elderly subjects, infections, interleukin 2 mRNA, zinc, oxidative stress, tumor necrosis factor α, interleukin 1β

INTRODUCTION

The essentiality of zinc and its deficiency in humans were recognized in the early 1960s (1, 2). Dietary zinc intake declines with advancing age in both developing and developed countries (3, 4). Zinc deficiency and susceptibility to infections due to cell-mediated immune dysfunction have been reported to occur in the elderly (3, 4).

Oxidative stress has been recognized as an important contributing factor in several chronic diseases attributed to aging, such as atherosclerosis and related cardiovascular disorders, mutagenesis and cancer, neurodegeneration, and immunologic disorders and even in the aging process itself (5). Together, $O^{\cdot}2^{-}$, H_2O_2, and $^{\cdot}OH$ are known as reactive oxygen species, and they are continuously produced in vivo under aerobic conditions. Multiple roles of zinc as an antioxidant in cell cultures and animal models have been observed (5–8). The use of zinc in the management of oxidative stress in the elderly has not, however, been reported.

Inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin (IL) 1β, generated by activated monocytes and macrophages, are also known to generate greater amounts of reactive oxygen species (9, 10). In addition, chronic inflammatory processes have been implicated in high cardiovascular mortality in elderly subjects (11, 12). TNF neutralization in the treatment of septic shock (13, 14) and the use of IL-1 receptor antagonist (IL-1ra) and TNF antibody in the treatment of rheumatoid arthritis (15, 16) suggest that these cytokines are important in the pathogenesis of the above disorders. Increases in inflammatory cytokines TNF-α and IL-1β have been associated with cutaneous leishmaniasis (17), and increases in lipid peroxidation products were associated with zinc deficiency in children with chronic giardiasis (18).

Zinc supplementation to healthy human subjects aged 20–50 y reduced the concentrations of the oxidative stress–related byproducts malondialdehyde (MDA), 4-hydroxyalkenals (HAE), and 8-hydroxydeoxyguanine in the plasma; inhibited the ex vivo induction of TNF-α and IL-1β mRNA in mononuclear cells (MNCs); and provided protection against TNF-α–induced nuclear factor–$\kappa\beta$ activation in isolated MNCs (19). We previously provided evidence that, in the promyelocytic leukemia cell line

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HL-60, which differentiates to the monocyte and macrophage phenotype in response to phorbol-12-myristate-13-acetate, zinc increases the expression of A20 and the binding of A20 transactivating factor to DNA, which results in the inhibition of induced nuclear factor–κB activation (19). Nuclear factor–κB is involved in the gene expression of TNF-α and IL-1β in monocytes and macrophages in humans and HL-60 cells, and the effect of zinc in inhibiting the gene expression of TNF-α and IL-1β is cell specific (20–25).

Because zinc deficiency and susceptibility to infections due to cell-mediated immune dysfunctions have been observed in the elderly, we hypothesized that zinc supplementation would reduce the incidence of infection in the elderly. To understand the mechanism by which zinc may affect cell-mediated immune functions, we used reverse transcriptase (RT)–polymerase chain reaction (PCR) analysis to assess phytohemagglutin-induced expression of IL-2 mRNA in isolated MNCs obtained from elderly subjects before and after supplementation. Because zinc supplementation to younger adults reduced the generation of inflammatory cytokines and decreased oxidative stress markers (19), we also hypothesized that zinc supplementation to the elderly not only would increase the generation of IL-2 mRNA in MNCs but also would decrease the generation of TNF-α and IL-1β and decrease oxidative stress markers.

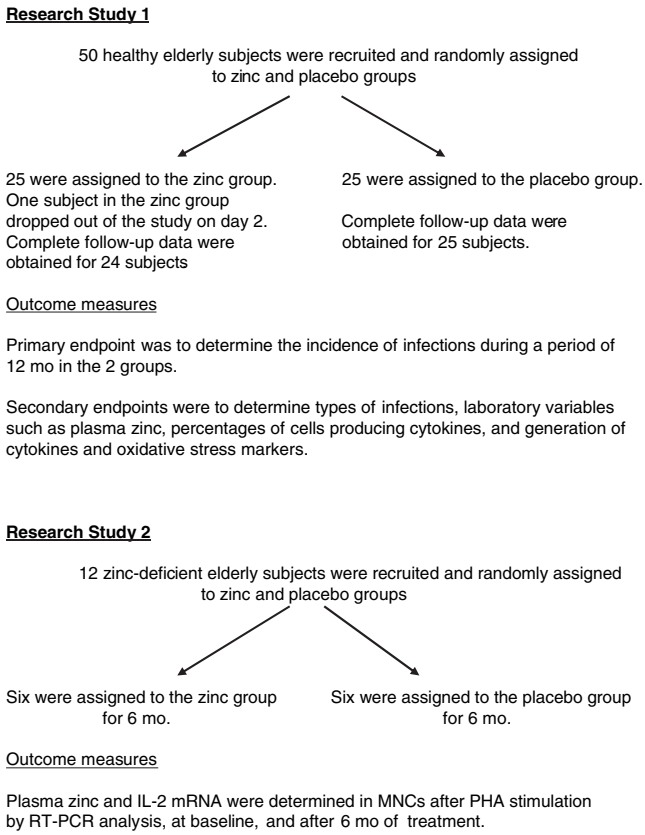

FIGURE 1. The plan of the study. IL, interleukin; mRNA, messenger RNA; MNCs, mononuclear cells; PHA, phytohemagglutin; RT-PCR, reverse transcriptase–polymerase chain reaction.

SUBJECTS AND METHODS

Subjects

All subjects provided written informed consent. The study protocol was approved by the Human Investigation Committee of Wayne State University and was in accord with the Helsinki Declaration as revised in 1983.

Young adults

Thirty-one young adults ($\bar{x} \pm$ SD age: 32.1 $\pm$ 13.1 y; range: 18–54 y) were recruited for assays of plasma zinc, intercellular adhesion molecule, vascular endothelial cellular adhesion molecules, E-selectin, nitric oxide (NO), MDA, and inflammatory cytokines generated ex vivo, so that their values could be compared with those of the older subjects in the current study. These studies were done only once.

Twenty-eight of these subjects were white, 2 were African American, and 1 was American Indian. Twelve were men and 19 were women. These subjects were affiliated with Wayne State University, and several were medical students. They were all free of any chronic illness, and none had any infection during the 6-mo observation period.

Elderly subjects

A diagram of the study protocol is shown in **Figure 1**. We recruited 50 healthy elderly adults of both sexes (aged 55–87 y) and all ethnic groups from St Patrick's Senior Citizen Center (Detroit, MI) to participate in a randomized, placebo-controlled trial of the efficacy of zinc with respect to the incidence of infections and the effect on ex vivo–generated inflammatory cytokines and plasma concentrations of markers of oxidative stress. One participant in the zinc group dropped out on day 2. We therefore had complete data on 49 participants.

A complete chart review of each elderly subject was done by the research nurse to determine his or her potential eligibility for the study. Exclusion criteria were as follows: life expectancy (as estimated by the physician) of $<$8 mo; progressive neoplastic disease; severe cardiac dysfunction (New York Heart Association Class IV); significant kidney disease (blood urea nitrogen $>$40 mg/dL or creatinine $>$2.0 mg/dL); significant liver disease (known active hepatitis or cirrhosis) or transferrin concentrations $>$25% above the upper normal laboratory values; or serum alkaline phosphatase concentrations $>$200 IU. We excluded those persons who were self-supplementing with zinc, who were not mentally competent, or who did not understand the study information and could not provide informed consent.

The purpose of St Patrick's Senior Citizen Center is to empower the elderly to live independently and with dignity and good quality of life. The center provides programs of health education and classes in art, computers, and a biweekly exercise program. Seniors are offered breakfast and lunch 7 d/wk. Transportation to and from their homes (if necessary) is provided daily and for weekly outings. Thus, the subjects from St Patrick's were healthier than elderly persons living in nursing homes.

Intervention

Elderly subjects were randomly assigned in pairs to the zinc-supplemented or the placebo group with the use of envelopes that each contained 2 smaller envelopes (1 assigning a subject to zinc treatment and 1 assigning a subject to placebo). The persons caring for the patient, involved in drawing blood, or running laboratory analyses were blinded to the assignment. In the zinc group, 1 subject was 80 y old, 1 subject was 87 y old, 15 subjects

were <70 y old, and 7 were 70–80 y old. In the placebo group, 1 subject was 82 y old, 15 subjects were <70 y old, and 9 subjects were 70–80 y old.

Each day for 12 mo, subjects in the zinc-supplemented group received 1 capsule of zinc gluconate (15 mg elemental zinc) orally 1 h before breakfast and 2 capsules before going to bed (≥2 h after dinner or last meal). Subjects in the placebo group received placebo capsules in the same manner. Both zinc and placebo capsules were supplied by Labcatal Laboratories (Paris, France). The zinc content of capsules was checked and certified by Labcatal Laboratories.

Outcome measures

Our primary endpoint was to determine the incidence of infections in the 2 groups of subjects. To eliminate the effect of seasonal variations, participants were followed for a period of 12 mo. A nurse practitioner evaluated study subjects who appeared to have infections. Because a diagnosis of infection occasionally is difficult to establish in the elderly, given such subjects' atypical presentation of disease, subtle clinical manifestations, and cognitive impairment, the practice guidelines for evaluation of fever and infections in long-term care facilities was used as our basis (26). The nurse-practitioner was blinded to the treatment assignment. All subjects were given an oral glass thermometer (Becton Dickinson, San Jose, CA) and a pocket calendar, and they were instructed to record infection symptoms and temperature on the calendar on a daily basis. All of the subjects had typical presentations, such as fever, sore throat, upper respiratory tract infection (URI), and common cold. Secondary endpoints were types of infection and laboratory variables such as plasma zinc, percentages of cells producing cytokines, and generation of cytokines and plasma oxidative stress markers.

Preliminary study

An additional 24 subjects were recruited from St Patrick's Senior Citizens Center for a preliminary study. For 23 of these subjects, the ages ranged from 56 to 76 y, and 1 subject was 83 y old. Twelve were African American, and 12 were white. Eleven were men, and 13 were women. They were followed for 1 y for incidence of infection. With the use of the definition for infection as outlined above, the mean (±SD) incidence of infections was 1.4 ± 0.95/y in each of 24 zinc-nonsupplemented elderly subjects. We chose a sample size of 50 for the current study so that we could detect a 50% reduction in the incidence of infections in the zinc-supplemented group, with a SD of 0.95, a 2-sided P value of 0.05, and an approximate power of ≥80%.

Plasma zinc and copper

Plasma zinc was assayed by methods established in our laboratory that used flameless atomic absorption spectrophotometry with a Zeeman background corrector (SpectraAA 220Z; Varian Optical Spectroscopy Instruments, Victoria, Australia (19). Samples were digested in zinc-free nitric acid and diluted with zinc-free water before analysis. Reference standards included bovine liver (National Bureau of Standards) and pooled plasma previously analyzed by flame atomic absorption. The normal values for healthy persons are 110 ± 10 μg/dL, and values of <90 μg/dL (2 SDs below the mean in the current study) are considered to be in the deficient range. Plasma copper was also analyzed by flameless atomic absorption spectrophotometry.

Percentage of cells positive for specific cytokines

Whole blood (0.5 mL) was mixed with RPMI-1640 media supplemented with 10% fetal bovine serum and 10 μg brefeldin-A/mL and then incubated at 37 °C for 4 h in the presence of selected stimulators. For T helper 1 (Th1) cytokines [IL-2 and interferon (IFN) γ], we used phorbol-12-myristate-13-acetate (25 ng/mL) and ionomycin (1 μg/mL); for T helper 2 (Th2) cytokines (IL-4 and IL-10), we used 25 μg concanavalin/mL; and for inflammatory cytokines (IL-1β and TNF-α), we used 2 μg lipopolysaccharide/mL (LPS; Sigma Aldrich, St Louis, MO). The source of the LPS was *Escherichia coli* 0111:B4. After incubation, samples were labeled with fluorescence-labeled anti-CD3 (for Th1 and Th2 cytokines) or anti-CD14 (for IL-1β and TNF-α), lysed, and then fixed with 0.25 mL of 3.7% formaldehyde in phosphate-buffered saline. Fixed cells were permeabilized with 0.1% saponin (Sigma Chemical Co, St Louis, MO) and labeled with antibodies directed against specific cytokines. All fluorescence-labeled antibodies were obtained from CalTag Laboratories (Burlingame, CA). Data for samples were collected at the flow cytometry core facility at the Wayne State University Karmanos Cancer Institute, and the results were analyzed by using CELL QUEST software (version 3.2; Becton Dickinson).

Ex vivo generation of cytokines

MNCs were isolated from 10 mL heparinzed whole blood by using Histopaque 1077 density gradient (Sigma Chemical Co) and then resuspended in RPMI-1640 medium plus 10% fetal bovine serum (1×10^6/mL). Cells were stimulated with phytohemagglutinin for 48 h for the generation of Th1 and Th2 cytokines and with LPS for 24 h for the generation of inflammatory cytokines (25). Supernatants were removed and stored at −20 °C until they were assayed with the use of an enzyme-linked immunosorbent assay for IL-1β, IL-2, IL-4, IL-10, IFN-γ, and TNF-α (R&D Systems, Minneapolis, MN).

Markers of oxidative stress

Plasma lipid peroxidation by-products MDA and HAE were measured by using a thiobarbituric acid lipid peroxidation assay kit (Oxford Biochemical Research, Oxford, MI); plasma 8-hydroxydeoxyguanine was assessed with the use of an enzyme-linked immunosorbent assay (Japan Institute for the Control of Aging, Shizuoka, Japan), and total plasma NO was assessed by using an NO kit (Oxford Biomedical) at baseline and after 6 mo of supplementation.

Reverse transcriptase–polymerase chain reaction for interleukin 2 mRNA

We selected 12 zinc-deficient subjects for the study of reverse transcriptase–PCR for IL-2 mRNA. They were a separate group of subjects and not a part of the main study. The inclusion and exclusion criteria were the same as those described in detail for elderly subjects. These subjects were recruited from the senior center; they provided written informed consent and were randomly assigned to receive either zinc or placebo according to the protocol. The mean age of the 6 subjects in the zinc group was 65 ± 9.1 y (range: 56–83 y), and that of the 6 subjects in the placebo group was 67.5 ± 6.6 y (range: 61–81 y; $P = 0.6$). The zinc group included 4 women and 2 men; the placebo group included 3 women and 3 men. The zinc group included 4 whites, 1 African American, and 1 Chinese; the placebo group included

4 whites and 2 African Americans. The mean plasma zinc concentrations at baseline in the zinc and placebo groups were 86.38 ± 3.6 and 88 ± 0.82 μg/dL, respectively ($P = 0.35$).

We compared the baseline data for IL-2 mRNA and plasma zinc concentrations in these 12 zinc-deficient subjects with the data in a separate group of12 zinc-sufficient subjects who were not a part of the main study. These subjects were also recruited from the senior center, were ambulatory, and were free of any chronic illness; they provided written informed consent. Their mean age was 65 ± 5.8 y (range: 59–83 y). This group included 3 men and 9 women and 8 whites, 3 African Americans, and 1 American Indian. Their mean plasma zinc concentration at baseline was 94.5 ± 2.54 μg/dL, whereas that in the 12 zinc-deficient subjects was 85.4 ± 2.54 μg/dL ($P = 0.0001$). At baseline, the IL-2 mRNA of the zinc-sufficient subjects was 0.59 ± 0.05 and that of the zinc-deficient subjects was 0.39 ± 0.06 ($P = 0.0001$).

Isolated MNCs from each subject were stimulated with 10 μg/mL PHA-p for 24 h to induce IL-2 mRNA. Total RNA was extracted by using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. cDNA was prepared from 2 μg total RNA by RT at 42 °C for 30 min in a 20-μL reaction and synthesized in the presence of Moloney murine leukemia virus RT (2.5U) by using 2.5 μ mol oligo-d (thymidine) primer/L and reaction conditions as described by the manufacturer (Invitrogen). The reaction was stopped by heating the samples to 99 °C for 5 min. A GeneAMP RNA PCR kit (Applied Biosystems, Foster City, CA) was used for cDNA amplification. For the IL-2 cDNA PCR reaction, cDNA was amplified in a total of 50 μL in the presence of 25 pmol/L of each oligo nucleotide 5' TGT ACA GGA TGC AAC TCC TG and 3' CAA TGG TTG CTG TC TCA TCA G. Reaction conditions were melting for 45 s at 94 °C, annealing for 45 s at 60 °C, and extension for 120 s at 72 °C for 35 cycles, which were followed by a final extension for 7 min at 72 °C in a thermal cycler (Perkin-Elmer, Wellesley, MA).

Statistical analysis

Demographic differences between the zinc and placebo groups were examined by *t* test (age) and chi-square test (sex and ethnicity). For other variables, *t* tests were used to compare group differences when variables were normally distributed. If distributions were not normal, the group differences were compared by using the nonparametric Wilcoxon rank-sum test. Chi-square tests (Fisher's exact test: 2 × 2 frequency table) were used to compare the incidence of infections in the zinc and placebo groups. In both the zinc and placebo groups, the changes in laboratory variables from baseline to after intervention were compared by using a paired *t* test (27). Multivariate repeated-measures analyses were used to examine measures over time. All statistical analyses were conducted with JMP software (version 5.0; SAS Institute Inc, Cary, NC) on a Macintosh Powerbook G4 computer (Apple Computers, Cupertino, CA).

RESULTS

The demographic characteristics of the elderly participants in the zinc-supplemented and placebo-supplemented groups are shown in **Table 1**. Neither the ages of the 2 groups or the number of men and women participating in each group differed significantly. An equal number of African Americans and whites participated in the 2 groups. No other variables differed significantly between the groups.

TABLE 1
Demographic characterization of elderly study participants

Variable	Zinc group ($n = 24$)	Placebo group ($n = 25$)
Age (y)	65 ± 9[1]	67 ± 6
Sex (*n*)		
Male	8	8
Female	16	17
Ethnicity (*n*)		
Black	6	6
White	17	19
Hispanic	1	0
Use of medications (*n*)	8	9
Types of medications (*n*)[2]		
Antihypertensive	3	7
Procardia	2	—
Celebrex	1	2
Proventil and Brethine	1	—
Anticonvulsant	1	3
Lipitor	1	2
Antidepressant (Zoloft)	2	1
Synthroid	—	1
Influenza vaccine (*n*)	19	18
Pneumonia vaccine (*n*)	9	8
Chronic disease (*n*)		
Hypothyroid	1	1
Hypertension	3	3
Atrial fibrillation	1	—
Coronary artery disease	—	2
Smoker ≥1 pack/d	4	4
Alcohol (regular use)	3	4

[1] $\bar{x}$ ± SD (all such values).

[2] Procardia; Pfizer Laboratories, Division of Pfizer Inc, New York, NY; Celebrex; GD Searle & Co, Division of Pfizer Inc; Proventil; Schering Corp, Kenilworth, NJ; Brethine; AstraZeneca/Pharma Inc, Wilmington, NC; Lipitor; Parke-Davis, Division of Warner-Lambert Co, Phizer Inc; Zoloft; Phizer Inc; Synthroid; Abbott Laboratories, Abbott Park, IL.

A comparison of baseline data between the younger subjects and the elderly subjects is shown in **Table 2**. Plasma zinc was lower and the percentage of cells producing IL-1β and TNF-α and the generated concentrations of these cytokines were significantly higher in the elderly subjects. Intercellular adhesion molecules, vascular endothelial cell adhesion molecules, and E-selectin in the plasma also were significantly higher in the elderly. IL-10 generated by Th2 cells, which are known to produce a negative effect on IL-2 generated by Th1 cells) was significantly higher in the elderly. The oxidative stress markers also were significantly higher in the elderly than in the younger adults.

The effect of supplementation on clinical variables is shown in **Table 3**. The mean incidence of infections per subject in 12 mo was significantly ($P < 0.01$) lower in the zinc-supplemented group (0.29 ± 0.46) than in the placebo group (1.4 ± 0.95; effect size: 1.46). When the infections were categorized separately as URI (which included rhinitis, sinusitis, and bronchitis), tonsillitis, common cold, cold sores, eye infection and flu, a significantly lower incidence of fever and a nonsignificant trend toward a lower incidence of the common cold were observed in the zinc-supplemented group than in the placebo group (28). The diagnosis of common cold was based on cough, sore throat, hoarseness, rhinorrhea, sinus tenderness, tachypnea, or rales. Seventeen subjects in the zinc-supplemented group had no evidence of any

TABLE 2
A comparison of selected variables in young adults (18–54 y old) and in older subjects (>55 y old)[1]

Variables	Young adults	Older subjects	P^2
Plasma zinc (μg/dL)	101.4 ± 10.0 (31)[3]	94.3 ± 11.4 (49)	0.046
Plasma ICAM-1 (ng/mL)	538 ± 112.7 (25)	652.6 ± 169.8 (47)	0.001
Plasma VCAM-1 (ng/mL)	1766 ± 480.4 (25)	2209 ± 890.5 (46)	0.008
Plasma E-selectin (ng/mL)	32.2 ± 13.1 (19)	84.6 ± 47.6 (69)	<0.001
Plasma NO (μmol/L)	42.7 ± 10.9 (24)	55.6 ± 14.7 (36)	<0.001
Plasma MDA (μmol/L)	0.36 ± 0.10 (16)	0.49 ± 0.15 (34)	<0.001
IL-1β			
(% of cells)	8.5 ± 9.2 (28)	17.4 ± 23.5 (48)	0.023
Generated (pg/mL)	679.5 ± 110.9 (31)	938.3 ± 423.3 (28)	0.004
TNF-α			
(% of cells)	10.18 ± 10.86 (22)	18.25 ± 20.5 (48)	0.035
Generated (pg/mL)	1522 ± 390 (26)	1882 ± 722.6 (24)	0.036

[1] ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular endothelial cellular adhesion molecule 1; NO, nitric oxide; MDA, malondialdehyde; IL, interleukin; TNF-α, tumor necrosis factor α.
[2] *t* Test.
[3] $\bar{x}$ ± SD; *n* in parentheses (all such values).

infection during the study, but only 3 subjects in the placebo group had no evidence of infection.

In the zinc-supplemented group, the total incidence of infections was 7 (2 from January through March, 0 from April through June, 3 from July through September, and 2 from October through December). In the placebo group, the total incidence of infections was 35 (8 from January through March, 11 from April through June, 7 from July through September, and 9 from October through December).

With time (12 mo of supplementation), the plasma zinc concentration in the subjects in the zinc group increased significantly, whereas that in the placebo group trended lower (**Table 4**). Also with time (12 mo of supplementation), the ex vivo generation of TNF-α decreased significantly in the zinc-supplemented subjects and increased significantly in the placebo group (**Table 5**). The reduction in TNF-α concentration was maximal at the end of 6 mo. Ex vivo generation of IL-10 decreased nonsignificantly with time in the zinc group. The percentage of cells positive for TNF-α, IL-1β, or IL-10 did not change significantly with time in either the placebo or zinc-supplemented group.

The changes in concentrations of plasma molecular markers of oxidative stress (MDA + HAE and 8-oHdG) between baseline and at the end of 6 mo of zinc supplementation showed a greater (and significant) decrease in the zinc-supplemented group than in the placebo group (**Table 6**). A nonsignificantly lower concentration of NO was observed in the zinc group at the end of 6 mo. We did not repeat these studies at the end of 12 mo of supplementation.

In MNCs isolated from zinc-deficient elderly subjects, zinc supplementation increased the ex vivo PHA-induced IL-2 mRNA expression and plasma zinc concentration above the values found in the zinc-deficient subjects who were given placebo ($P < 0.05$; **Table 7**). At baseline, the plasma zinc and IL-2 mRNA concentrations did not differ significantly between the zinc and placebo group subjects (Table 7).

Group × time interactions were significant for IL-2 mRNA ($P = <0.0001$) and plasma zinc ($P = <0.0088$) concentrations.

TABLE 3
Effect of zinc and placebo supplementation on clinical variables[1]

	Subjects affected in 1 y		
Variables	Zinc group ($n = 24$)	Placebo group ($n = 25$)	P^2
	%		
Infection	29	88	<0.001
Upper respiratory tract infection	12	24	0.136
Tonsillitis	0	8	0.255
Common cold	16	40	0.067
Cold sores	0	12	0.124
Flu	0	12	0.124
Fever	0	20	0.027
One infection each/y	29	52	
Two infections each/y	0	24	
Three infections each/y	0	8	
Four infections each/y	0	4	
Received antibiotics	8	48	

[1] Each subject could appear in >1 subcategory of infections.
[2] Chi-square Fisher's exact test.

TABLE 4
Effect of zinc ($n = 24$) and placebo ($n = 25$) supplementation on plasma zinc and copper concentrations[1]

	Baseline	At 12 mo	P^2
Plasma zinc (μg/dL)			0.0002
Zinc group	92.9 ± 9.45[3]	104 ± 16.69	
Placebo group	95.7 ± 13.09	88.5 ± 9.66	
Plasma copper (μg/dL)			0.750
Zinc group	182.2 ± 50.5[3]	210.7 ± 60.7	
Placebo group	193.4 ± 61.6	215.4 ± 58.7	

[1] No significant differences in plasma zinc or plasma copper were found between the 2 groups at baseline (*t* test).
[2] *P* value for change in groups over time (time × group interaction) (multivariate repeated-measures analyses).
[3] $\bar{x}$ ± SD (all such values).

TABLE 5
Effect of zinc and placebo supplementation on the generation of cytokines[1]

	Baseline	At 6 mo	At 12 mo	P^2
Percentage of cells positive for selected cytokine[3]				
TNF-α				
Zinc group	18 ± 18[4]	19 ± 19	18 ± 18	0.060
Placebo group	17 ± 21	24 ± 24	38 ± 34	
IL-1β				
Zinc group	20 ± 24	23 ± 24	23 ± 26	0.240
Placebo group	14 ± 23	10 ± 11	24 ± 28	
IL-10				
Zinc group	10 ± 11	7 ± 7	8 ± 8	0.170
Placebo group	6 ± 6	11 ± 15	12 ± 18	
Cytokines generated ex vivo (pg/mL)[5]				
TNF-α				
Zinc group	1897 ± 1004	1344 ± 544	1411 ± 786	0.018
Placebo group	1728 ± 498	1923 ± 782	2698 ± 785	
IL-1β				
Zinc group	892 ± 372	984 ± 373	766 ± 295	0.137
Placebo group	878 ± 188	881 ± 206	955 ± 223	
IL-10				
Zinc group	1916 ± 1277	952 ± 785	934 ± 873	0.056
Placebo group	917 ± 608	858 ± 483	1018 ± 834	

[1] TNF-α, tumor necrosis factor α; IL, interleukin. No significant differences (*t* test) were found in the percentage of cells positive for TNF-α, IL-1β, or IL-10 or in generated TNF-α and IL-1β between the 2 groups at baseline. The generated IL-10 concentration was, however, higher in the zinc group than in the placebo group ($P = 0.015$) at baseline, the explanation for which is unclear.

[2] *P* value for change in groups over time (time × group interaction) (multivariate repeated-measures analyses).

[3] $n = 24$ in both the zinc and placebo groups.

[4] $\bar{x} \pm$ SD (all such values).

[5] $n = 12$ and 14 in the zinc and placebo groups, respectively. Means within group are repeated measures. The repeated measures by group (zinc and placebo) for TNF-α showed a significant change ($P = 0.012$ and 0.042, respectively), which means that, over time, TNF-α changed from baseline in both groups.

In the zinc-supplemented group, both of these variables increased during the 6-mo period, whereas they did not change in the placebo-treated group (Table 7). During the 6-mo observation period, 2 subjects in the zinc-treated group had one episode of bronchitis each. In the placebo-treated group, 4 subjects had an infection: 1 subject each had laryngitis, flu, URI, and common cold.

DISCUSSION

Our study showed that zinc supplementation administered to the elderly population resulted in a significant decrease in the incidence of infection. Many animal studies show that zinc deficiency decreases resistance to a range of bacterial, viral, fungal, and parasitic pathogens (29), probably because of the immune impairment induced by zinc deficiency. The baseline plasma

TABLE 6
Effect of zinc and placebo supplementation on plasma oxidative stress markers[1]

	Baseline	At 6 mo	P^2
MDA+HAE (μmol/L)			
Zinc group	1.66 ± 0.34[3]	1.35 ± 0.18	0.0002
Placebo group	1.70 ± 0.30	1.71 ± 0.35	
8-OHdG (ng/mL)			
Zinc group	0.63 ± 0.16	0.50 ± 0.14	0.030
Placebo group	0.66 ± 0.13	0.68 ± 0.13	
Nitric oxide (μmol/L)			
Zinc group	87.34 ± 8.08	79.01 ± 10.96	0.180
Placebo group	89.43 ± 11.72	86.74 ± 9.28	

[1] $n = 13$ and 11 subjects in the zinc and placebo groups, respectively. MDA, malondialdehyde; HAE, 4-hydroxyalkenals; 8-OHdG, 8-hydroxydeoxyguanine. No significant differences (*t* test) in oxidative stress markers were found between the 2 groups at baseline.

[2] *P* value for change in groups over time (time × group interaction) (multivariate repeated-measures analyses).

[3] $\bar{x} \pm$ SD (all such values).

TABLE 7
Effect of zinc and placebo supplementation on interleukin (IL) 2 mRNA and plasma zinc concentrations in zinc-deficient elderly subjects[1]

	Baseline	At 6 mo	P^2
IL-2 mRNA[3]			
Zinc group	0.38 ± 0.07[4]	0.63 ± 0.03	<0.001
Placebo group	0.40 ± 0.05	0.39 ± 0.04	
Plasma zinc (μg/dL)			
Zinc group	84.0 ± 3.03	97.6 ± 5.98	<0.0088
Placebo group	86.8 ± 2.04	89.2 ± 3.06	

[1] $n = 6$ subjects in each group. No significant difference (*t* test) in IL-2 mRNA was found between the 2 groups at baseline. Despite the random assignment of zinc-deficient subjects into zinc or placebo group, the plasma zinc concentration was significantly lower in the zinc group than in the placebo group ($P = 0.016$).

[2] *P* value for change in groups over time (time × group interaction) (multivariate repeated-measures analyses).

[3] Relative expression of IL-2 mRNA/18S RNA.

[4] $\bar{x} \pm$ SD (all such values).

zinc concentrations in our elderly subjects were low, which suggested that a marginal zinc deficiency was present in this group. Thus, the enhancing effect on the immune response of providing zinc should translate into improved host defense and increased resistance to pathogens in zinc-deficient subjects. According to our plasma zinc criteria, ≈35% of the elderly subjects in the current study were considered zinc-deficient. We observed, however, that zinc supplementation also decreased concentrations of oxidative stress markers and showed antiinflammatory responses in subjects who were not zinc deficient as judged by plasma zinc concentrations at baseline, which suggested that zinc had a therapeutic effect in this regard similar to the effect we had earlier reported in healthy volunteers (19).

Zinc deficiency not only adversely affects the production of IL-2 and IFN-γ but also decreases the IL-12 production from macrophages (AS Prasad, personal observations, 2002). IFN-γ along with IL-12 is required for optimal phagocytic activity of macrophages (parasites, viruses, and bacteria). We previously observed that, in zinc-deficient persons, ex vivo generation of IL-1β is increased, which suggests that zinc deficiency, per se, may activate monocytes and macrophages to generate inflammatory cytokines. These inflammatory cytokines, in turn, are known to generate oxidative stress. Zinc supplementation decreased not only the production of inflammatory cytokines but also that of oxidative stress markers, as we previously reported (19, 29). Thus, the current study provides an important link between zinc supplementation, increased IL-2 mRNA production, decreased incidence of infections, decreased generation of inflammatory cytokines, and decreased oxidative stress in the elderly subjects.

Numerous studies have shown the benefits of zinc supplementation with respect to infectious diseases in human populations (29). Controlled trials of zinc supplementation in infants and children showed a 25–30% reduction in the incidence and duration of acute and chronic diarrhea and a reduction of up to 50% in the incidence of pneumonia (30–32). *Schistosoma mansoni* egg counts observed in children given zinc supplements were lower than those in children who received placebo (33). In addition, *Acrodermatitis enteropathica* patients have fewer infections when given supranormal amounts of zinc supplementation (4). IL-2 production in zinc-deficient patients with sickle cell disease was low, and oral zinc supplementation (75 mg zinc as acetate) resulted in a decreased incidence of infections, fewer days in the hospitals, and correction of ex vivo IL-2 generation (34). Although the effect of zinc on the common cold remains controversial, in our studies in patients with common cold, the duration and severity of cold symptoms were ≈50% less in zinc-treated subjects than in placebo group subjects (35).

Zinc has several key roles relating to cell signaling, cell activation, gene expression, protein synthesis, and apoptosis (36). Zinc is crucial to the normal development of immune cells, and it plays an important role in maintaining the activity of a range of immune cells, including neutrophils, monocytes, macrophages, natural killer cells, and B and T cells. Zinc-deficient subjects have a greater susceptibility to a variety of pathogens (36). Results from our previous research indicate that nearly 30–35% of the upper middle-class elderly population in the Detroit area may be zinc deficient, as judged by their lymphocyte zinc concentrations (37). Low thymulin activity and low IL-2 production have been reported in zinc-deficient elderly subjects (36). Other investigators have observed that zinc supplementation to elderly subjects increased the numbers of circulating T cells in association with improved delayed-type hypersensitivity reactions and immunoglobulin G antibody responses to tetanus toxoid (38).

We have presented evidence for a significant increase in IL-2 mRNA in zinc-deficient elderly subjects after zinc supplementation, a finding consistent with our previous reports in experimental human model studies and in cell culture studies in the HUT-78 cell line (19, 39). We conclude that zinc is involved in increasing IL-2 production in zinc-deficient elderly subjects by increasing the gene expression of IL-2. We have also observed a decrease in IL-10 production in zinc-supplemented elderly subjects, and this decrease may also have an effect of increasing IL-2 production (40).

Results from the current study show that elderly subjects are oxidatively stressed and that zinc is an effective antiinflammatory as well as an antioxidant agent. The administration of 45 mg elemental zinc/d did not reduce plasma copper concentrations in zinc-supplemented elderly subjects. Inasmuch as zinc is nonmutagenic and relatively nontoxic (except for induction of copper deficiency when the therapeutic dose of zinc is >50 mg elemental zinc/d and is used for >12 wk), a long-term trial of zinc in the elderly should be undertaken to determine zinc's role in preventing many chronic disorders that have been related to oxidative stress and chronic inflammatory cytokines such as TNF-α, IL-1β, and IL-8 (13–16). Most important, we show here that the ex vivo generation of TNF-α from isolated MNCs is significantly decreased in elderly subjects after zinc supplementation. We also observed in the placebo group that TNF-α increased with time, which suggests that this increase may be an effect of increasing age. A long-term, still ongoing trial has been conducted for the past 10 y in patients with age-related macular degeneration who received 80 mg elemental zinc as oxide and 2 mg Cu to prevent copper deficiency (41). Zinc alone has decreased the incidence of blindness due to age-related macular degeneration in 25% of these elderly subjects, and, most important, increased longevity has also been reported in those treated with zinc (41, 42). These observations are truly exciting and deserve further exploration.

The limitation of the current study is that the number of subjects in the trial was small. We hope that our results will stimulate larger zinc trials in the elderly.

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