

It is worth pointing out that the larger trials (VISP, HOPE2, VORVIT, and HOST) were conducted in subjects with advanced vascular disease. In these trials the subjects were also given a variety of drugs and other treatments that may have obscured the potential beneficial effects of the B vitamin intervention. Advanced vascular disease is in theory accompanied by depletion of thioretinaco ozonide from cellular membranes (6). Intervention with B vitamins could not be expected effectively to counteract depletion of this substance, which is implicated in oxidative metabolism (7). Future human trials with this substance in subjects with advanced vascular disease would be expected to be more effective than intervention with folic acid, vitamin B-6, and vitamin B-12 because thioretinaco ozonide requires synthesis from homocysteine thiolactone, retinoic acid, cobalamin, and ozone. If future studies confirm this prediction, homocysteine that is bound to plasma proteins, as determined by current assay methods, is correctly considered to be a marker of disease caused by the underlying cellular deficiency of thioretinaco ozonide.

No conflicts of interest were reported.

Kilmer S McCully

Pathology and Laboratory Medicine Service
Veterans Affairs Boston Healthcare System
West Roxbury, MA 02132
E-mail: Kilmer.mccully@med.va.gov

REFERENCES

1. Jamison RL, Hartigan P, Kaufman JS, et al. Veterans Affairs Site Investigators. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007;298:1163–70.
2. Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *JAMA* 2006;296:2720–6.
3. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007;369:1876–82.
4. Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin intervention for stroke prevention trial. an efficacy analysis. *Stroke* 2005;36:2404–9.
5. Wald DS, Morris JK, Law M, Wald NJ. Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. *BMJ* 2006;333:1114–7.
6. McCully KS. Chemical pathology of homocysteine. I. Atherogenesis. *Ann Clin Lab Sci* 1993;23:477–93.
7. McCully KS. Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine thiolactone metabolism. *Ann Clin Lab Sci*. 1994;24:27–59.

Erratum

Meydani SN, Barnett JB, Dallal GE, et al. Serum zinc and pneumonia in nursing home elderly. *Am J Clin Nutr* 2007;86:1167-73.

The following important information should have been included in footnote 2 to the title of our article: the study was also supported by HRCA/Harvard Research Nursing Home grant PO1 AG004390.

The following information should have been included in the acknowledgements section on page 1172: we thank D. Lipsitz for facilitating the recruitment of subjects and for assisting with the study at the Hebrew Rehabilitation Center.

Erratum

Soenen S, Westerterp-Plantenga S. No differences in satiety or energy intake after high-fructose corn syrup, sucrose, or milk preloads. *Am J Clin Nutr* 2007;86:1586–94.

In the first sentence of the Design section of the abstract, the phrase “4800-mL drinks” should have read “four 800-mL drinks.”



Serum zinc and pneumonia in nursing home elderly^{1–3}

Simin N Meydani, Junaidah B Barnett, Gerard E Dallal, Basil C Fine, Paul F Jacques, Lynette S Leka, and Davidson H Hamer

ABSTRACT

Background: Zinc plays an important role in immune function. The association between serum zinc and pneumonia in the elderly has not been studied.

Objective: The objective was to determine whether serum zinc concentrations in nursing home elderly are associated with the incidence and duration of pneumonia, total and duration of antibiotic use, and pneumonia-associated and all-cause mortality.

Design: This observational study was conducted in residents from 33 nursing homes in Boston, MA, who participated in a 1-y randomized, double-blind, and placebo-controlled vitamin E supplementation trial; all were given daily doses of 50% of the recommended dietary allowance of essential vitamins and minerals, including zinc. Participants with baseline ($n = 578$) or final ($n = 420$) serum zinc concentrations were categorized as having low ($<70 \mu\text{g/dL}$) or normal ($\geq 70 \mu\text{g/dL}$) serum zinc concentrations. Outcome measures included the incidence and number of days with pneumonia, number of new antibiotic prescriptions, days of antibiotic use, death due to pneumonia, and all-cause mortality.

Results: Compared with subjects with low zinc concentrations, subjects with normal final serum zinc concentrations had a lower incidence of pneumonia, fewer (by almost 50%) new antibiotic prescriptions, a shorter duration of pneumonia, and fewer days of antibiotic use (3.9 d compared with 2.6 d) ($P \leq 0.004$ for all). Normal baseline serum zinc concentrations were associated with a reduction in all-cause mortality ($P = 0.049$).

Conclusion: Normal serum zinc concentrations in nursing home elderly are associated with a decreased incidence and duration of pneumonia, a decreased number of new antibiotic prescriptions, and a decrease in the days of antibiotic use. Zinc supplementation to maintain normal serum zinc concentrations in the elderly may help reduce the incidence of pneumonia and associated morbidity. *Am J Clin Nutr* 2007;86:1167–73.

KEY WORDS Serum zinc, nursing home elderly, pneumonia, mortality, antibiotic use

INTRODUCTION

Pneumonia is a major public health problem in the elderly (1). An important predisposing factor to the increased incidence of infections such as pneumonia in the elderly is the age-associated decline in immune function (2). Such changes in immune response with age, in addition to malnutrition, contribute to the increased frequency and severity of pneumonia and to the mortality due to pneumonia in the elderly (1–4).

Zinc has been shown to play an important role in the regulation of the T cell-mediated function (5–7). Zinc deficiency has been shown to cause thymus involution and to depress lymphocyte proliferation, interleukin-2 (IL-2) production, delayed-type hypersensitivity skin responses, and antibody response to T cell-dependent antigens (5, 8). Similar defects in T cell function have been observed with aging (2). Several investigators have reported low zinc status or decreased intakes in elderly subjects (9–11). Furthermore, low zinc status in the elderly has been shown to contribute to age-associated dysregulation of the immune response (3, 12), and zinc supplementation has been shown to improve T cell-mediated function in the elderly (9, 12–15). In children, low concentrations of circulating zinc have been shown to be associated with an increased risk of respiratory morbidity (16), and zinc supplementation has been shown to reduce both the risk and duration of pneumonia and deaths due to pneumonia in children (17, 18). The association between serum zinc and pneumonia in the elderly, however, has not been studied.

From April 1998 to August 2001, a randomized controlled trial was carried out to investigate the effect of vitamin E supplementation on respiratory infections in an elderly nursing home population (19). We found a high proportion ($\approx 30\%$) of nursing home elderly with low serum zinc concentrations at baseline and after 1 y of follow-up, despite the fact that all participants received 50% of the recommended

¹ From the Nutritional Immunology Laboratory (SNM, JBB, LSL, and DHH), the Biostatistics Unit (GED), and the Nutritional Epidemiology Program (PJF), Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; the Department of Pathology, Sackler Graduate School of Biochemical Sciences, Tufts University, Boston, MA (SNM); the Friedman School of Nutrition Science and Policy at Tufts University (SNM, JBB, GED, PFJ, and DHH); the Nutrition/Infection Unit, Department of Public Health and Family Medicine, Tufts University School of Medicine, Boston, MA (JBB); the Travel and Tropical Medicine Practice, Boston, MA (BCF); the Department of Medicine, Boston University School of Medicine, Boston, MA (DHH); and the Center for International Health and Development, Boston University School of Public Health, Boston, MA (DHH).

² Supported by the NIA, National Institutes of Health grant 1R01-AG13975; the US Department of Agriculture agreement 58-1950-9-001, and a grant for preparation of the study capsules from Hoffmann-La Roche Vitamins and Fine Chemicals Division (currently DSM).

³ Address reprint requests and correspondence to SN Meydani, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: simin.meydani@tufts.edu.

Received January 31, 2007.

Accepted for publication May 30, 2007.

dietary allowance (RDA) of essential vitamins and minerals, including zinc, during the trial. Because recently published studies in children (17, 18) have shown zinc supplementation to be beneficial in reducing morbidity and mortality from pneumonia and past research has shown the negative effect of zinc deficiency on immune function in the elderly, we examined the relations between serum zinc concentration and the incidence and duration of pneumonia, the number of new antibiotic prescriptions, the days of antibiotic use, death due to pneumonia, and all-cause mortality in elderly nursing home residents.

SUBJECTS AND METHODS

Study design and intervention

A total of 617 subjects was enrolled ($\approx 33\%$ enrolled in each of 3 successive years) into a randomized, double-blind, placebo-controlled trial of the effect of 1 y of vitamin E supplementation (200 IU/d) on respiratory infections in a nursing home population (19). The Tufts–New England Medical Center Institutional Review Board approved the study protocol and the informed consent form. For subjects who were not capable of giving informed consent, such as those with dementia, a proxy identified by the nursing home staff was contacted for written informed consent. In cases in which either the staff or the investigators were uncertain as to a subject's ability to give informed consent, informed consent was sought from both the subject and the proxy (both were required for participation).

Nursing home residents have a heterogeneous intake of micronutrients (20), some of which are necessary for proper immune function. To reduce variability in the vitamin E trial, all subjects received a daily capsule containing 50% of the RDA (21) for essential micronutrients, including zinc. Fifty percent of the RDA was selected because few subjects meeting our eligibility criteria would have dietary intakes $< 50\%$ of the RDA for micronutrients (22). The exact composition of the essential nutrients mix per capsule given to the subjects was as follows: vitamin A (400 μg), thiamine (0.6 mg), riboflavin (0.6 mg), niacin (6.0 mg), pyridoxine (0.9 mg), folate (100 μg), cyanocobalamin (1 μg), vitamin C (30 mg), vitamin D (100 μg), vitamin E (200 IU for those in the treatment group, and 4 IU in the control group), iron (5 mg), selenium (25 μg), iodine (75 μg), zinc (7 mg), and copper (0.8 mg). Zinc was given to study subjects in the sulfate form.

At baseline and at follow-up, $\approx 30\%$ of subjects were found to be of marginal zinc status based on the presence of low serum zinc concentrations. Other micronutrient deficiencies were much less prevalent at baseline (from 0% to 10%), and their prevalence did not change significantly at follow-up (19).

Selection of study participants

Detailed information on the screening and recruitment of participants was described previously (19). The eligibility criteria included age ≥ 65 y, life expectancy > 6 mo, no anticipated discharge within 3 mo, not room-bound for the past 3 mo, body mass index (BMI; in kg/m^2) ≥ 16 , serum albumin ≥ 3.0 g/dL, ability to swallow pills, and willingness to receive influenza vaccine and provide informed consent. Exclusion criteria included active neoplastic disease, tube feeding, kidney dialysis, intravenous or urethral catheters for the past 30 d, tracheostomy

or chronic ventilator use, chronic steroid treatment > 10 mg/d, use of immunosuppressive drugs, use of antibiotics within the previous 2 wk, and more than the RDA level of supplements of selenium, zinc, β -carotene, fish oil, and vitamins E, C, and B-6.

The current analyses included all enrolled subjects with baseline ($n = 578$) or end-of-study zinc measurements ($n = 420$, including 7 subjects with only end-of-study zinc values). For the purposes of this study, subjects were categorized by serum zinc concentrations on the basis of cutoffs of < 70 $\mu\text{g}/\text{dL}$ to indicate low serum zinc concentrations and ≥ 70 $\mu\text{g}/\text{dL}$ to indicate normal serum zinc concentrations (23). Although there is no consensus that zinc status can be easily defined by serum zinc values, it is the most widely used biochemical indicator of zinc status and is the only biochemical indicator of zinc status for which adequate reference data are available (23, 24). In addition, several studies in the elderly have shown that serum zinc concentrations do respond to zinc supplementation (13, 14, 25).

Outcomes

Pneumonia-related outcomes included incidence of and number of days with pneumonia, number of new antibiotic prescriptions for pneumonia, days of antibiotic use for treatment of pneumonia, and death due to pneumonia. We also examined all-cause mortality.

Data collection

Information regarding subject characteristics, baseline diseases and medications, and vaccination history was obtained from medical records. Fasting blood samples were collected at baseline and at study completion for clinical chemistries, complete blood count, and nutritional status as previously described (19). Serum samples were collected into trace metal-free tubes, and serum zinc concentrations were measured with a Perkin-Elmer flame atomic absorption spectrometer (26) at the Nutrition Evaluation Laboratory (NEL), Human Nutrition Research Center on Aging at Tufts University (HNRCA). Specifically for the validation of the zinc procedure, the NEL used reference material from the National Institute of Standards and Technology (NIST). Commercially available controls (Lyphochek Assayed Chemistry Control Levels 1 & 2, Bio-Rad Laboratories, Irvine, CA; NIST certified material, Bi-Level Trace Metal QC Check Sample, VHG Labs, Inc, Manchester, NH) were used in the daily operational procedures for zinc. The interassay CV for zinc is 5.5–6.5%, and the intraassay CV is 3.5–4.5%. The laboratory reference range is 70–130 $\mu\text{g}/\text{dL}$.

Study nurses were trained by a study physician to identify relevant respiratory symptoms and to perform a focused physical examination of the respiratory tract. Supervised practice evaluations were repeated throughout the study to reinforce the nurses' clinical skills and to ensure consistency of the pneumonia data collection. The study nurses collected information weekly relating to infection, including symptoms of respiratory infections, temperature, respiratory and heart rate, and a physical examination focused on the respiratory system. The nurses reviewed each participant's chart for documentation of laboratory analyses, radiography, medications, micronutrient supplementation, weight, and nursing or physician descriptions of symptoms and signs relating to pneumonia and other respiratory infections.

At the end of the study, data collected from the subjects were randomly assigned, by nursing home, to the 2 study physicians

for diagnosis of infections. Infection data from any one subject were evaluated by only one physician, except for 18 subjects whose records were used to determine the concurrence of diagnoses between physicians.

Diagnosis of pneumonia

Details on how the study physicians evaluated the data to determine incidence and duration of pneumonia were described previously (19). Briefly, clinical definitions of pneumonia were developed on the basis of accepted definitions (27). To increase the specificity of the definitions, a diagnosis of pneumonia had to include at least one physical sign and thus could not be made on symptoms alone. An episode was considered resolved when all symptoms ceased. A new infection was defined as one occurring after 7 symptom-free days. Pneumonia symptoms could include cough with or without sputum production, chest pain, dyspnea, and fever. Signs of infection included an elevated temperature (≥ 38 °C), tachycardia, tachypnea, abnormal breath sounds, and dullness to percussion of the chest. The diagnosis required radiologic findings of one or more new pulmonary infiltrates.

Statistical analysis

The demographic and clinical characteristics of participants in the low and normal serum zinc groups were compared by using Student's *t* test for independent samples (continuous measures) and Fisher's exact test (categorical measures).

As noted above, all subjects received a capsule containing 50% of the RDA for essential micronutrients, including zinc. Because of this supplementation, the baseline zinc concentrations might not reflect the usual zinc status during the entire study period. Therefore, we also used zinc concentrations measured at the final study visit as a marker of zinc status. However, analyses based on final zinc concentrations were necessarily limited to those who survived or completed the trial.

Rate ratios and their CIs for incidence of pneumonia and number of new antibiotic prescriptions per year were modeled by using Poisson regression with the natural logarithm of time as an offset (28). None of the deviance statistics from the Poisson regressions exceeded 1, which suggests that the variability in response was in keeping with the Poisson distribution. Multiple linear regression analyses were used to determine the association between serum zinc and duration of pneumonia and days of antibiotic use due to pneumonia. Cox proportional hazards models using baseline zinc concentrations were fitted to determine the hazards ratio for deaths due to pneumonia and all-cause mortality. Except for the proportional hazards analyses, the analyses were performed by using both baseline and final zinc concentrations as predictors. Although the original study did not show a significant difference in the rate of pneumonia between the vitamin E and placebo groups (19), we nevertheless controlled for allocation to treatment and placebo groups in these analyses. We also adjusted for year of enrollment, age, sex, baseline BMI, current smoking status, diabetes mellitus, and chronic obstructive pulmonary disease (including bronchial asthma), which have been shown to be associated with an increased risk of pneumonia or pneumonia-associated death (1, 29, 30). Analyses of the comparison of those with low compared with those with normal final serum zinc concentrations were also controlled for change in BMI between baseline and follow-up values. Additionally, in separate analyses, we also controlled for

baseline serum albumin and change in serum albumin concentrations between baseline and follow-up in these models. Two-sided observed significance levels ($P < 0.05$) were considered to be statistically significant. All calculations were performed by using SAS for WINDOWS (version 9.1.3; SAS Institute Inc, Cary, NC).

RESULTS

The demographic and clinical characteristics of participants with baseline zinc ($n = 578$) and those with final study zinc concentrations ($n = 420$) were not different (Table 1). The distribution of these characteristics was also not different from our original study population ($n = 617$) (19).

Elderly with low baseline serum zinc concentrations were significantly older ($P = 0.0001$), were more likely to have had congestive heart failure ($P = 0.047$), and had lower serum albumin concentrations ($P = 0.0006$) and white blood count ($P = 0.042$) (Table 1). Elderly with low final serum zinc concentrations were older ($P = 0.014$), had lower serum albumin concentrations ($P = 0.003$), and a lower percentage of lymphocytes ($P = 0.048$) and were more likely to have had coronary artery disease ($P = 0.042$) at baseline than were those with normal final zinc concentrations. All subjects received influenza vaccine, and there was no significant difference in the proportion of subjects who had pneumococcal immunization between those with low and those with normal serum zinc concentrations (19).

When participants with low or normal baseline zinc concentrations were compared, differences in the incidence and duration of pneumonia, the number of new antibiotic prescriptions, and the days of antibiotic use for pneumonia were not statistically significant (Table 2). Deaths due to pneumonia were 53% lower in those with normal than in those with low baseline serum concentrations of zinc, but this association was not statistically significant ($P = 0.198$). The all-cause mortality rate was 39% lower in those with normal than in those with lower baseline zinc concentrations ($P = 0.049$) (Table 2). Controlling for congestive heart failure and other variables found to be significantly different between those with low and those with normal baseline serum zinc concentrations in the model did not materially change the statistical significance of the differences observed.

End-of-study serum zinc concentrations were strongly associated with the incidence and duration of pneumonia and with the number of new antibiotic prescriptions and days of antibiotic use (Table 3; $P \leq 0.004$ for all). The incidence of pneumonia and the total number of new antibiotic prescriptions used for the treatment of pneumonia were $\approx 50\%$ lower in those with end serum zinc concentrations ≥ 70 $\mu\text{g/dL}$ than in those with concentrations < 70 $\mu\text{g/dL}$. In addition, the duration of pneumonia was lower by 3.9 d and the days of antibiotic use by 2.6 d in those with end serum zinc in the normal range than in those with low concentrations (Table 3). Control for coronary artery disease and other variables found to be significantly different between those with low and those with normal final serum zinc concentrations in our multiple regression analyses model did not materially change the statistical significance of the differences observed. In addition, differences remained statistically significant after baseline serum albumin and change in serum albumin concentrations between baseline and follow-up were controlled for. The 310 subjects with normal end zinc concentrations at completion had 78 episodes of pneumonia, whereas the 110



TABLE 1

Characteristics of study participants by baseline and final serum zinc concentrations¹

Baseline characteristics	Baseline serum zinc			Final serum zinc		
	All (n = 563–578)	Normal ² (n = 386–396)	Low ³ (n = 177–182)	All (n = 412–420)	Normal ² (n = 305–310)	Low ³ (n = 105–110)
Age (y)	84.6 ± 7.6 ⁴	83.8 (72.3)	86.4 (7.9) ⁵	84.6 (7.5)	84.1 (7.1)	86.1 (7.7) ⁶
Female sex [n (%)]	419 (72)	283 (71)	136 (75)	314 (75)	227 (73)	87 (79)
White race [n (%)]	544 (94)	373 (94)	171 (94)	398 (95)	291 (94)	107 (97)
BMI (kg/m ²)	25.8 ± 5.2	25.9 (4.9)	25.5 (5.8)	26.0 (5.2)	26.0 (4.9)	26.0 (5.9)
Current smoker [n (%)]	43 (8)	31 (8)	12 (7)	30 (7)	24 (8)	6 (6)
Vitamin E supplementation [n (%)]	290 (50)	199 (50)	91 (50)	217 (52)	141 (45)	62 (56)
Serum albumin (g/dL)	3.76 ± 0.34	3.79 (0.32)	3.69 (0.36) ⁵	3.77 (0.34)	3.80 (0.33)	3.69 (0.33) ⁶
Total cholesterol (mg/dL)	197 ± 46	199 (46)	193 (45)	197 (45)	197 (46)	199 (43)
White blood count (cells/μL)	7.10 ± 2.30	7.23 (2.46)	6.81 (1.87) ⁵	6.96 (1.78)	7.01 (1.81)	6.82 (1.69)
Total lymphocyte count (cells/μL)	2.12 ± 1.41	2.18 (1.63)	1.98 (0.66)	2.10 (0.75)	2.15 (0.78)	1.98 (0.66)
Percentage lymphocytes (%)	30.1 ± 9.3	30.1 (9.0)	30.2 (10.1)	30.7 (9.0)	31.1 (9.1)	29.7 (8.8) ⁶
Pneumococcal vaccination [n (%)]	53 (9)	39 (9)	14 (8)	46 (11)	33 (11)	13 (12)
NSAID use [n (%)]	212 (37)	156 (39)	56 (31)	158 (38)	120 (39)	38 (35)
Obstructive lung disease [n (%)] ⁷	146 (25)	100 (25)	46 (25)	98 (23)	71 (23)	27 (25)
Coronary artery disease [n (%)]	194 (34)	123 (31)	71 (39)	131 (31)	88 (28)	43 (39) ⁶
Congestive heart failure [n (%)]	120 (21)	73 (18)	47 (26) ⁶	79 (19)	52 (17)	27 (25)
Hypertension [n (%)]	297 (51)	205 (52)	92 (51)	222 (53)	162 (52)	60 (55)
Diabetes mellitus [n (%)]	117 (20)	81 (20)	36 (20)	87 (21)	68 (22)	19 (17)
Malignancy [n (%)]	53 (9)	37 (9)	16 (9)	44 (10)	34 (11)	10 (9)
Dementia [n (%)] ⁸	286 (49)	202 (51)	90 (49)	207 (49)	149 (48)	58 (53)

¹ NSAID, nonsteroidal antiinflammatory drug.² Defined as ≥70 μg/dL.³ Defined as <70 μg/dL.⁴ $\bar{x} \pm SD$ (all such values).^{5,6} Significantly different from normal [Student's *t* test for independent samples (continuous measures) and Fisher's exact test (categorical measures)]:⁵ *P* < 0.01, ⁶ *P* < 0.05.⁷ Includes asthma, chronic obstructive pulmonary disease, and chronic bronchitis.⁸ Includes Alzheimer disease.

subjects with low end zinc concentrations had 51 episodes. The fraction of pneumonias occurring in the final 2 mo of the study was not significantly different between those subjects with normal (14 cases out of 78) and those with low (8 cases out of 51) end serum zinc concentrations, ie, 18% compared with 16%, respectively.

Thirty percent of the participants had marginal zinc status. However, given that there were other nutrients in the capsules provided, which might influence pneumonia incidence, we corrected for these nutrients in the same model that included zinc. Our findings with zinc remain robust despite the control for these nutrients. In addition, the mean changes in weight and BMI from

TABLE 2

Pneumonia, antibiotic use, and death by baseline serum zinc concentration

	Baseline serum zinc ¹		Rate ratio or mean difference (95% CI) ²	<i>P</i> ³
	≥70 μg/dL (n = 379)	<70 μg/dL (n = 174)		
Incidence of pneumonia (no./person-year)	0.34	0.37	0.87 (0.63, 1.21)	0.414
Duration of pneumonia (d/person-year)	4.32	5.65	−1.5 (−3.4, 0.4)	0.126
Number of new antibiotic prescriptions for pneumonia (no./person-year)	0.34	0.36	0.90 (0.65, 1.25)	0.539
Days of antibiotic use for pneumonia (d/person-year)	3.07	3.58	−0.7 (−2.0, 0.7)	0.330
Deaths due to pneumonia (no./person-year)	0.02	0.04	0.47 (0.15, 1.49)	0.198
All-cause mortality (no./person-year)	0.12	0.19	0.61 (0.37, 1.00)	0.049

¹ Crude values.

² Poisson regression analyses were used for incidence of pneumonia and number of antibiotic prescriptions, least-squares regression analyses for duration of pneumonia and of antibiotic use, and Cox proportional hazard regression for deaths. All analyses controlled for treatment (supplemented with vitamin E or not), age, sex, chronic obstructive lung disease, current smoking status, diabetes mellitus, year of enrollment (1998–2000), and baseline BMI; additional control for coronary artery disease, congestive heart failure, and white blood cell count, and, in separate models, for baseline serum albumin concentrations, did not materially affect the observed associations.

³ Derived from Poisson, least-squares, and Cox proportional hazard regression analyses.

TABLE 3
Pneumonia and antibiotic use by final serum zinc concentration

	Final serum zinc ¹		Rate ratio or mean difference (95% CI) ²	P ³
	≥70 μg/dL (n = 310)	<70 μg/dL (n = 110)		
Incidence of pneumonia (no./person-year)	0.25	0.46	0.52 (0.36, 0.76)	<0.001
Duration of pneumonia (d/person-years)	3.19	6.82	-3.9 (-6.2, -1.6)	<0.001
Number of new antibiotic prescriptions for pneumonia (no./person-years)	0.26	0.48	0.52 (0.36, 0.75)	<0.001
Days of antibiotic use for pneumonia (d/person-years)	2.50	4.85	-2.6 (-4.4, -0.9)	0.004

¹ Crude values.

² Poisson regression analyses were used for incidence of pneumonia and number of antibiotic prescriptions and least-squares regression analyses for duration of pneumonia and of antibiotic use. All analyses controlled for treatment (supplemented with vitamin E or not), age, sex, chronic obstructive lung disease, current smoking status, diabetes mellitus, year of enrollment (1998–2000), and baseline and change in BMI between baseline and follow-up; additional control for coronary artery disease, congestive heart failure, and percentage of lymphocytes, and, in separate models, for baseline serum albumin and change in serum albumin concentrations between baseline and follow-up, did not materially affect the observed associations.

³ Derived from Poisson and least-squares regression analyses.

baseline to follow-up between subjects with low and normal zinc concentrations were not significantly different; the mean (\pm SD) changes in weight were -0.81 ± 4.70 and -0.01 ± 4.39 kg, respectively, and in BMI were -0.35 ± 1.87 and -0.03 ± 1.65 , respectively.

DISCUSSION

We observed that elderly nursing home residents with low serum zinc had a higher risk of pneumonia, a longer duration of pneumonia episodes, a greater number of new antibiotic prescriptions, and more days of antibiotic use for the treatment of pneumonia at the end of 1 y of daily micronutrient supplementation with 50% of the RDA, including zinc. In addition, low baseline concentrations of serum zinc in our elderly nursing home population were associated with increased all-cause mortality.

Our finding of a significantly lower all-cause mortality rate (by 39%) in those with normal baseline serum zinc concentrations than in those with low baseline serum zinc concentrations suggests that zinc may play a crucial role in influencing mortality in the elderly. Severe zinc deficiency can impair immunity and increase susceptibility to infectious diseases, a major cause of mortality in the elderly (1, 3, 31, 32). Indeed, the risk of mortality was reduced by 27% in participants of the Age-Related Eye-Disease Study (aged 55–81 y) who received supplementation with 80 mg Zn/d (relative risk: 0.73; 95% CI: 0.61, 0.89) (33). Results from our study indicate that supplementation with <80 mg Zn/d might reduce mortality in the elderly. In addition, zinc supplementation has been shown to reduce overall mortality by as much as 51% in children with diarrhea and by as much as 68% in infants born full-term and those small-for-gestational age (34, 35). However, a recent zinc mortality trial in Zanzibar found a nonsignificant 7% reduction in the relative risk of all-cause mortality in children supplemented with zinc (36).

When baseline serum zinc status was used as a measure of zinc to determine whether low serum zinc affects susceptibility to pneumonia, the differences were not statistically significant. It may be that findings from baseline zinc concentrations were attenuated because of a higher risk of death among subjects with low baseline zinc concentrations or because of a loss of subjects due to serious illnesses, hospitalizations, or both. In support of

this finding, participants with low baseline serum zinc concentrations had a significantly shorter period to “death or first pneumonia” as a combined outcome than did those with adequate baseline serum zinc concentrations (data not shown). In addition, introducing a supplement that provided 50% of the RDA for zinc to all study subjects might make the baseline zinc concentration a biased estimate of usual zinc status during the follow-up period. It is expected that some subjects whose serum zinc concentrations were low or borderline at baseline might move into the normal range in response to the supplementation. Thus, baseline zinc concentrations may not reflect the true zinc status during the follow-up period. If normal zinc status were associated with a lower incidence of pneumonia, inclusion of subjects with normal zinc concentrations in the low baseline serum zinc group could result in a lower apparent incidence of pneumonia in that group and thereby attenuate the true association between zinc status and risk of pneumonia. This is supported by the observation that the elderly whose serum zinc concentrations responded to zinc supplementation, ie, low at baseline and adequate at the end and adequate at baseline and adequate at the end, had lower rates of pneumonia (0.22 and 0.26, respectively) than did those with low concentrations at baseline and at the end (0.41) and adequate at baseline and low at the end (0.57).

Because of the potential biases introduced by zinc supplementation, we performed similar analyses using final zinc concentrations, because this measure may better reflect the zinc status of subjects during the course of the study. We observed a strong association between low final zinc concentrations and an increased incidence and duration of pneumonia, the number of new antibiotic prescriptions, and days of antibiotic use for pneumonia treatment. As described in Results, the association between serum zinc concentration and pneumonia remained significant even when other micronutrients were included in the model. Furthermore, the lower incidence and morbidity of pneumonia observed in subjects with normal final zinc concentrations than in those with low final zinc concentrations was not due to differences between the 2 groups in changes in weight, BMI, or other micronutrients during the study period (19).

Zinc deficiency has been suggested to be a risk factor for immune deficiency and subsequent infection relapses in the elderly (12, 31, 37). Zinc is essential for membrane integrity, DNA

synthesis, and cell proliferation and is a cofactor to >300 enzymes (5, 38). Also, zinc is essential to the function of all highly proliferating cells in the human body, especially the immune system (39). Thymus atrophy, lymphopenia, and other defects in T cell function have been observed in both zinc deficiency and in the elderly (2, 5, 8).

Low zinc consumption has been reported in the elderly (10, 40–42). Bogden et al (42) reported that zinc ingestion was below the RDA in >90% of their 100 study subjects aged 60–89 y. Prasad et al (25) reported that ≈35% of the elderly in their study were zinc deficient. In addition, as mentioned above, we found below normal serum zinc concentrations in ≈30% of the nursing home elderly enrolled in our vitamin E supplementation study. This level of marginal zinc status persisted during the study period despite the provision of 50% of the RDA for zinc to the participants (19). Thus, our finding of a significant association between low serum zinc concentration and pneumonia could be of major importance to the health of the elderly, because poor zinc status would lead to an impaired immune response, which could in turn result in an increased susceptibility to infections in the elderly (9, 31, 37). Supplementation with 50% of the RDA for zinc appeared to benefit many of the participants with inadequate baseline zinc concentrations, but the results also showed that this level of supplementation was not adequate to maintain serum zinc concentrations in a significant proportion of the subjects.

Plasma or serum zinc concentrations are also known to decrease sharply in many infections (38, 43, 44). The decline is part of a set of metabolic reactions to infection known as the acute phase response (45). Although one could argue that lower serum zinc concentrations might be due to a higher incidence of pneumonia, or that reverse causality may be a possible explanation for these findings, this was unlikely in the present study because the acute effect of pneumonia on zinc is likely to be transient—≈2 wk if effective therapy is provided (46). Given the similar incidence of pneumonia in our participants in the last months of the study, transient suppression of serum zinc by the acute phase response was unlikely to be responsible for the low end-study zinc concentrations.

Zinc supplementation may play an important role in the prevention and/or modulation of infectious diseases in the elderly (3, 9, 14, 31). Various studies of zinc supplementation in the elderly (with supplements ranging between 25 and 100 mg/d in the sulfate, gluconate, or acetate form over 3–12 mo) have observed increased circulating zinc concentrations (13, 14, 25) and an enhanced immune status, including an improved cell-mediated immune response, serum thymulin activity, and IL-2 production, and an increased response to skin-test antigens (12, 15, 25, 47). Also, when cultures of white blood cells from elderly subjects were supplemented with 98 μg/dL (or 15 μmol/L) of zinc (the physiologic concentration), they produced interferon-α in amounts comparable with those from the younger subjects (48). In a randomized, double-blind, placebo-controlled clinical trial ($n = 81$), institutionalized elderly had a significant decrease in the mean number of respiratory infections 2 y after supplementation with micronutrients containing zinc and selenium, but not vitamins (49). In another larger ($n = 725$), randomized, double-blind, placebo-controlled intervention study, low-dose supplementation with zinc and selenium significantly increased the humoral response in institutionalized elderly after vaccination (50). The number of subjects without respiratory tract infections during the study was also found to be higher in the elderly who

received trace elements over a 2-y period (50). Although these studies suggest a protective effect of zinc against respiratory infections, the contribution from other nutrients and/or the synergistic effects with other nutrients present in the mixture could not be ruled out. A recent study by Prasad et al (25) showed that supplementation with 45 mg elemental Zn/d in the gluconate form for 12 mo in a small number of elderly ($n = 49$; 24 in the supplemented group and 25 in the placebo group) significantly reduced the incidence of all infections, including respiratory infections. The authors concluded that the results, although encouraging, need to be corroborated in a larger number of participants.

The results from our current study, in addition to these earlier findings, suggest that elderly with low serum zinc concentrations might benefit from zinc supplementation. Such a measure has the potential to reduce not only the number of episodes and duration of pneumonia and the number of new antibiotic prescriptions and days of antibiotic use due to pneumonia but also all-cause mortality in the elderly. An adequately powered randomized, double-blind, controlled trial seems to be the likely next step. Such a study is needed to determine the efficacy of zinc supplementation as a potential low-cost intervention to reduce morbidity and mortality due to pneumonia in this vulnerable population.

The authors' responsibilities were as follows—SNM: had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; SNM, BCF, DHH, and LSL: responsible for data acquisition; SNM, JBB, GED, BCF, PFJ, and DHH: responsible for the study concept and design, interpretation of the study findings, and the drafting of the manuscript; GED: responsible for the statistical analyses; SNM, JBB, BCF, PFJ, and DHH: helped to direct the analyses. All authors were responsible for the critical review and revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors declared that they had no conflict of interest. The funding organizations had no input concerning the design or conduct of the study; the collection, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript.

REFERENCES

1. LaCroix AZ, Lipson S, Miles TP, White L. Prospective study of pneumonia hospitalizations and mortality of U.S. older people: the role of chronic conditions, health behaviors, and nutritional status. *Public Health Rep* 1989;104:350–60.
2. Miller RA. The cell biology of aging: immunological models. *J Gerontol* 1989;44:B4–8.
3. Mocchegiani E, Giacconi R, Muzzioli M, et al. Zinc, infections and immunosenescence *Mech Ageing Dev* 2000;121:21–35. (Published erratum appears in *Mech Ageing Dev* 2001;122:353.)
4. Riquelme R, Torres A, el-Ebiary M, et al. Community-acquired pneumonia in the elderly. Clinical and nutritional aspects. *Am J Respir Crit Care Med* 1997;156:1908–14.
5. Fraker PJ, King LE, Laakko T, et al. The dynamic link between the integrity of the immune system and zinc status. *J Nutr* 2000;130(suppl):1399S–406S.
6. Allen JI, Perri RT, McClain CJ, Kay NE. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J Lab Clin Med* 1983;102:577–89.
7. Oleske JM, Westphal ML, Shore S, et al. Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Its correction. *Am J Dis Child* 1979;133:915–8.
8. Beck FW, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. *Am J Physiol* 1997;272:E1002–7.
9. Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M. Zinc deficiency in elderly patients. *Nutrition* 1993;9:218–24.
10. Sandstead HH, Henriksen LK, Greger JL, et al. Zinc nutriture in the

- elderly in relation to taste acuity, immune response, and wound healing. *Am J Clin Nutr* 1982;36:1046–59.
11. Lindeman RD, Clark ML, Colmore JP, Lindeman RD, Clark ML, Colmore JP. Influence of age and sex on plasma and red-cell zinc concentrations. *J Gerontol* 1971;26:358–63.
 12. Wagner PA, Jernigan JA, Bailey LB, Nickens C, Brazzi GA. Zinc nutrition and cell-mediated immunity in the aged. *Int J Vitamin Nutr Res* 1983;53:94–101.
 13. Bogden JD, Oleske JM, Lavenhar MA, et al. Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. *J Am Coll Nutr* 1990;9:214–25.
 14. Kajanachumpol S, Srisurapanon S, Supanit I, et al. Effect of zinc supplementation on zinc status, copper status and cellular immunity in elderly patients with diabetes mellitus. *J Med Assoc Thailand* 1995;78:344–9.
 15. Duchateau J, Delepesse G, Vrijens R, Collet H. Beneficial effects of oral zinc supplementation on the immune response of old people. *Am J Med* 1981;70:1001–4.
 16. Bahl R, Bhandari N, Hambidge KM, Bhan MK. Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. *Am J Clin Nutr* 1998;68(suppl):414S–7S.
 17. Brooks WA, Santosham M, Naheed A, et al. Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomised controlled trial. *Lancet* 2005;366:999–1004.
 18. Brooks WA, Yunus M, Santosham M, et al. Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial. *Lancet* 2004;363:1683–8.
 19. Meydani SN, Leka LS, Fine BC, et al. Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA* 2004;292:828–36. (Published erratum appears in *JAMA* 2004;292:1305.)
 20. Drinka PJ, Goodwin JS, Drinka PJ, Goodwin JS. Prevalence and consequences of vitamin deficiency in the nursing home: a critical review. *J Am Geriatr Soc* 1991;39:1008–17.
 21. National Research Council (US), Subcommittee on the Tenth Edition of the RDAs, National Institutes of Health (US), National Research Council (US), Committee on Dietary Allowances. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
 22. Fiatarone M, Fiatarone M. Nutrition in the geriatric patient. *Hospital Practice (Office Edition)* 1990;25:38–40.
 23. Institute of Medicine (US), Panel on Micronutrients. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc: a report of the Panel on Micronutrients. Washington, DC: National Academy Press, 2002.
 24. Hotz C, Peerson JM, Brown KH, Hotz C, Peerson JM, Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). *Am J Clin Nutr* 2003;78:756–64.
 25. Prasad AS, Beck FW, Bao B, Fitzgerald JT, Snell DC, Steinberg JD, Cardozo LJ. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr* 2007;85(3):837–44.
 26. Smith JC Jr, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clin Chem* 1979;25:1487–91.
 27. McGeer A, Campbell B, Emori TG, et al. Definitions of infection for surveillance in long-term care facilities. *Am J Infect Control* 1991;19:1–7.
 28. Ramsey FL, Schafer DW. The statistical sleuth: a course in methods of data analysis. 2nd ed. Pacific Grove, CA: Duxbury/Thomson Learning, 2002.
 29. Mandell LA, Bartlett JG, Dowell SF, et al. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clin Infect Dis* 2003;37:1405–33.
 30. Koivula I, Sten M, Makela PH. Risk factors for pneumonia in the elderly. *Am J Med* 1994;96:313–20.
 31. Mocchegiani E, Muzzioli M, Gaetti R, et al. Contribution of zinc to reduce CD4+ risk factor for 'severe' infection relapse in aging: parallelism with HIV. *Int J Immunopharmacol* 1999;21:271–81.
 32. Janssens J-P, Krause K-H. Pneumonia in the very old. *Lancet Infect Dis* 2004;4:112–24.
 33. Clemons TE, Kurinij N, Sperduto RD, Group AR. Associations of mortality with ocular disorders and an intervention of high-dose antioxidants and zinc in the Age-Related Eye Disease Study. *Arch Ophthalmol* 2004;122:716–26. (AREDS report no. 13.)
 34. Baqui AH, Black RE, El Arifeen S, et al. Effect of zinc supplementation started during diarrhoea on morbidity and mortality in Bangladeshi children: community randomised trial. *BMJ* 2002;325:1059.
 35. Sazawal S, Black RE, Menon VP, et al. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial. *Pediatrics* 2001;108:1280–6.
 36. Sazawal S, Black RE, Ramsan M, et al. Effect of zinc supplementation on mortality in children aged 1–48 months: a community-based randomized placebo-controlled trial. *Lancet* 2007;369:927–34.
 37. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* 1998;68(suppl):447S–63S.
 38. Rink L, Kirchner H, Rink L, Kirchner H. Zinc-altered immune function and cytokine production. *J Nutr* 2000;130(suppl):1407S–11S.
 39. Ibs KH, Rink L, Ibs K-H, Rink L. Zinc-altered immune function. *J Nutr* 2003;133(suppl):1452S–6S.
 40. Briefel RR, Bialostosky K, Kennedy-Stephenson J, et al. Zinc intake of the U.S. population: findings from the third National Health and Nutrition Examination Survey, 1988–1994. *J Nutr* 2000;130(suppl):1367S–73S.
 41. Schmuck A, Roussel AM, Arnaud J, et al. Analyzed dietary intakes, plasma concentrations of zinc, copper, and selenium, and related antioxidant enzyme activities in hospitalized elderly women. *J Am Coll Nutr* 1996;15:462–8.
 42. Bogden JD, Oleske JM, Munves EM, et al. Zinc and immunocompetence in the elderly: baseline data on zinc nutrition and immunity in unsupplemented subjects. *Am J Clin Nutr* 1987;46:101–9.
 43. 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.
 44. Duggan C, MacLeod WB, Krebs NF, et al. Plasma zinc concentrations are depressed during the acute phase response in children with falciparum malaria. *J Nutr* 2005;135:802–7.
 45. Kushner I, Kushner I. The phenomenon of the acute phase response. *Ann NY Acad Sci* 1982;389:39–48.
 46. Halm EA, Fine MJ, Marrie TJ, et al. Time to clinical stability in patients hospitalized with community-acquired pneumonia: implications for practice guidelines. *JAMA* 1998;279:1452–7.
 47. Fortes C, Forastiere F, Agabiti N, et al. The effect of zinc and vitamin A supplementation on immune response in an older population. *J Am Geriatr Soc* 1998;46:19–26.
 48. Cakman I, Kirchner H, Rink L. Zinc supplementation reconstitutes the production of interferon-alpha by leukocytes from elderly persons. *J Interferon Cytokine Res* 1997;17:469–72.
 49. Girodon F, Lombard M, Galan P, et al. Effect of micronutrient supplementation on infection in institutionalized elderly subjects: a controlled trial. *Ann Nutr Metab* 1997;41:98–107.
 50. Girodon F, Galan P, Monget AL, et al. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: a randomized controlled trial. *MIN. VIT. AOX. geriatric network Arch Intern Med* 1999;159:748–54.