Micronutrient Supplementation and Immune Function in the Elderly

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Immunologic function, particularly cell-mediated immunity, declines with age, contributing to the increased incidence of infectious diseases in the elderly. Nutrition may play a pivotal role in maintaining immune competence in older adults. Most studies to date have focused on micronutrient deficiencies and supplementation, sometimes using “mega-dose” formulations. Multivitamin/mineral supplements or specific micronutrients such as zinc and vitamin E may be of value; however, data suggest there is likely a therapeutic range for many micronutrients, and oversupplementation may be harmful. Specific alterations of dietary lipids may also be useful for modulating immune responses in the elderly. This review summarizes the prevalence of vitamin and mineral deficiencies in older adults and highlights the outcomes of trials of micronutrient supplementation to augment immune function in the elderly.

Older age has frequently been associated with an increased risk of infection [1, 2]. Many factors contribute to this phenomenon; the presence of comorbid illness and a decline of typical defenses, so-called immune senescence, are usually cited as major risk factors. Immune senescence is characterized by specific cytokine changes that favor Th2 T-helper responses (antibody production, including autoantibody production) while suppressing Th1 responses (cytotoxic T cell and macrophage activation, i.e., cell-mediated immunity). Nutritional factors may play a critical role in the maintenance of immune function and be particularly important in the elderly [3].

Most studies on nutrition in the elderly have focused on the prevalence of micronutrient (vitamin and mineral) deficiencies and the role of supplementation in maintaining immune function. While this is a useful starting point, the implicit presumption that supplementation is without risk (i.e., if some is good, more is better) has recently been called into question by two antioxidant-supplement trials [4, 5]. These clinical trial failures emphasize the need to define micronutrient actions at the cellular level before rationally designing clinical trials. In addition, most supplementation trials have focused on in vitro measures of immune function, rarely being powered to include clinical endpoints that validate surrogate markers.

This article reviews the currently available data on the prevalence of micronutrient deficiency in the elderly and from published trials of vitamin and mineral supplementation in older adults and examines the potential deleterious effects of oversupplementing with certain micronutrients.

Prevalence of Micronutrient Deficiencies in the Elderly

While micronutrient deficiencies are considered rare in developed countries, the elderly represent a population at markedly increased risk because of nursing home domiciliary setting, low outdoor activity levels, and poor access to foods with adequate nutritional content.

Vitamin A (retinol) has many effects on immune function, including many of the limbs of immunity that decline with age [6–8]. The prevalence of retinol deficiency in older adults is dependent upon how one measures retinol. A French nursing home study [9] indicated that 55% of the elderly have inadequate dietary intake of retinol, but a more specific measure of body retinol stores, the relative dose response [10], which measures the amount of retinol excreted after an oral loading dose, indicated 21% were retinol-deficient. Retinol deficiency can also be measured by changes in corneal cells (impression cytology) or serum levels. By these two criteria, 6% and 2%, respectively, of nursing home residents in that study were retinol-deficient [9].

The World Health Organization (WHO) cut-off value for retinol deficiency based on serum levels is set by the amount required to prevent ocular changes (<0.70 μmol/L). The level required for optimal immune function might be considerably higher (>1.05 μmol/L) [6, 11, 12]; thus, estimates of retinol (or other vitamin/mineral) deficiency on the basis of the WHO standard definitions may significantly underestimate the problem.

Vitamin D deficiency is also quite prevalent in specific elderly populations. Nursing home residents and homebound elderly persons rarely get adequate sunlight exposure, and intolerance of vitamin D–fortified dairy products is common. The increased awareness of osteoporosis and its prevention may alleviate some of this problem, but in a study in the United...
States published in 1995 [13], 54% of homebound and 38% of nursing home–resident elderly persons were found to have serum vitamin D levels below normal (i.e., <25 nmol/L).

Two antioxidant micronutrients, zinc and vitamin E, have also been shown to have significant effects on immune function, and deficiencies of these micronutrients may be important for immune function in the elderly. Probably because of changes in dietary preferences, income, and total calories, zinc consumption declines with age [14], falling below the recommended dietary allowance (RDA) of 12–15 mg/d in many older adults [14, 15]. In a study of 118 healthy, ambulatory elderly persons in the Detroit area [15], mean zinc intake was 9.06 mg/d, but serum zinc levels (mean ± SD) were comparable to those of young, healthy control subjects (110.6 ± 13.4 μg/dL and 108.3 ± 12.1 μg/dL, respectively). However, zinc levels within granulocytes and lymphocytes were significantly lower than those of control subjects.

Similar limitations are present in the data concerning the prevalence of vitamin E deficiency in the elderly. Vitamin E deficiency is often defined by intake data rather than serum or cellular levels, a practice that may inaccurately reflect vitamin E status [16]. In Chandra’s study of free-living, healthy elderly persons [17], 8.3% of the subjects were found to be vitamin E–deficient (defined as a serum level less than the 95% CI for “normal” subjects [12–48 μmol/L]). However, in a study by Meydani et al. [18], subjects with serum vitamin E levels >34.8 μmol/L had significantly enhanced immune responses.

The discrepancies between serum and cellular levels of zinc and in defining appropriate cutoffs for vitamin E deficiency make it difficult to define the prevalence of “deficiency” in older adults. This is similar to the obstacles described above for determining the prevalence of vitamin A deficiency and in the use of inappropriate cutoff values for immune responses. These difficulties, in part, have led to the common practice of enrolling “all comers” in many of the vitamin and mineral supplementation trials involving the elderly, rather than restricting supplementation to only those deemed to be “deficient.”

Supplementation Trials

Many vitamin and/or mineral supplementation trials have been performed in older adults (table 1). Two major study designs have been employed: pretest/post-test, where individuals serve as their own control subjects, and randomized, placebo-controlled trials. With a few notable exceptions, these studies enrolled few subjects, primarily measured immune responses as endpoints, and were not powered to utilize clinical endpoints.

Multivitamin/mineral supplementation studies. Three randomized, placebo-controlled trials of multivitamin/mineral supplementation in elderly subjects have been reported, and all demonstrated some enhancement of surrogate markers of immune response (natural killer [NK] cell activity, delayed-type hypersensitivity [DTH] responses, or lymphocyte cytokine production; table 1).

The only interventional trial in the elderly to demonstrate a significant effect on a clinical endpoint was a multivitamin and mineral supplement trial published by Chandra [17]. In that study, patients were randomized to receive placebo or a daily oral supplement of retinol, β-carotene, thiamine, riboflavin, niacin, pyridoxine, folate, vitamin B12, vitamin C, vitamin D, vitamin E, iron, zinc, copper, selenium, iodine, calcium, and magnesium. Baseline rates of deficiency, based on determinations of serum levels of each vitamin and mineral, were similar in the two groups prior to supplementation.

After 12 months, the supplemented group had a significantly lower percentage of patients deficient in vitamin A, β-carotene, pyridoxine, vitamin C, iron, and zinc, while the percentage of subjects deficient for these nutrients did not change in the placebo group. Immune responses (CD4 T-cell percentage, NK cell activity, T-cell mitogenic responses, and IL-2/IL-2 receptor expression) were enhanced in the supplemented group. In addition, infection-related illness (as diagnosed by the blinded principal investigator) was substantially reduced in the supplemented group. The mean number of days of illness due to infection was 23 for supplement recipients vs. 48 for placebo recipients (P = .002). Furthermore, antibiotic use occurred for a mean of 18 days in the supplemented group vs. 32 days in the placebo group (P = .004).

β-Carotene/vitamin A supplementation studies. Long-term administration of vitamin A can be associated with hepatotoxicity. Therefore, vitamin A is rarely utilized in supplementation studies, except as a single high-dose supplement or in daily dosing with an amount similar to the RDA. There have been two sizable studies of vitamin A supplementation in nursing home patients: one employed high single doses [30] and the other used daily supplementation with or without zinc [28]. Both studies demonstrated no clinical effect, and one raised concerns that vitamin A supplementation may be harmful, on the basis of the finding of reduced numbers of CD3 and CD4+ T cells in vitamin A recipients [28].

β-Carotene, a precursor of vitamin A that is not associated with hepatotoxicity, is often used in supplementation trials. The effect of β-carotene supplementation on NK cell activity was extensively investigated as part of the Physicians’ Health Study [31]. This trial is particularly noteworthy for its long duration of follow-up (144 months). β-Carotene administration was associated with enhanced NK cell activity in elderly men (aged 65–86 years) but not in younger men (aged 51–64 years). This was true despite the fact that there was no clear effect of β-carotene on release of immunomodulators (IL-2 or prostaglandin E2 [PGE2]) from peripheral blood mononuclear cells. The clinical significance of β-carotene-enhanced NK cell activity is unknown.

Zinc supplementation studies. Zinc supplementation in the elderly has been attempted with use of several forms of zinc, in doses ranging from 15 mg q.d. of zinc acetate to
Table 1. Summary of data concerning supplementation trials and immune response in the elderly.

<table>
<thead>
<tr>
<th>Type of study, reference</th>
<th>No. of subjects</th>
<th>Duration (mo)</th>
<th>Supplements (dose or dosage)</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized, placebo-controlled</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[19]</td>
<td>47</td>
<td>12</td>
<td>Multivitamin/mineral</td>
<td>↑ NK cells; slowed decline of CD4⁺ cells</td>
</tr>
<tr>
<td>[20]*</td>
<td>30</td>
<td>1</td>
<td>Vitamin C (100 mg), vitamin E (50 mg), vitamin A (8,000 U)</td>
<td>↑ T cells, CD4⁺ cells, CD4/CD8 cell ratio, lymphocyte responses</td>
</tr>
<tr>
<td>[17]</td>
<td>96</td>
<td>12</td>
<td>Multivitamin/mineral</td>
<td>↑ IL-2, lymphocyte responses; ↓ no. of days with illness</td>
</tr>
<tr>
<td>[21]</td>
<td>56</td>
<td>6, 12</td>
<td>Multivitamin/mineral</td>
<td>↑ DTH responses at 12 mo</td>
</tr>
<tr>
<td>[22]</td>
<td>32</td>
<td>1</td>
<td>Vitamin E (800 mg)</td>
<td>↑ DTH responses, IL-2 responses; ↓ PGE₂ production</td>
</tr>
<tr>
<td>[23]</td>
<td>80</td>
<td>1, 4, 4.5</td>
<td>Vitamin E (60, 200, 800 U)</td>
<td>↑ DTH responses at all 3 doses; ↑ mitogen responses only in the 800-U group</td>
</tr>
<tr>
<td>[24]</td>
<td>20</td>
<td>1</td>
<td>Vitamin C (500-mg injection)</td>
<td>↑ DTH responses</td>
</tr>
<tr>
<td>[25]</td>
<td>20</td>
<td>3</td>
<td>β-Carotene (15, 30, 45, 60 mg)</td>
<td>↑ CD4⁺ and NK cells, no. of IL-2 receptors</td>
</tr>
<tr>
<td>[26]</td>
<td>103</td>
<td>3</td>
<td>Zinc acetate (15, 100 mg)</td>
<td>No change in DTH responses or lymphocyte mitogen responses</td>
</tr>
<tr>
<td>[27]³</td>
<td>63</td>
<td>12</td>
<td>Zinc acetate (15, 100 mg)</td>
<td>↑ DTH responses, NK cell activity, and lymphocyte proliferative responses</td>
</tr>
<tr>
<td>[28]³</td>
<td>118</td>
<td>3</td>
<td>Vitamin A (retinol palmitate, 800 mg), zinc sulfate (25 mg)</td>
<td>Retinol: ↑ CD3, CD4 cells; zinc: ↑ CD4⁺, cytotoxic T cells (CD3⁺, CD16⁺, and CD56⁺ cells)</td>
</tr>
<tr>
<td>[29]</td>
<td>84</td>
<td>1</td>
<td>Zinc sulfate (220 mg b.i.d.)</td>
<td>No change in antibody responses to influenza vaccine</td>
</tr>
<tr>
<td>[30]</td>
<td>109</td>
<td>Single dose</td>
<td>Vitamin A (200,000 U)</td>
<td>No change in infection rates or antibiotic use</td>
</tr>
<tr>
<td>[31]³</td>
<td>21</td>
<td>144</td>
<td>β-Carotene (50 mg q.o.d.) or ASA (325 mg q.o.d.) or both or neither</td>
<td>↑ NK cell activity without altering NK cell percentage, IL-2, or IL-2 receptors</td>
</tr>
<tr>
<td>[18]</td>
<td>88</td>
<td>8</td>
<td>Vitamin E (60, 200, or 800 mg q.d.)</td>
<td>↑ DTH responses, antibody titers to new T-cell dependent antigens; no effect on booster or T-cell-independent responses</td>
</tr>
<tr>
<td>Pretest/post-test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[32]</td>
<td>21</td>
<td>2</td>
<td>Commercial formula</td>
<td>↑ DTH responses</td>
</tr>
<tr>
<td>[33]</td>
<td>158</td>
<td>. . .</td>
<td>Vitamin C (400 mg)</td>
<td>↑ IgG, IgM, and C-3 (complement) levels</td>
</tr>
<tr>
<td>[34]</td>
<td>5</td>
<td>1</td>
<td>Zinc sulfate (55 mg)</td>
<td>↑ DTH responses</td>
</tr>
<tr>
<td>[35]</td>
<td>13</td>
<td>6</td>
<td>Zinc gluconate (30 mg)</td>
<td>↑ IL-1, DTH responses</td>
</tr>
<tr>
<td>[36]</td>
<td>8</td>
<td>4.5</td>
<td>Elemental zinc (30 mg b.i.d.)</td>
<td>↑ Lymphocytes, polymorphonuclear neutrophils, DTH responses</td>
</tr>
<tr>
<td>Other (nonrandomized, no placebo control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[37]</td>
<td>58</td>
<td>3</td>
<td>Macronutrient or micronutrient</td>
<td>↑ DTH responses</td>
</tr>
<tr>
<td>[38]</td>
<td>15</td>
<td>1, 2</td>
<td>Vitamin B₆ (50 mg)</td>
<td>↑ CD4⁺ cells and lymphocyte responses</td>
</tr>
<tr>
<td>[39]</td>
<td>30</td>
<td>1</td>
<td>Zinc sulfate (220 mg)</td>
<td>↑ T lymphocytes and DTH responses; no change in mitogen responses</td>
</tr>
</tbody>
</table>

NOTE. ASA = aspirin; DTH = delayed-type hypersensitivity; NK = natural killer; PGE₂ = prostaglandin E₂; ↑ = increase in; ↓ = decrease in.
* Subjects were hospitalized.
³ Study involved multiple comparison periods.
³ Partial crossover design.
² Factorial design.

220 mg b.i.d. of zinc sulfate (table 1). In most of these studies, zinc was administered with other vitamins or a multivitamin/mineral supplement. Again, no clinical endpoints have been evaluated in these trials, only surrogate markers of immune response. Several studies indicate zinc may enhance the number of CD3⁺, CD4⁺, CD16⁺, and CD56⁺ lymphocytes, NK cell activity, or DTH responses in elderly subjects [27, 28, 34], whereas others demonstrate no effect upon
DTH responses [26] or antibody production in response to influenza vaccine [29].

**Vitamin E supplementation studies.** Although many studies of vitamin E supplementation have been published (table 1), one particularly well done and comprehensive study was recently published by Meydani and colleagues [18]. In that study, vitamin E supplementation (200–800 mg/d) was associated with a 65% increase in DTH responses and enhanced antibody responses to primary immunization with a T-cell-dependent antigen (hepatitis B). However, there was no significant influence on response to T-cell-independent vaccine (pneumococcal vaccine) or on booster responses to a T-cell-dependent antigen (tetanus).

**Cautions Regarding Micronutrient Supplementation in the Elderly**

Several recent studies indicate the need for caution in designing future nutritional supplementation trials. There is strong epidemiological evidence that dietary intake of β-carotene and retinoids is associated with a decreased risk of certain types of cancer [39–41]. In fact, secondary prevention trials suggested that retinol supplementation could decrease the risk of cancer relapse [42]. On the basis of these findings and the presumption that β-carotene acted through its antioxidant effects (thus, if some is good, more is likely to be better), primary prevention trials enrolling large numbers of subjects were begun. Two of these trials [4, 5] have now been stopped prematurely because of an increased incidence of lung cancer in β-carotene recipients.

This example points out that many of the mechanisms underlying the activity of specific micronutrients are poorly understood. In addition to its antioxidant capacities, β-carotene is a retinol precursor. Retinol and other retinoid metabolites of β-carotene are tightly controlled by feedback mechanisms in the body and influence the transcription of hundreds of gene products [43]. It is unclear what role β-carotene supplementation might play in altering those complex relationships at the tissue or subcellular level. Furthermore, dietary studies indicating high intake of a particular nutrient do not distinguish occasional high-level consumption from chronic moderate- to high-level consumption. Clearly, ingesting 350 mg of β-carotene once a week with Sunday dinner could have markedly different physiological effects than those of daily supplementation with 50 mg of β-carotene.

The need to “right dose” rather than “mega-dose” a micronutrient is illustrated by findings concerning selenium. Selenium, in moderate concentrations, is an antioxidant that has been shown in several recent trials [44, 45] to reduce cancer risk, perhaps in part through augmentation of immune responses. However, at higher concentrations, selenium can have pro-oxidant activity. In vitro studies have shown that immune responses at increasing concentrations of selenium closely parallel the responses seen in vivo with increasing dietary intake (figure 1 [46]). Thus, supplementation of selenium may cause more harm than benefit in some subjects.

**Lipid Mediators of Immune Responses and Immune Senescence**

Other specific nutrients may play an important role in modulating immune responses in the elderly. Inflammatory and immune responses are initiated by a cascade of mediators that are either upregulated or downregulated in response to a foreign antigen. Eicosanoids, lipid end-products of arachidonic acid, play a critical role in this response. There is strong evidence that eicosanoid production may be altered with age and influence downstream responses. PGE₂ is an eicosanoid produced from arachidonic acid via cyclooxygenase activity. Macrophage- and splenocyte-derived PGE₂ levels are greatly increased with age [47]. Furthermore, PGE₂ favors production of Th2-associated cytokines (IL-4 and IL-5) while suppressing Th1-associated cytokines (IL-2 and IFN-γ), a pattern analogous to immune senescence [48, 49]. Inhibition of cyclooxygenase can restore PGE₂ levels to normal [47, 50].

Manipulations of dietary lipid intake can have a significant effect on immune function, perhaps via altered eicosanoid production, but dietary lipids can also influence membrane fluidity (and thereby access to receptors) [51–54] and other physiological functions. Many examples of altering immune responses via dietary lipid intake can be found in animal models [55], but perhaps the best example in humans utilized a common dietary supplement, N-3 fatty acid (fish oil) enrichment [56]. Subjects in that trial were placed on the National Cholesterol Education Program-2 diet, with or without fish oil supplementation.

Fish oil (N-3 fatty acids) intake was associated with a 35%–45% decrease in mononuclear cell production of IL-1, TNF, and IL-6 and a 24% reduction in lymphocyte proliferation. Furthermore, DTH responses were also reduced by 45% (P = .009). Thus, alterations of dietary intake of specific lipids can clearly influence immune responses in humans. This offers the possibility that specific dietary lipid combinations could upregulate immune responses (perhaps in anergic elderly persons) or downregulate immune responses (perhaps in cases of rheumatoid arthritis or other autoimmune diseases) in elderly subjects. Such an approach has already been suggested for managing some inflammatory diseases in young adults [57].

The mechanism of vitamin E–mediated augmentation of immune responses in the elderly may involve altered eicosanoid production. Vitamin E, in doses greater than the current RDA, can suppress production of the immunosuppressive eicosanoid PGE₂ [47, 50].

**Conclusions**

Impaired immune responses are common in older adults, and immune senescence likely contributes to the increased inci-
Figure 1. Effect of selenium concentration (ppm = parts per million) on in vitro and in vivo measures of immune response. Both selenium deficiency and toxicity impair immune function (reprinted with permission from [46]).

dence of infectious diseases in the elderly. While some have questioned the role of nutritional deficiency in the impaired immune response of healthy elderly subjects [58, 59], a large body of evidence suggests that micronutrients play a role in maintaining immune competence in older adults. Furthermore, supplementation with a variety of vitamins or multivitamin/mineral preparations may reverse some of the changes associated with impaired immune responses in the elderly. The potential deleterious effects of oversupplementation must be weighed against the beneficial effects demonstrated in some preliminary nutritional-supplement trials. A better understanding of the beneficial mechanisms of specific vitamin, mineral, or dietary lipid intake on immune responses could help identify nutritional strategies that may mitigate immune senescence.

References
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50. Beharka AA, Wu D, Han SN, Meydani SN. Macrophage prostaglandin production contributes to the age-associated decrease in T cell function which is reversed by the dietary antioxidant vitamin E. Mech Ageing Dev 1997; 93:59−77.


