Original Research

The Effects of a Multivitamin/Mineral Supplement on Micronutrient Status, Antioxidant Capacity and Cytokine Production in Healthy Older Adults Consuming a Fortified Diet

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Background: Inadequate micronutrient intake among older adults is common despite the increased prevalence of fortified/enriched foods in the American diet. Although many older adults take multivitamin supplements in an effort to compensate, studies examining the benefits of this behavior are absent.

Objective: To determine whether a daily multivitamin/mineral supplement can improve micronutrient status, plasma antioxidant capacity and cytokine production in healthy, free-living older adults already consuming a fortified diet.

Methods: An eight-week double-blind, placebo-controlled clinical trial among 80 adults aged 50 to 87 years (mean=66.5±8.6 years).

Results: Multivitamin treatment significantly increased ($p<0.01$, compared to placebo) plasma concentrations of vitamins D (77 to 100 nmol/L), E (27 to 32 μmol/L), pyridoxal phosphate (55.1 to 75.2 nmol/L), folate (23 to 33 nmol/L), B12 (286 to 326 pmol/L)), C (55 to 71 μmol/L), and improved the riboflavin activity coefficient (1.23 to 1.15), but not vitamins A and thiamin. The multivitamin reduced the prevalence of suboptimal plasma levels of vitamins E ($p=0.003$), B12 ($p=0.004$), and C ($p=0.08$). Neither glutathione peroxidase activity nor antioxidant capacity (ORAC) were affected. No changes were observed in interleukin−2, −6 or −10 and prostaglandin E_2, proxy measures of immune responses.

Conclusions: Supplementation with a multivitamin formulated at about 100% Daily Value can decrease the prevalence of suboptimal vitamin status in older adults and improve their micronutrient status to levels associated with reduced risk for several chronic diseases.

INTRODUCTION

Aging is accompanied by a variety of physiological, psychological, economic and social changes that compromise nutritional status and/or affect nutritional requirements [1]. For these reasons, the diets of many older adults do not currently meet the recommended intake levels of several essential vitamins and minerals [2, 3]; thus, low micronutrient status is often reported in this population [4–6]. Nutritional status surveys of the elderly indicate a low to moderate prevalence of frank nutrient deficiencies, but an increased risk of malnutrition, along with evidence of subclinical deficiencies having a direct impact on physiologic function [7–10].

Overt micronutrient deficiencies have been reported as prevalent in nursing home populations, and recommendationsd

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have been proferred that all institutionalized older adults receive a multivitamin/mineral supplement for general nutritional prophylaxis [11–13]. Trials conducted in long-term hospitalized elders with modest doses of antioxidant vitamins have demonstrated their capacity to improve the status of vitamins C and E, β-carotene and the activities of glutathione peroxidase and/or superoxide dismutase after six months [14–16]. In healthy, free-living older adults, clinical trials using multivitamin/mineral supplements have demonstrated improved nutrient status in as little as two months [17, 18]. Significant effects on immune response outcomes such as infectious disease episodes and delayed-hypersensitivity skin test responses have been noted after 12 months of intervention [19, 20].

The amount of any particular nutrient required to prevent a deficiency is inherently defined in the Recommended Dietary Allowances (RDA) [21]. This standard is being revised to include nutrient intakes associated with reductions in the risk for chronic disease, values often higher than those necessary to prevent deficiency [22, 23]. Many older adults are unable to consume sufficient, let alone optimal, levels of certain nutrients solely by diet, and a modified food guide pyramid for people over 70 years recommends the use of dietary supplements to bridge this gap [24].

Dietary supplement use by Americans over 50 years ranges from 31% to 56% [25]. The most often consumed supplement is a multivitamin preparation [26]. The effects of multivitamin supplementation in this population have not been examined since the advent of mandatory folate fortification of flour, voluntarily fortification of common foods such as vitamin C in milk, calcium and vitamin E in orange juice, and 'superfortified' breakfast cereals. Thus we examined whether a multivitamin/mineral supplement formulated at about the Daily Value (DV) can improve micronutrient status, antioxidant capacity and immune function (via cytokine production as a proxy) in healthy, older adults consuming a fortified diet.

MATERIALS AND METHODS

Subjects

Healthy, free-living older adults ≥50 years residing in the Greater Boston area were recruited by newspaper advertisements, direct mailings and clinic postings. Volunteers were excluded if they were smokers, used dietary supplements regularly for three months prior to screening, were taking medications known to interfere with folate metabolism, had established diseases of the gastrointestinal tract, liver and/or kidney, or any disability which would impede full participation in the study. On the basis of these criteria, 272 men and women were eligible for an initial blood screening visit. Since homocysteine is a functional indicator of low vitamin status, and the mean homocysteine concentration at the initial screening visit was 7.8 ± 2.0 μmol/L, participants with a total plasma homocysteine concentration above the mean (≥ 8.0 μmol/L) were invited to participate in the clinical trial. Of the 92 participants eligible for the trial, six had blood chemistry measures outside standard reference ranges, three developed medical conditions undetected during the initial screening visit, one lost interest, one was unwilling to refrain from dietary supplements, and one had gastrointestinal complaints, leaving a total of 80 subjects who completed the study. The age range of study subjects was 50 to 87 years (mean$=66.5 \pm 8.6$ years).

The study design was approved by the Human Investigation Research Committee of Tufts University and the New England Medical Center. All subjects signed a written informed consent agreement before participating.

Experimental Design

The protocol was designed as a double-blind, placebo-controlled clinical trial of an effervescent multivitamin/mineral preparation formulated at about 100% DV for most nutrients (Table 1). After gender stratification, subjects were randomized

Table 1. Composition of the effervescent multivitamin/mineral supplement

Vitamins	Amount	% DV	Minerals	Amount	% DV
Vitamin A	5000 IU	100	Boron	0.15 mg	*
Vitamin D3	400 IU	100	Calcium	162 mg	16
Vitamin E	30 IU	100	Chromium	65 μg	54
Vitamin K1	25 μg	31	Iodine	150 μg	100
Vitamin C	250 mg	416	Manganese	3.5 mg	175
Thiamin	1.5 mg	100	Molybdenum	160 μg	213
Riboflavin	1.7 mg	100	Nickel	5 μg	*
Vitamin B6	2 mg	100	Phosphorus	109 mg	10
Vitamin B12	6 μg	100	Potassium	80 mg	2
Niacinamide	20 mg	100	Selenium	20 μg	29
Biotin	30 μg	10	Tin	10 μg	*
Folate	400 μg	100	Vanadium	10 μg	*
Pantothenic Acid	10 mg	100	Zinc	15 mg	100
			Silicon	2 mg	*

* Daily Value (%DV) not established.

to receive either supplement or placebo. The placebo was composed of the same, non-nutritive base ingredients found in the supplement, i.e., citric acid, sodium bicarbonate, sweeteners, flavoring and coloring agent. During the seven days prior to the intervention, all subjects were given placebo to test their ability to comply with the protocol and required to give two overnight fasting blood samples (on day −7 and day 0) to determine baseline values for the micronutrients of interest. Fasting blood samples were again collected for analyses on days 49 and 56. The duplicate 'before' and 'after' measures were intended to ensure precision in the results, especially for those nutrients where the magnitude of change was anticipated to be small.

Subjects were asked to return all opened foil packets in which the tablets were contained as well as any unused tablets as a measure of compliance. Although subjects were asked to consume their usual dietary patterns, the Willett food frequency questionnaire [27] was administered both before and after the clinical trial to account for any significant changes in nutritional status not attributable to the supplement.

Micronutrient Analyses

All micronutrient analyses were performed using validated methods for assessing nutrient status (Table 2). The enzyme activity coefficient assays for thiamin, riboflavin and vitamin B6 measure activity coefficient or A.C. units.

Oxygen Radical Absorbance Capacity

The ORAC assays were carried out on a COBAS FARA II spectrofluorometric analyzer (Roche Diagnostic System, Inc., Branchburg, NJ) as described previously [28]. Assays were performed in duplicate on each blood sample, and all samples for each subject were analyzed within the same run.

Cytokine Production and Prostaglandin E_2 Activity

Cytokines IL-2, 6, 10, and prostaglandin E_2 (PGE_2) were assayed using commercially available immunoassay kits (Quantikine, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Statistical Analyses

All statistical analyses were performed with SPSS v8.0 (SPSS, Inc., Chicago IL). Prior to formal analysis, a logarithmic transformation was applied to concentrations of folate, vitamin B6 and vitamin B12 in order to achieve homogeneity of variance and linearity of regressions, but untransformed values were used to construct tables and graphs of summary statistics. Tests of repeated measures ANOVA were used to determine statistically significant changes in plasma nutrient concentrations, ORAC values and cytokine production between placebo and supplemented groups. Student's *t* test was used to compare baseline characteristics between the placebo and supplemented groups. The Wilcoxon-Mann-Whitney test was used to determine whether nutrient concentration changes from suboptimal to optimal categories were different in the placebo and supplemented groups. Summaries are expressed as means ± standard deviation (SD), and two-sided observed significance levels (p values) <0.05 are considered statistically significant.

RESULTS

Baseline characteristics of the study participants are presented in Table 3. No significant differences were observed between the placebo and supplemented groups with respect to age, gender and BMI. Although all participants were apparently healthy, the number of most frequently reported chronic illnesses were distributed evenly between the two groups. Baseline plasma micronutrient concentrations were also not significantly different between groups (Table 4).

No significant differences in kilocalorie, macronutrient or dietary micronutrient intake were detected at baseline (Table 5). Dietary consumption of vitamins A and C were more than twofold higher than recommended (RDA) levels, while intake

Table 2. Micronutrient analysis methods

Analyte	Method	Reference
Vitamin A	HPLC	[66]
25-OH-vitamin D	Protein-binding assay	[67]
α-tocopherol	HPLC	[66]
γ-tocopherol	HPLC	[66]
Thiamin (A.C.)*	Enzyme activity coefficient	[68]
Riboflavin (A.C.)*	Enzyme activity coefficient	[68]
Vitamin B6 (A.C.)*	Enzyme activity coefficient	[68]
Pyridoxal phosphate	Radio-enzymatic assay	[69–73]
Vitamin B12	Radioimmunoassay	Unpublished**
Folate	Radioimmunoassay	Unpublished**
Vitamin C	HPLC	[74]
Glutathione peroxidase	UV, kinetic assay	[75]

* Activity coefficient.

** Quantaphase II B12/Folate Radioassay; BioRad Laboratories, Hercules, CA.

Table 3. Characteristics of study participants

Subject Characteristics	Placebo Group	Supplemented Group
Males	26	28
Females	13	13
Age (range 50 to 87 years)[a]	66.5 ± 8.1	66.6 ± 9.1
BMI (kg/m^2)[a]	27.1 ± 3.8	27.9 ± 4.7
Chronic Illnesses Reported		
Hypertension	15	18
Hypercholesterolemia	10	10
Coronary Artery Disease	5	8
Rheumatoid Arthritis	4	7
Hypothyroid	4	3

[a] No significant differences were detected, $\bar{x}\pm$SD.

of the B-vitamins thiamin, riboflavin, B6 and B12 were all at or above their respective recommended levels. Intakes of vitamins D and folate were lower than recommended levels but within 74% and 86%, respectively, of current recommendations for this age group. Vitamin E consumption in both groups was approximately one third of the RDA.

Baseline measures of glutathione peroxidase activity (in both plasma and red blood cells), ORAC, cytokine and PGE_2 also did not differ between groups (Table 6).

Fat-Soluble Vitamins

Following the eight-week treatment, subjects taking the supplement had significant elevations in the plasma status of vitamins D (25(OH)D) and E (α-tocopherol) ($p<0.01$) of 30% and 21%, respectively (Table 4). No significant interaction between vitamin D change and season of study entry was noted (ANCOVA, $p=0.37$). Plasma γ-tocopherol decreased 12% ($p<0.001$) in the supplemented group, and no difference in mean plasma vitamin A (retinol) was detected in either group. Although vitamin K was included in the supplement, plasma concentrations were not measured. No significant changes in fat-soluble vitamin status were detected in the placebo group. Dietary intake of the fat-soluble vitamins did not change in either group during the intervention.

Although the prevalence of suboptimal vitamin A status (<2.5 μmol/L) [29] in the group receiving the multivitamin (78%) was not reduced by the supplement, mean dietary intake was 12,137 IU (>2X RDA), and all subject baseline levels were well above the cutoff point for the category associated with low risk of deficiency (>1.05 μmol/L) [22]. After the intervention, one placebo group subject had a drop in plasma retinol sufficient to reclassify him into the moderate risk category (0.35−1.05 μ mol/L). Supplementation reduced the prevalence of low vitamin D status (<37.5 nmol/L) [30] from 7% to 0% and suboptimal α-tocopherol (<30 μmol/L) [29] from 73% to 49%. In the placebo group, the prevalence of low vitamin D status was reduced from 13% to 8%. The prevalence of suboptimal α-tocopherol increased from 80% to 82% in the placebo group. Statistical significance was achieved only for α-tocopherol (Wilcoxon-Mann-Whitney test, $p=0.003$). The change in plasma retinol concentration after supplementation ranged from −0.4 to +0.5 μmol/L; the change in 25(OH)D ranged from −6.2 to +61.2 nmol/L; and the change in α-tocopherol ranged from −0.7 to +20.*1* μmol/L.

Water-Soluble Vitamins

After eight weeks of treatment, supplemented subjects showed significant improvement in the plasma concentrations of vitamins B6, B12, C, riboflavin, pyridoxal phosphate and folate (Table 4). Activity coefficient assays for riboflavin and vitamin B6 decreased 7% and 6% respectively, and no significant change in the status of thiamin was detected. Activity coefficient generating assays for thiamin, riboflavin and vitamin B6 are inversely proportional to their respective changes in plasma concentration. Plasma concentrations of pyridoxal phosphate, folate, vitamins B12 and C were increased by 36%, 42%, 14% and 29%, respectively. No significant changes in water-soluble vitamin status were found in the placebo group, although vitamin B12 decreased 4%. Dietary intake of the water-soluble vitamins did not change in either group during the intervention.

Supplementation reduced the prevalence of suboptimal plasma vitamin C concentrations (<50 μmol/L) [29] from 29% to 5%, suboptimal folate (<15 nmol/L) [31] from 15% to 5%, suboptimal vitamin B12 (<258 pmol/L) [32] from 42% to 27% and low vitamin B6 (<20 nmol/L) [33] from 7% to 0%. In the placebo group, the prevalence of suboptimal vitamin C status was reduced from 41% to 33% and suboptimal vitamin B12 increased from 67% to 80%. Only the change in vitamin B12 achieved statistical significance ($p=0.004$), although the vitamin C change approached it ($p=0.08$). The change in plasma vitamin C concentration after supplementation ranged from −21.6 to +78.9 μmol/L, the change in folate concentration ranged from −8 to +25 nmol/L, the change in PLP concentration ranged from −136.4 to +105.0 nmol/L, and the change in vitamin B12 concentration ranged from −24 to +141 pmol/L.

Antioxidant Capacity

As shown in Table 6, neither plasma nor red blood cell glutathione peroxidase activity changed in either the supplement or placebo group after eight weeks of treatment. No significant differences in ORAC were detected in either group after treatment.

Cytokine Production and PGE_2

No significant differences in the production of cytokines IL-2, 6, 10 and PGE_2 activity were detected in either group after eight weeks of treatment (Table 7).

Table 4. Changes in plasma micronutrient status following 8 week intervention[a]

	Placebo Group			Supplemented Group		
	Before	After	Difference	Before	After	Difference
Fat-Soluble Vitamins						
Vitamin A (μmol/L)	2.15 ± 0.44	2.11 ± 0.46	−0.04 ± 0.20	2.11 ± 0.46	2.16 ± 0.51	0.05 ± 0.21
Vitamin D, 25OHD (nmol/L)	75 ± 36	77 ± 30	0.8 ± 17.7	77 ± 29	100 ± 27	22.5 ± 16.2[b]
Vitamin E(α) (μmol/L)	26 ± 8	25 ± 7	−0.7 ± 3.5	27 ± 9	32 ± 11	5.7 ± 4.9[b]
Vitamin E(γ) (μmol/L)	4.5 ± 1.5	4.6 ± 1.5	0.1 ± 0.9	4.9 ± 3.1	4.3 ± 3.1	−0.6 ± 0.9[c]
Water-Soluble Vitamins						
Thiamin (A.C.)*	1.09 ± 0.07	1.08 ± 0.08	−0.01 ± 0.06	1.09 ± 0.08	1.07 ± 0.06	−0.02 ± 0.08
Riboflavin(A.C.)*	1.26 ± 0.10	1.26 ± 0.11	0.002 ± 0.06	1.23 ± 0.11	1.15 ± 0.07	−0.08 ± 0.08[b]
Vitamin B6 (A.C.)*	1.77 ± 0.16	1.76 ± 0.19	−0.002 ± 0.14	1.73 ± 0.23	1.62 ± 0.18	−0.11 ± 0.12[b]
Pyridoxal Phosphate (nmol/L)	42.6 ± 18.3	43.5 ± 18.4	0.9 ± 12.6	55.1 ± 42.1	75.2 ± 39.9	20.1 ± 40.8[c]
Vitamin B12 pmol/L)	232 ± 115	225 ± 112	−7.5 ± 29.9	286 ± 83	326 ± 89	39.5 ± 41.0[b]
Folate (nmol/L)	23 ± 6	24 ± 6	0.4 ± 5.2	23 ± 8	33 ± 8	9.7 ± 7.1[b]
Vitamin C (μmol/L)	55 ± 19	55 ± 18	0.1 ± 13.5	55 ± 20	71 ± 16	15.7 ± 17.9[b]

[a] $\bar{x} \pm SD$

[b] $p<0.01$, differences: supplement *vs.* placebo

[c] $p<0.001$, differences: supplement *vs.* placebo

* Activity coefficient

Table 5. Baseline dietary intake (average daily values)[a]

Nutrient	Placebo Group	Supplemented Group
Energy (kcal)	2098 ± 888	2059 ± 925
Protein (g)	91 ± 38	85 ± 34
Carbohydrate (g)	276 ± 131	280 ± 144
Fat (g)	38 ± 19	31 ± 14
Vitamin A (IU)	11,918 ± 6316	12,356 ± 7987
Vitamin D (IU)	300 ± 196	294 ± 136
Vitamin E (IU)	10.1 ± 5.2	10.9 ± 7.9
Thiamin (mg)	1.57 ± 0.63	1.65 ± 0.77
Riboflavin (mg)	1.92 ± 0.86	1.99 ± 0.82
Vitamin B6 (mg)	2.38 ± 0.96	2.53 ± 1.23
Folate (μg)[b]	342 ± 143	392 ± 212
Vitamin B12 (μg)	7.21 ± 4.90	6.14 ± 2.77
Vitamin C (mg)	168 ± 91	179 ± 102

[a] No significant between group differences were detected, $\bar{x} \pm SD$

[b] Not adjusted for folate in fortified foods

DISCUSSION

Among a group of apparently healthy, free-living older adults consuming an adequate and fortified diet, eight weeks of daily supplementation with a multivitamin/mineral preparation formulated at about 100% DV for most vitamins significantly improved vitamin status, except for vitamin A and thiamin, but had no effect on measures of antioxidant defenses and cytokine production. As the dietary intake of vitamins did not change over the course of the intervention, the change in vitamin status can be attributed to the supplement. Baseline plasma vitamin concentrations were comparable to those found in other multivitamin intervention studies conducted in independently living older adults [17–19] and higher than levels found in institutionalized populations [15]. Nutrient status changes after two months of supplementation were of the same magnitude as those found in other studies of non-institutionalized elders with similarly formulated multivitamin preparations [17, 18].

Chavance *et al.* [18] examined older adults in France given either multivitamin or placebo for four months. After two months plasma folate increased 33 nmol/L compared to the 10 nmol/L change observed here; however, baseline concentrations were lower in the French subjects (16 *vs.* 23 nmol/L) who were not consuming fortified foods. Baseline vitamin C status was lower in the French group (30 *vs.* 55 μmol/L), and the change in vitamin C status after two months was also lower (12 *vs.* 16 μmol/L); however, their formulation contained half the amount of vitamin C (120 mg). The plasma α-tocopherol concentrations in the French cohort were slightly higher at baseline (32 *vs.* 27 μmol/L); however, their change in status after two months was similar to ours (+6 *vs.* +5 μmol/L). After four months only plasma vitamin C and folate continued to rise after the first two-month interval in the French subjects. All other plasma micronutrients remained close to their concentrations determined at two months.

Mann *et al.* [17] examined older American adults administered either multivitamin or placebo for four months. After two months no significant changes were noted in the status of vitamins A and E. Their baseline α-tocopherol levels were higher (26–31 μmol/L) than observed in this study and improved after four months with 30 IU to 33–34 μmol/L. Although the supplement used by Mann *et al.* [17] contained more vitamin C (300 mg), the mean plasma vitamin C concentration was lower at two months, but the same by four months, as achieved with this protocol.

In addition to reducing the prevalence of poor nutrient status, the multivitamin appears to have increased several vitamins into a more optimal range relevant to reducing the risk

Table 6. Effects of treatment on antioxidant capacity

Measure	Placebo Group		Supplemented Group	
	Before	After	Before	After
Glutathione peroxidase:				
Plasma (U/L)	243 ± 40	235 ± 40	246 ± 45	246 ± 43
RBC (U/g HGB)	20.2 ± 6.7	20.3 ± 6.9	21.3 ± 5.7	21.5 ± 5.7
ORAC (μmol Trolox equivalent)	750 ± 264	740 ± 232	766 ± 190	762 ± 196

Table 7. Effects of treatment on cytokine production

Measure	Placebo Group			Supplemented Group		
	Before	After	N	Before	After	N
IL-2 receptor (pg/mL)	1049 ± 571	1002 ± 530	38	963 ± 371	962 ± 334	38
IL-6 (pg/mL)	2.0 ± 1.4	2.4 ± 2.2	37	2.4 ± 2.3	2.5 ± 2.3	36
IL-10 stimulated (pg/mL)	851 ± 721	806 ± 601	18	1032 ± 878	970 ± 661	21
PGE_2 stimulated (pg/mL)	3806 ± 4420	2923 ± 2804	28	2701 ± 2303	2806 ± 3246	27

of chronic disease. Gey *et al.* [29] defined the 'optimal' plasma concentrations for vitamins A, C, and E through cross-cultural studies examining plasma nutrient levels and risk for cardiovascular diseases and cancer. Values 20% to 50% lower than the target thresholds for either vitamin C (>50 μmol/L), E (>30 μmol/L) or retinol (>2.5 μmol/L) were found to approximately double the relative risk of CVD and cancer, respectively [34, 35]. Although the mean plasma vitamin A concentration in the present study is considered less than optimal, Garry *et al.* [36] suggests that years of regular supplement use are required to cross that threshold. The mean plasma vitamin C concentration after multivitamin supplementation increased within the boundaries of the third quintile of plasma vitamin C described in the Sayhoun *et al.* survey of 747 older Americans [37]. Their third quintile was associated with a 0.51 relative risk of heart disease and 0.64 relative risk of overall mortality versus those in the lowest quintile. Elevating the mean vitamin E concentration to >28 μmol/L shifted subjects from the fourth to fifth quintiles of plasma vitamin E, as described by Riemersma *et al.* [38] for a population of 504, which was associated with a 64% reduced risk of angina pectoris. Similarly, this shift was sufficient to place our subjects in the group at lowest risk of CHD mortality as determined in the Vitamin Substudy of the WHO/MONICA project [34].

Improving 25-hydroxyvitamin D (25(OH)D) concentrations above the level associated with subclinical deficiency (<37.5 nmol/L) may reduce the risk of developing skeletal fractures due to secondary hyperparathyroidism, lower serum calcium and phosphate levels, higher serum alkaline phosphatase and osteoporosis [39–41]. However, parathyroid hormone (PTH) levels become minimal when 25(OH)D concentrations exceed 100 nmol/L [42, 43]. In studies showing osteoporosis fracture prevention with vitamin D and calcium supplementation, mean 25(OH)D concentrations exceeded 100 nmol/L [42, 44, 45]. Multivitamin supplementation was able to elevate the mean plasma 25(OH)D concentration to the 100 nmol/L level.

Improving the status of folate, vitamins B6 and B12 is effective in reducing plasma homocysteine [46–53], which, in turn, is associated with a reduced risk for vascular disease [54, 55]. The multivitamin intervention increased plasma levels of these B-vitamins sufficiently to have a significant effect in lowering total plasma homocysteine concentrations [56]; however, low plasma levels of folate, B6 and B12 are also associated with an increased risk for heart disease independent of plasma homocysteine concentration [57, 58]. Multivitamin supplementation reduced the prevalence of suboptimal plasma vitamin B12 (>258 pmol/L, $p=0.004$), but did not shift the mean to the lowest risk quartile (i.e., >335 pmol/L) for coronary atherosclerosis as described by Siri *et al.* [57]. Eliminating suboptimal plasma pyridoxal phosphate concentrations may have an impact on the risk for atherosclerosis considering the report by Robinson *et al.* [58] of a 76% increased risk for vascular disease at suboptimal PLP levels.

Supplemental vitamin E treatment has been reported to enhance cell-mediated and humoral immune responses [59]. Chandra *et al.* [20] found that after one year of multivitamin supplementation, older adults had significantly enhanced *in vitro* lymphocyte proliferative responses to mitogens, interleukin 2 (IL-2) production and IL-2 receptor release, natural killer cell activity, antibody responses to influenza vaccine, and a lower incidence of infectious diseases. Immunological responses were greatest among the subjects with the lowest nutrient status at baseline. In contrast to our subjects, this cohort presented with a marked prevalence of deficiencies, e.g., in vitamin C (23%), vitamin A (13%), β-carotene (17%), and vitamin E (8%). Bogden *et al.* [19] determined delayed hypersensitivity skin test responses after one year of multivitamin supplementation in older adults and found no change at six months, but significant increases after 12 months. After four months of multivitamin supplementation, Chavance *et al.* [18] observed no significant difference in the incidence of reported infectious episodes between supplement and placebo groups.

Direct comparisons are not possible between these studies because of the different outcome parameters and duration of treatment. However, it is noteworthy that multivitamin interventions shorter than six months appear not to impact immune function.

Chao *et al.* [60] observed no change in total ORAC in young men assigned to one of four antioxidant treatments (2000 RE vitamin A as β-carotene, 500 mg vitamin C, 440 mg α-TE vitamin E, or all combined with 100 μg selenium and 30 mg zinc) or placebo, and subjected to strenuous activity for 28 days despite increases in measures of oxidative stress (breath pentane, serum blood lipid peroxide, urine malondialdehyde and 8-hydroxydeoxyguanosine) in all groups. In contrast, Cao *et al.* found that non-protein plasma ORAC increased in older women one to two hours after consuming 1250 mg vitamin C [61]. Within a biological system, the total ORAC assay measures the total antioxidant capacity of all known nonenzymatic water- and lipid-soluble antioxidants, including β-carotene, glutathione, methionine, uric acid, bilirubin, phenolic acids, flavanols, flavonols, flavones, isoflavones, flavanones, anthocyanins, in addition to vitamin C and α-tocopherol [28, 62, 63]. Plasma proteins and lipoproteins account for about 85% to 90% of the overall peroxyl-radical trapping capacity, while one half of the non-protein ORAC value in humans is attributed to uric acid [64]. The change in plasma antioxidant vitamins in this study is either insufficient to affect ORAC due to their low contribution to the non-protein plasma oxidant capacity in fasting plasma, or uric acid status may have masked any changes in ORAC at the antioxidant concentrations achieved here. The activity of glutathione peroxidase, another component of antioxidant defense mechanisms, was unaffected by the multivitamin. This response might not be unexpected as the subjects were not selenium deficient, and the supplement contained only 20 μg of selenium. Similarly, Meydani *et al.* found no change in glutathione peroxidase activity with vitamin E supplementation [65].

CONCLUSION

Supplementation with a multivitamin supplement can improve micronutrient status in healthy, older Americans to levels above those obtained with a fortified diet. This increase in nutritional status reduces the prevalence of suboptimal plasma vitamin concentrations and will shift blood levels of key nutrients into ranges associated with reduced risk for several chronic diseases.

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