

Accelerated Article

The Effects of Dietary Selenium on the Immune System in Healthy Men

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ABSTRACT

Eleven men were fed foods naturally high or low in selenium for 120 d. Selenium intake was stabilized at 47 $\mu\text{g}/\text{d}$ for 21 d, then changed to either 13 or 297 $\mu\text{g}/\text{d}$ for 99 d, leading to significantly different blood selenium and glutathione peroxidase concentrations. Serum immunoglobulins, complement components, and primary antibody responses to influenza vaccine were unchanged. Antibody titers against diphtheria vaccine were 2.5-fold greater after reinoculation in the high selenium group. White blood cell counts decreased in the high-selenium group and increased in the low-selenium group, resulting primarily from changes in granulocytes. Apparent increases in cytotoxic T-lymphocytes and activated T-cells in the high-selenium group only approached statistical significance. Lymphocyte counts increased on d 45 in the high-selenium group. In vitro proliferation of peripheral lymphocytes in autologous serum in response to poke-weed mitogen was stimulated in the high-selenium group by d 45 and remained elevated throughout the study, whereas proliferation in the low selenium group did not increase until d 100. This study indicates that the immune-enhancing properties of selenium in humans are the result, at least in part, of improved activation and proliferation of B-lymphocytes and perhaps enhanced T-cell function.

Index Entries: Selenium; secondary immune response; leukocytes; lymphocytes; white blood cells; granulocytes; blastogenesis; antibody titers; mitogens.

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INTRODUCTION

The importance of selenium to optimal functioning of the immune system has been well established. Selenium supplementation in experimental animals has been associated with increases in natural-killer (NK) cell activity, cytotoxic T-cell activity; interleukin-2 (IL-2) receptor expression, T-cell proliferation, lymphokine-activated killer cell activity, delayed-type hypersensitivity (DHS) skin response, and vaccine-induced immunity to malaria (1). In vitro studies of selenium supplementation have observed decreased NF- κ B activation, decreased expression of IL-6, IL-8, and tumor necrosis factor (TNF) mRNA following ultraviolet (UV) treatment, increased apoptosis in tumor cells, increased phytohemagglutinin response in lymphocytes, increased killing by macrophages, and increased target killing by cytotoxic T-cells (1). Selenium deficiency in animals has been associated with decreased antibody production by lymphocytes, increased virulence of Coxsackie virus, decreased neutrophil chemotaxis and candidacidal activity, increased CD4⁺ T-cells, and decreased CD8⁺ and CD4⁻/CD8⁻ thymocytes (1).

The evidence that dietary selenium plays a significant role in human immune function is more sparse. Supplementation with selenium (usually as sodium selenite) has been reported to increase proliferation of peripheral blood lymphocytes in response to pokeweed mitogen (2) and phytohemagglutinin (PHA) (3); increase the expression of high-affinity IL-2R (3); decrease the incidence of hepatitis B virus infection and primary liver cancer (4); and increase cytotoxic lymphocyte-mediated tumor cytotoxicity and NK cell activity (5). Selenium deficiency in humans has been associated with dilated cardiomyopathy (6), osteoarthropathy (7), skeletal myopathy (8), and (in iodine-deficient populations) cretinism (9). Decreased tissue selenium levels have been observed in male infertility (10), pancreatitis (11), Behcet's disease (an inflammatory disorder of unknown etiology) (12), rheumatoid arthritis (13), and in the heart (14) and serum (15) of AIDS patients. Low serum selenium has been associated with increased mortality in HIV-positive drug-users (RR = 19.9, $p < 0.0001$) (16) and HIV-infected children (RR = 5.96, $p = 0.02$) (17).

At the molecular level, selenium is an essential component of several enzymes important to optimal immune function. Selenoenzymes such as glutathione peroxidase and thioredoxin reductase are effective antioxidants that help protect immune cells from oxidative damage from reactive oxygen species released to kill engulfed bacteria (18). Thioredoxin reductase is also the source of reduced thioredoxin, an essential cofactor for signal transduction pathways involved in immune cell activation and differentiation (19), and mediates the cell death effects of the combination of β -interferon and retinoic acid via the JAK-STAT pathway (20). Sequence analysis of the HIV genome has suggested that the virus may code for several viral selenoproteins (21), including a selenium-dependent glutathione peroxidase (22). A similar analysis of the human

T-lymphocyte genome suggests that selenoproteins may be encoded in the +1 reading frame overlapping the human CD4, CD8, and HLA-DR genes (23). Many more selenoproteins of unknown functions have been observed in animals (24) that have not been confirmed in humans, suggesting the possibility that selenium may affect the immune function by mechanisms not yet anticipated.

We fed 11 men a controlled diet of conventional foods with naturally high or low selenium contents for 120 d while confined in a metabolic research unit to identify the effects in humans of dietary selenium as it occurs naturally in foods. In this report, we present results describing the effects of these diets on immune status, functions, and responses.

SUBJECTS AND METHODS

Subjects.

Twelve healthy male volunteers were recruited for this study from a pool of 148 candidates who passed an initial telephone screening. Exclusion criteria were the following: weight for height greater than 125% of ideal (25); use of selenium supplements or selenium-containing shampoos; abnormal electrocardiogram, blood cell counts, clinical chemistries or semen analysis; HIV infection; use of illegal drugs; habitual use of tobacco or alcohol; chronic use of medications; history of psychiatric illness; and history of thyroid or heart disease, syphilis, hepatitis, diabetes, hypertension, or hyperlipidemia. One subject in the high selenium group withdrew from the study after 60 d for personal reasons unrelated to the study, and his data are not included. The baseline characteristics of the 11 subjects who completed the study are shown in Table 1. There were no significant differences between the groups with respect to any of these characteristics.

The subjects were confined in a metabolic research unit for 120 d under 24 h supervision by staff members. Subjects participated in two required 2-mile walks per day and were always escorted by staff members when out of the metabolic research unit. No other forms of exercise were permitted. The study protocol was approved by the Human Subjects Review Committees of the University of California at Davis and the US Department of Agriculture. The protocol was reviewed with the study volunteers and their informed consent was obtained in writing prior to the study, in accordance with the Common Federal Policy for Protection of Human Research Subjects.

Experimental Diets and Treatments.

Subjects were fed a diet composed of conventional foods, based on beef and rice as staples, with nonfat milk powder as a protein supplement. To increase the intake of micronutrients, one multivitamin, multimineral

Table 1
Baseline Characteristics of Subjects Eating the Low-Selenium
and High-Selenium Diets

	Low selenium group (n = 6)	range	High selenium group (n = 5)	range
	mean \pm SD		mean \pm SD	
Age (y)	31 \pm 9	26 – 45	35 \pm 7	20 – 44
Height (cm)	181.2 \pm 4.2	174 – 185	178.1 \pm 5.8	170 – 184
Weight (kg)	74.9 \pm 9.8	66 – 90	73.5 \pm 12.6	60 – 94
Energy intake (MJ/d)	11.8 \pm 1.4	10.9 – 14.6	10.9 \pm 1.0	10.0 – 14.0
BMI (kg/m ²)*	22.8 \pm 3.3	19 – 27	23.3 \pm 4.4	18 – 29
Body fat (kg)	12.1 \pm 4.9	6.2 – 21	13.8 \pm 11.7	2.8 – 31
Plasma Se (μ mol/L)	1.49 \pm 0.10	1.33 – 1.62	1.34 \pm 0.24	1.15 – 1.67
Plasma GPx (U/mg)‡	2.0 \pm 0.4	1.3 – 2.5	1.8 \pm 0.3	1.6 – 2.2
Serum T ₃ , nmol/L	1.57 \pm 0.25	1.1 – 1.8	1.82 \pm 0.36	1.5 – 2.3
Serum TSH, mU/L	1.69 \pm 0.30	1.2 – 2.1	2.25 \pm 0.81	1.5 – 2.6

*BMI-body mass index (weight/height²).

‡Glutathione peroxidase specific activity (enzyme units per milligram protein).

supplement tablet, free of selenium (Unicap M, Upjohn Co., Kalamazoo, MI), was administered to each subject each day. The total diet (food plus supplements) contained at least 100% of the recommended dietary allowance (RDA) (26) for all nutrients except magnesium (56%), calcium (72%), and selenium (27) (Table 2). The diet was fed in three daily meals and an evening snack, in a repeating cycle of eight daily menus, using the same quantities of rice, beef, and powdered milk every day. Foods for each meal were individually weighed to the nearest gram. All meals were consumed completely under the direct observation of staff members. Plates were cleaned with rubber spatulas, cups and glasses were rinsed with distilled water, and the residues were consumed.

For the first 21 d, all subjects were fed a diet that provided 47 μ g/d of selenium at the average energy intake of 11.7 MJ/d to adapt the subjects to the experimental diet and stabilize their body weights. The initial energy requirement for each subject was estimated from the Harris-Benedict equation, and the energy intake of each subject was subsequently adjusted as needed to compensate for any changes in body weight. When energy intakes were changed, all components of the diet were adjusted proportionally such that the relative composition of the diet did not change.

On d 22, after blocking into six pairs matched for blood selenium concentrations, the subjects were randomized to either the low-selenium diet (13 μ g/d at 11.7 MJ/d) or the high-selenium diet (297 μ g/d at 11.7 MJ/d) for the remaining 99 d. The only difference between the experimental

Table 2
Diet Composition

	Daily intake (per 11.7 MJ)	RDA
Protein	68.5 g (10.6% of energy)	63 g
Carbohydrate	357 g (55% of energy)	n.a.
Fat	99.2 g (34.4% of energy)	n.a.
saturated fat*	32.0 g	n.a.
monounsaturated fat*	35.7 g	n.a.
polyunsaturated fat*	25.8 g	n.a.
Fiber*	6.1 g	n.a.
Cholesterol*	253 mg	n.a.
Selenium (stabilization diet)	47 µg	55 µg
Selenium (low selenium diet)	13 µg	55 µg
Selenium (high selenium diet)	297 µg	55 µg
Iodine*	280 µg	150 µg
Calcium	572 mg	800 mg
Iron	28.3 mg	10 mg
Magnesium	195 mg	350 mg
Phosphorus	1013 mg	800 mg
Zinc	28.4 mg	15 mg
Copper	2.93 mg	1.5–3 mg‡
Manganese	3.68 mg	2–5 mg‡
Potassium	2645 mg	1875–5625 mg‡

Note: Unless otherwise indicated, values are from analyses of composites of foods from each experimental diet. Contributions from the daily multivitamin, multimineral supplement are included.

*Dietary component estimated from food composition tables (28).

‡Estimated Safe and Adequate Daily Dietary Intake (29).

diets was the geographic origin of the rice and beef staples, which were obtained from regions with either very high or very low soil selenium; all other components of the three diets were identical. Subjects and the analysts were blinded to which subjects were eating which diets.

A metabolic tracer experiment was conducted beginning on d 110, using a stable isotope of selenium. On this day only, all subjects were fed the low selenium diet and were administered an oral dose of $\text{Na}_2^{74}\text{SeO}_3$ (10 µg selenium for the low-selenium group or 300 µg selenium for the high-selenium group) with the morning meal. Because the amounts and chemical form of selenium given to the subjects on d 110 were different from the selenium in the foods, measurements made after d 110 (DHS skin responses and serum antibody responses to rechallenges with diptheria and tetanus vaccines) may have been affected by the stable isotope administration.

Laboratory Measurements

Blood samples were collected between 0700 and 0800, after an overnight fast of 12 h into evacuated tubes containing heparin (in vitro proliferation assays), or EDTA (blood cell counting and lymphocyte phenotyping), or without anticoagulants (serum). Complete blood counts, lymphocyte phenotypes, serum immunoglobulins, and complement fractions were determined as previously reported (30). After centrifugation, erythrocyte, serum, and plasma samples were immediately frozen and stored at -70°C until analyzed. Selenium was measured by fluorescence-derivatization high-performance liquid chromatography (HPLC) (31). Selenium-dependent glutathione peroxidase activity and total protein were determined by automated colorimetric methods (32,33).

Isolation and Culture of PBMNCs

Peripheral blood mononuclear cells (PBMNC) were isolated by using Histopaque-1077 (Sigma Chemical Co, St. Louis, Mo) and maintained in culture as previously reported (34). The culture medium used was RPMI-1640 (Gibco, Grand Island, NY) with L-glutamine (2 mmol/L), fetal bovine serum (100 mL/L), penicillin (100 kU/L), streptomycin (100 mg/L), and gentamicin (20 mg/L). One hundred microliters of the culture medium containing 1×10^5 PBMNCs was seeded in each well of a 96-well culture plate. An additional 100 μL of culture medium with or without the mitogens was added to each well. The T-cell mitogens used in this study were phytohemagglutinin (PHA) and Concanavalin A (Con A) and the B-cell mitogen was pokeweed. Pokeweed, PHA, and Con A were purchased from Sigma Chemical Co. Each mitogen was used at two concentrations; the concentrations (mg/L) of the mitogens were PHA 5 and 10, Con A 10 and 20, and pokeweed 1.0 and 2.0. PBMNCs were cultured for 72 h; [^3H]thymidine, 37 kBq in 50 μL , was added to each well during the last 6 h. Thymidine incorporation into cellular DNA (1 Bq/1000 cell) was used as the index of PBMNC proliferation.

Determination of NK Cell Activity

Natural-killer cell activity was determined using the nonadherent PBMNC and the ^{51}Cr -labeled K-562 cells at effector: target cell ratios of 100: 1, 50: 1, 25: 1, 12.5: 1, 6.2: 1, and 3.1: 1 as previously described (35). Six wells were used for each effector cell concentration and for the spontaneous and maximal release (caused by 3% centrime) of ^{51}Cr . After 4 h incubation at 37°C in 5% CO_2 , the plates were centrifuged and aliquots of supernatant collected to determine the ^{51}Cr released. Percent lysis was calculated as

$$\% \text{Lysis} = \frac{(\text{Experimental CPM} - \text{Spontaneous CPM})}{(\text{Maximum CPM} - \text{Spontaneous CPM})} \times 100$$

DHS Skin Responses

During the baseline period (d 8–11) and at the end of the study (d 113–116), DHS skin response to seven recall antigens was assayed by intradermally injecting 0.1 mL of each antigen solution into the forearm. The antigens used were tuberculin purified-protein derivative (one or five international test units), mumps (four complement-fixing test units), tetanus toxoid (1 : 100, [v/v] dilution of a solution containing 4 flocculation units/0.5 mL), candida (1 : 100 [v/v] dilution), trichophyton (1 : 30 [v/v] dilution), streptokinase streptase (100 and 200 kU/L), and coccidioidin (bioequivalent to US reference coccidioidin 1 : 100; provided by the Office of Biologics, Food and Drug Administration). The antigens were diluted with a diluent containing, per liter, 3 mL normal human serum and 9 g sodium chloride. Tuberculin purified-protein derivative, mumps, and tetanus toxoid were supplied by Connaught Laboratories Inc. (Swiftwater, PA). Candida (*Dermatophyton* 0), trichophyton, and the antigen diluent were obtained from Hollister Stier (Spokane, WA). Streptokinase streptase and coccidioidin were purchased from Behringwerke Ag (Marburg/Lahn, Germany), and Berkeley Biologicals (Berkeley, CA), respectively. The response to these antigens was determined by measuring mean induration diameters (mm) at 48 ± 2 h, and again at 72 ± 2 h, after injections. Data are reported as the sum of induration diameters for positive responses (induration score) and the number of positive responses to the seven antigens (antigen score).

Humoral Immune Responses

A diphtheria–tetanus vaccine was administered to all subjects on d 5 and again on d 102 to assess the effect of selenium on the secondary immune response to previously administered antigens. A multivalent influenza vaccine was also administered on d 102 to assess selenium's effect on the primary immune response to a novel antigen. Specific antibody titers were measured immediately before and 7 d and 14 d after inoculation with the vaccines, using the hemagglutination inhibition assay (36).

Statistical Analysis

For measurements repeated more than twice, the baseline value was subtracted from the value at each time-point to calculate within-subject changes, and repeated measures analysis of variance was used to test for significant effects of dietary selenium and time. When the selenium main effect or the selenium \times time interaction was significant, the Student–Newman–Keuls multiple comparison test was used to identify significant differences between the groups at individual time-points. For measurements obtained only twice (during baseline and at the end of the study), within-subject changes were compared between groups with a two-tailed

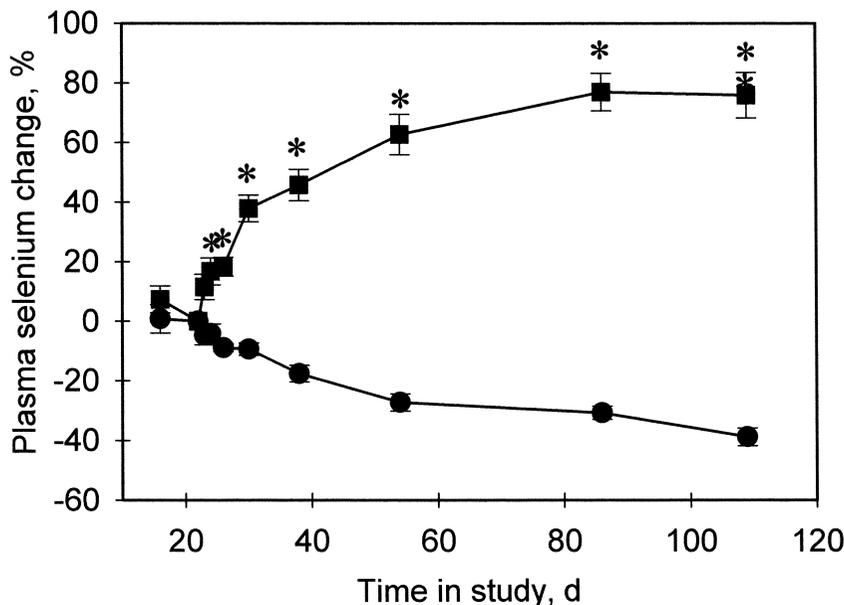


Fig. 1. Changes in blood plasma selenium. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

t-test. Measurements obtained only at the end of the study were compared between groups with a two-tailed *t*-test without any correction. Statistical tests were performed with SigmaStat software (SPSS, Chicago, IL). A probability of 0.05 or less was considered significant.

RESULTS

The high- and low-selenium diets caused significant changes in circulating selenium concentrations, which increased by 77% and decreased by 39% in plasma (Fig. 1) and increased by 70% and decreased by 27% in erythrocytes (Fig. 2). The final blood selenium concentrations were $116 \pm 11 \mu\text{g/L}$ and $278 \pm 21 \mu\text{g/L}$ in erythrocytes and $72.4 \pm 9.5 \mu\text{g/L}$ and $187 \pm 23 \mu\text{g/L}$ in plasma for the low-selenium and high-selenium groups, respectively. These changes in blood selenium concentrations were accompanied by corresponding but smaller changes in glutathione peroxidase activities (data not shown). Selenium and glutathione peroxidase were not measured in white blood cells.

Serum immunoglobulins were largely unaffected by the experimental diets, although IgM declined by about 10% in both groups (Table 3). Complement fraction C4 declined slightly in both groups, but C3 was unaffected in either group (Table 3). These parameters remained within

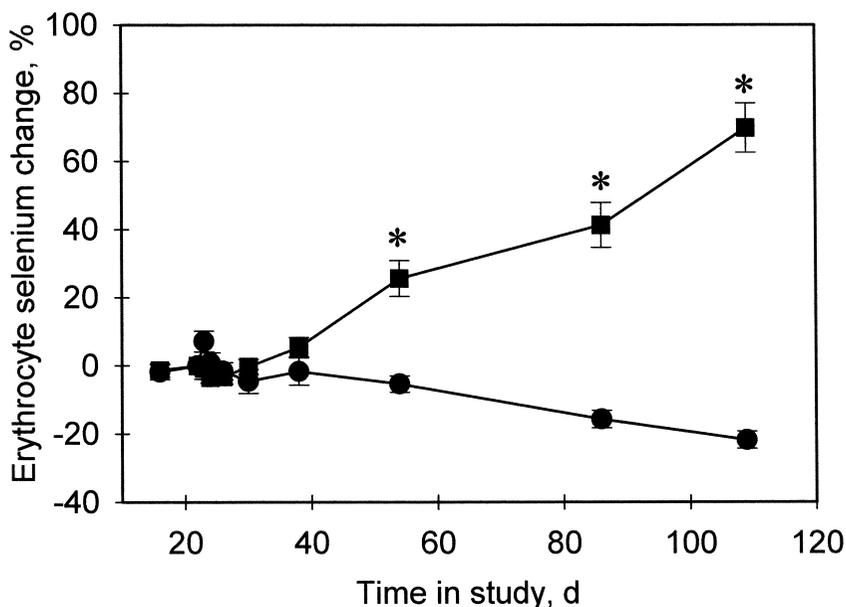


Fig. 2. Changes in erythrocyte selenium. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

clinically normal ranges for healthy adults throughout the study. The primary immune response of specific serum antibodies to an initial challenge with influenza vaccine at the end of the study was not different between groups (Table 3). However, dietary selenium did seem to boost the secondary immune response to diphtheria vaccine when rechallenged at the end of the study. The increased diphtheria antibody response from selenium was not quite significant in the repeated measures analysis of variance of the raw data ($p = 0.08$), but could be seen clearly when the data were expressed as ratios. The mean within-subject ratio of specific antibody titers 14 d after reinoculation (d 116) to titers 14 d after the initial challenge at baseline (d 19) was significantly greater ($p=0.031$, t -test of log-transformed data) in the high-selenium group (2.7 ± 1.8 -fold vs 0.9 ± 0.6 -fold). The titers of tetanus-specific antibodies exceeded the range of the assay in most samples, so no differences could be detected.

The mean white blood cell count decreased by 5% in the high-selenium group and increased by 10% in the low-selenium group (Table 4 and Fig. 3). Lymphocyte counts increased transiently in the high-selenium group, with a maximum 17% increase at d 45 (Fig. 4), but both groups ended with similar, slight overall increases in lymphocytes (Table 3). Granulocytes accounted for most of the changes in white blood cell counts, decreasing by 9% in the high-selenium group and increasing by 12% in the low-selenium group (Table 4 and Fig. 5). Erythrocyte counts,

Table 3
Effects of Low-Selenium and High-Selenium Diets on Humoral Immune System

	Low selenium group (n = 6)		High selenium group (n = 5)		Statistical analysis*		
	Baseline value† (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se × Time (p)
IgA, mg/dL	260 ± 131	260 ± 126	217 ± 52	204 ± 40	—	—	—
IgG, mg/dL	1086 ± 125	1144 ± 249	1025 ± 243	962 ± 174	—	—	—
IgM, mg/dL	132 ± 47	123 ± 49	101 ± 52	89 ± 38	—	0.009	—
C3, mg/dL	112 ± 15	112 ± 14	107 ± 17	109 ± 23	—	—	—
C4, mg/dL	23.8 ± 5.2	20.5 ± 5.4	20.7 ± 2.7	18.7 ± 3.0	—	<0.001	—
Pre-inoculation influenza A titre	n.a.	640 ± 313	n.a.	461 ± 115	—	n.a.	n.a.
Post-inoculation influenza A titre	n.a.	1960 ± 1230	n.a.	2250 ± 1800	—	n.a.	n.a.
Pre-inoculation influenza B titre	n.a.	1200 ± 114	n.a.	1850 ± 1480	—	n.a.	n.a.
Post-inoculation influenza B titre	n.a.	4430 ± 3010	n.a.	3380 ± 2980	—	n.a.	n.a.
Pre-inoculation diphtheria titre	1550 ± 2000	14,700 ± 20,300	2100 ± 1800	12,400 ± 16,400	—	—	—
Post-inoculation diphtheria titre	14,100 ± 14,600	16,600 ± 18,900	15,400 ± 14,500	23,600 ± 16,800	—†	—	0.08†

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

†Average value during 21-d baseline period.

‡The secondary immune response to diphtheria vaccine (mean within-subject fold change in titers from d 19 to d 116) was significantly greater in the high selenium group (2.7-fold vs 0.9-fold, $p = 0.031$, t -test of log-transformed ratios).

Table 4
Effects of Low-Selenium and High-Selenium Diets on Complete Blood Cell Counts and Blood Chemistry

	Low selenium group (n = 6)			High selenium group (n = 5)			Statistical analysis*		
	Baseline value† (mean ± SD)	Final value (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se × Time (p)	
White blood cells, thou/cu mm	4.1 ± 0.75	4.5 ± 0.76	5.8 ± 1.4	6.1 ± 1.3	5.8 ± 1.4	—	0.017	0.021	
Lymphocytes, thou/cu mm	.66 ± 0.30	1.78 ± 0.16	2.14 ± 0.53	2.04 ± 0.37	2.14 ± 0.53	—	0.019	0.007	
Granulocytes, thou/cu mm	2.08 ± 0.64	2.3 ± 0.71	3.3 ± 1.05	3.61 ± 0.97	3.3 ± 1.05	—	—	0.001	
Platelets, thou/cu mm	253 ± 48	245 ± 52	274 ± 69	281 ± 76	274 ± 69	—	—	—	
Erythrocytes, million/cu mm	5.1 ± 0.52	4.9 ± 0.45	4.7 ± 0.68	4.9 ± 0.62	4.7 ± 0.68	—	—	—	
Hemoglobin, g/dL	14.9 ± 1.3	14.2 ± 1.00	13.8 ± 1.07	14.2 ± 0.59	13.8 ± 1.07	—	<0.001	—	
Hematocrit, %	44.5 ± 4.0	41.8 ± 3.3	40.7 ± 2.9	42.2 ± 1.8	40.7 ± 2.9	—	<0.001	—	

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

†Average value during 21-d baseline period.

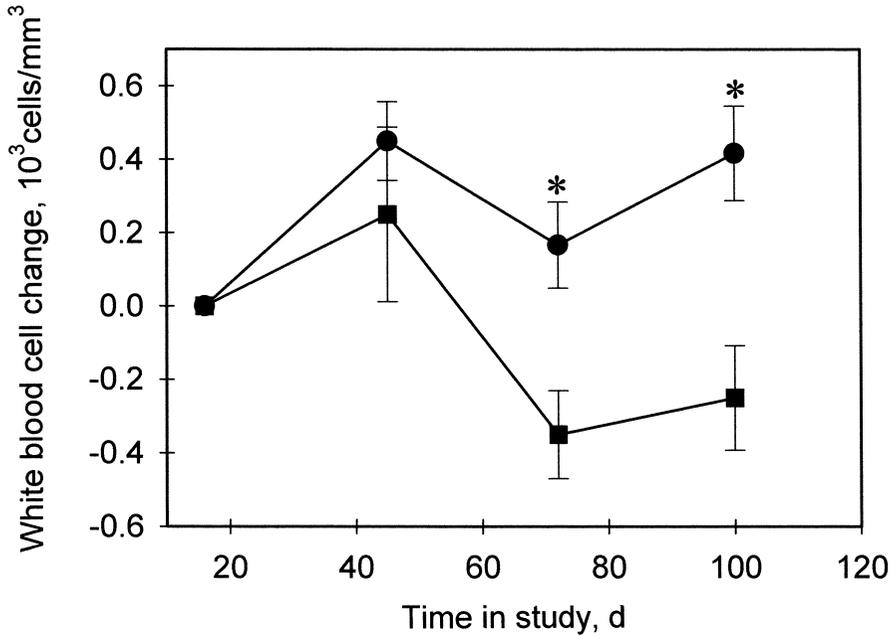


Fig. 3. Changes in white blood cell count. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

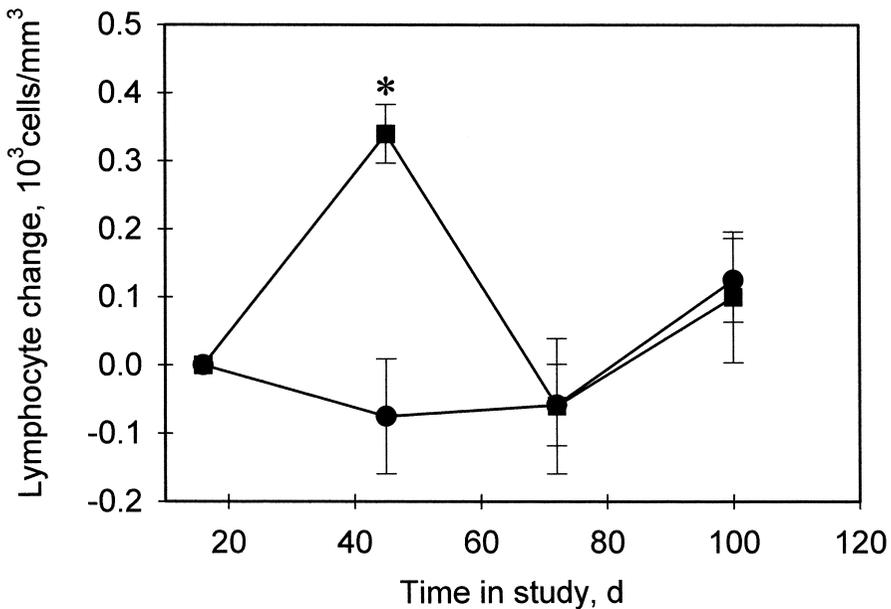


Fig. 4. Changes in lymphocyte count. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

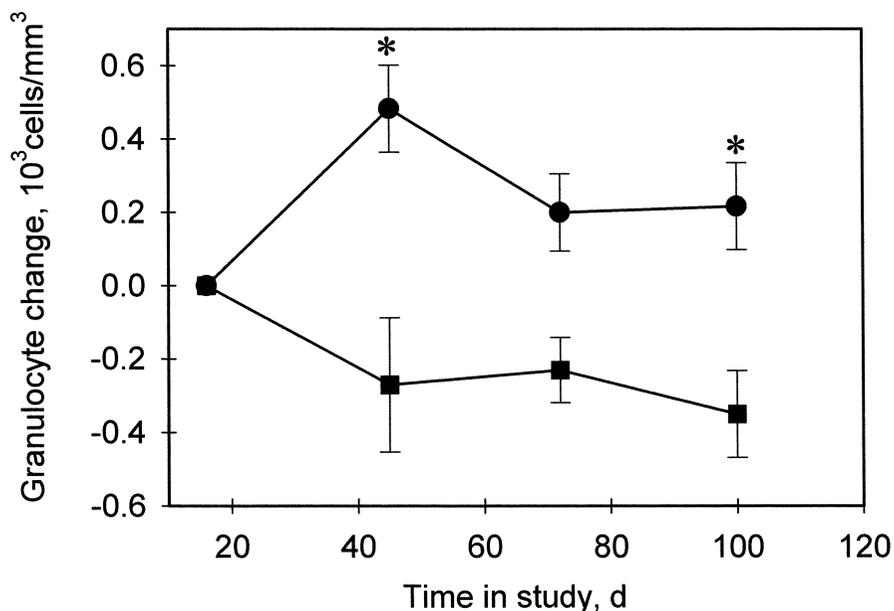


Fig. 5. Changes in granulocyte counts. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

hemoglobin concentrations, and hematocrit dropped slightly in both groups during the study, probably as a result of the repeated blood samplings (approx. 725 mL in 120 d). Lymphocyte phenotypes were of similar abundance in both groups and, for the most part, were not affected by dietary selenium (Table 5). T-Cells carrying the HLA-DR antigen tended to increase by about 20% in the high-selenium group (Fig 6.), but the difference only approached statistical significance ($p=0.088$). Cytotoxic T-lymphocytes seemed to increase in the high-selenium group (Fig. 7), but the trend was not statistically significant ($p=0.10$).

In vitro proliferation of PBMNCs in response to mitogens was not affected greatly by dietary selenium. When cultured in heterologous serum from a donor pool, no effects of selenium were observable (Table 6). However, PBMNCs from the high-selenium group that were cultured in serum from the same subject and stimulated with pokeweed mitogen proliferated more than cells from the low-selenium group at d 45 and 72 (Table 7). By d 100, this difference had disappeared, and both groups ended with similar 50–60% overall increases in proliferation in response to pokeweed mitogen (Figs. 8 and 9). This early increase in proliferation in the high-selenium group was observed with either 1 mg/L or 2 mg/L concentrations of pokeweed mitogen, but only when cultured with autologous serum. PBMNCs cultured with Concanavalin A at 10 or 20 mg/L

Table 5
Effects of Low-Selenium and High-Selenium Diets on Peripheral Blood Lymphocyte Phenotypes

	Low selenium group (n = 6)		High selenium group (n = 5)		Statistical analysis*		
	Baseline value [†] (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se × Time (p)
B (CD19 ⁺), 10 ⁶ /L	222 ± 70	251 ± 60	307 ± 72	294 ± 97	—	—	—
T (CD3 ⁺), 10 ⁶ /L	1177 ± 157	1290 ± 51	1502 ± 295	1582 ± 437	—	—	—
T helper (CD3 ⁺ 4 ⁺), 10 ⁶ /L	715 ± 122	791 ± 54	928 ± 148	950 ± 215	—	—	—
T suppressor (CD3 ⁺ 8 ⁺), 10 /L	415 ± 47	446 ± 58	498 ± 206	593 ± 368	—	0.032	—
NK cells (CD3 ⁻ 16 ⁺ 56 ⁺), 10 ⁶ /L	218 ± 138	196 ± 111	201 ± 71	261 ± 205	—	—	—
Cytotoxic T (CD3 ⁺ 16 ⁺ 56 ⁺), 10 ⁶ /L	14 ± 10	7.8 ± 5.8	40 ± 29	50 ± 42	—	0.004	—
Activated T (HLA-DR ⁺), 10 ⁶ /L	101 ± 46	95 ± 31	262 ± 221	322 ± 322	—	0.080	0.088
NK activity, % lysis [†]	44 ± 14	42 ± 21	45 ± 2.8	53 ± 19	—	—	—

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

[†]Average value during 21-d baseline period.

[‡]Effector : target cell ratio was 50 : 1. No significant changes were observed at the other effector : target cell ratios tested.

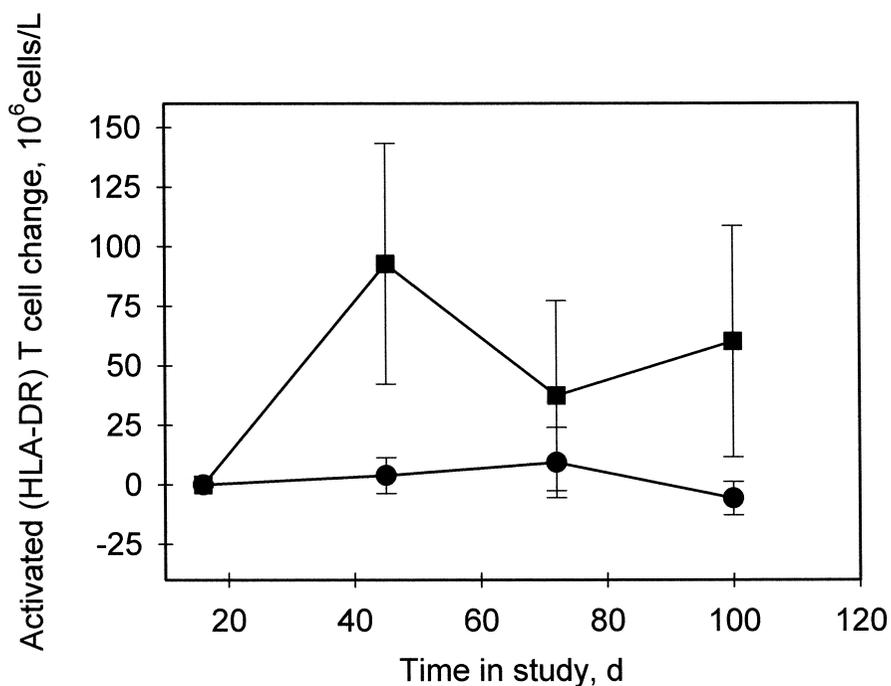


Fig. 6. Changes in HLA-DR antigen on peripheral blood lymphocytes measured by fluorescence-activated cell sorting. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●).

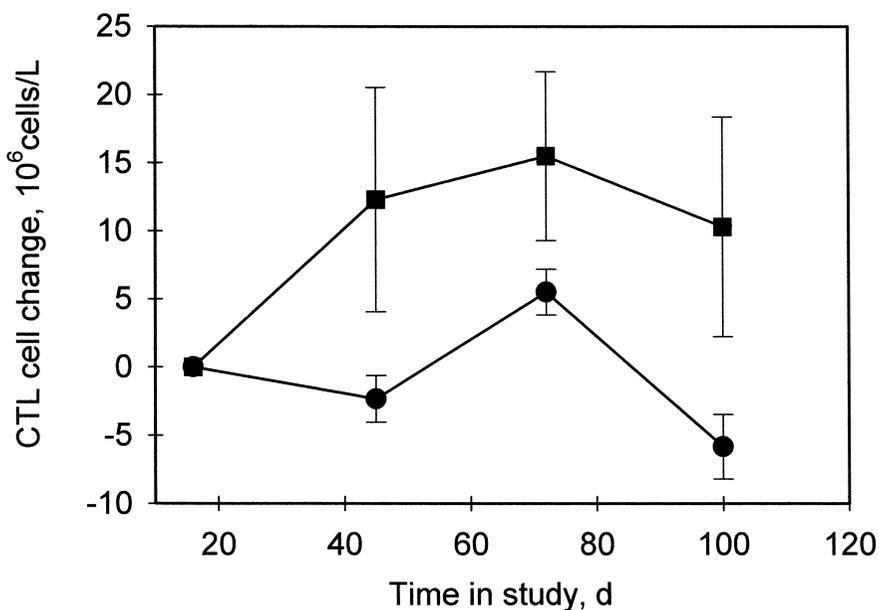


Fig. 7. Changes in cytotoxic T-lymphocytes. $CD3^+,56^+$ cells were measured by fluorescence-activated cell sorting. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●).

Table 6
Effects of Low-Selenium and High-Selenium Diets on Mitogen-Stimulated In Vitro Proliferation of PBMCs with Autologous Serum

	Low selenium group (n = 6)		High selenium group (n = 5)		Statistical analysis*		
	Baseline value† (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se × Time (p)
Phytohaemagglutinin 5, Bq/ 1000 cells	10.0 ± 3.1	11.5 ± 3.3	9.7 ± 2.2	10.8 ± 2.4	—	—	—
Phytohaemagglutinin 10, Bq/ 1000 cells	13.0 ± 2.2	13.5 ± 2.2	12.0 ± 1.8	11.7 ± 1.1	—	—	—
Concanavalin A 10, Bq/ 1000 cells	4.9 ± 1.5	7.0 ± 0.9	4.2 ± 1.9	5.7 ± 1.5	—	<0.001	—
Concanavalin A 20, Bq/ 1000 cells	5.8 ± 1.6	7.9 ± 0.9	4.8 ± 2.0	6.8 ± 1.0	—	<0.001	—
Pokeweed mitogen 1, Bq/ 1000 cells	3.8 ± 1.2	6.2 ± 0.7	3.3 ± 1.4	5.3 ± 1.8	—	<0.001	0.018
Pokeweed mitogen 2, Bq/ 1000 cells	4.5 ± 1.6	6.8 ± 0.8	3.8 ± 1.6	5.8 ± 2.1	—	<0.001	0.003
Control, Bq/ 1000 cells	0.044 ± 0.010	0.037 ± 0.010	0.046 ± 0.011	0.036 ± 0.015	—	<0.001	—

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

†Average value during 21-d baseline period.

Table 7
Effects of Low-Selenium and High-Selenium Diets on Mitogen-Stimulated In Vitro Proliferation of PBMNCs with Heterologous Serum

Parameter	Low selenium group (n = 6)		High selenium group (n = 5)		Statistical analysis*		
	Baseline value† (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se× Time (p)
Phytohaemagglutinin 5, Bq/ 1000 cells	13.3 ± 2.0	15.1 ± 2.2	11.3 ± 1.3	11.4 ± 1.2	—	—	—
Phytohaemagglutinin 10, Bq/ 1000 cells	14.1 ± 2.5	14.5 ± 1.1	13.2 ± 1.5	12.3 ± 0.9	—	—	—
Concanavalin A 10, Bq/ 1000 cells	5.6 ± 1.8	7.7 ± 1.0	6.0 ± 2.3	5.9 ± 2.6	—	—	—
Concanavalin A 20, Bq/ 1000 cells	6.9 ± 1.9	9.0 ± 1.2	6.8 ± 2.5	6.7 ± 2.7	—	—	—
Pokeweed mitogen 1, Bq/ 1000 cells	3.2 ± 1.3	4.8 ± 1.2	3.5 ± 2.6	4.5 ± 1.3	—	0.004	—
Pokeweed mitogen 2, Bq/ 1000 cells	4.1 ± 1.7	5.6 ± 1.1	4.2 ± 2.9	5.0 ± 1.7	—	0.008	—
Control, Bq/ 1000 cells	0.09 ± 0.13	0.04 ± 0.01	0.07 ± 0.03	0.04 ± 0.01	—	—	—

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

†Average value during 21-d baseline period.

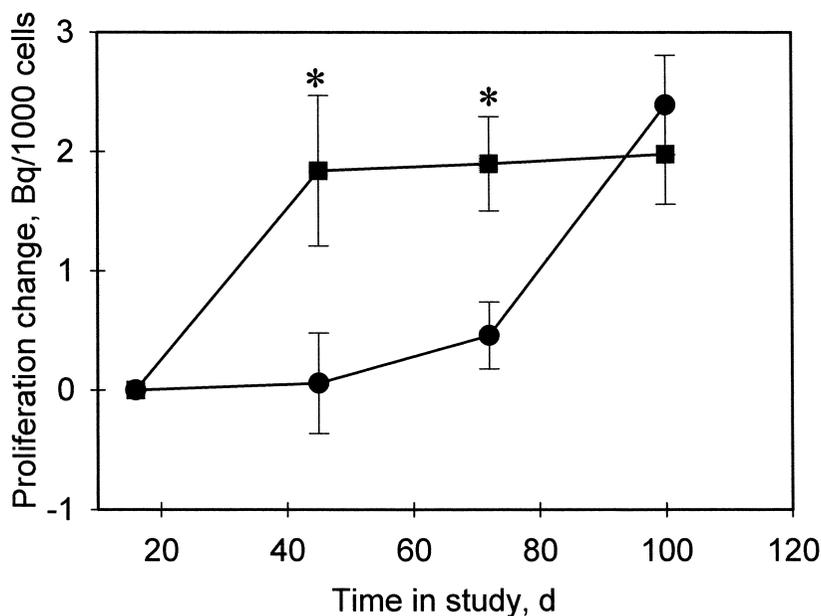


Fig. 8. Changes in lymphocyte proliferation with 1 mg/L pokeweed mitogen in autologous serum. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

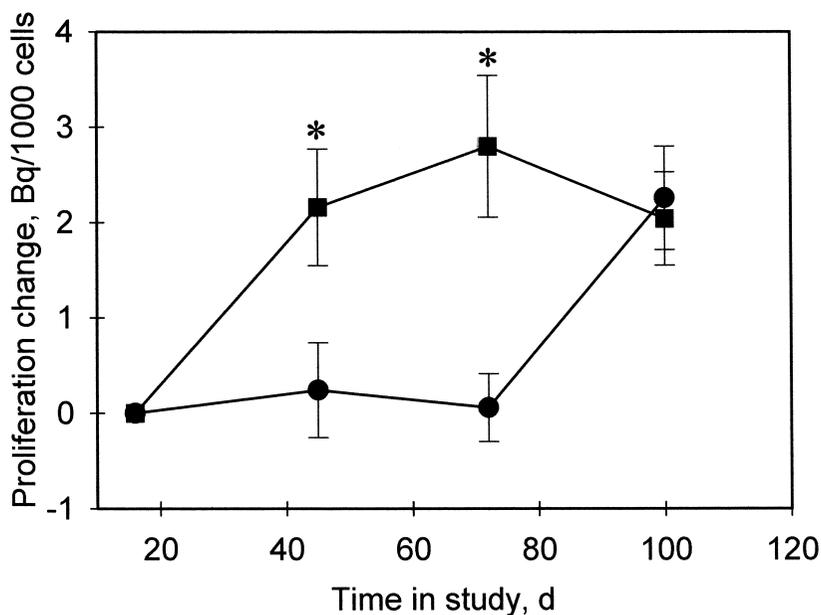


Fig. 9. Changes in lymphocyte proliferation with 2 mg/L pokeweed mitogen in autologous serum. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

displayed the same apparent trend toward earlier proliferative responses in the high-selenium group (not shown), but the differences were not significant in the repeated measures of analysis of variance (ANOVA).

Dietary selenium did not appear to affect DHS skin responses in this study (Table 8). However, there was a general trend toward decreasing responses in both groups during the study. The induration scores at 72 h after injection of the recall antigens at the end of the study were significantly decreased in both groups by an average of 31% compared to the baseline scores.

DISCUSSION

The overall health of the subjects did not change during the study. There was a small (0.9 kg) but statistically significant increase in mean body weight in the high-selenium group and a significant (0.4 kg) decrease in mean body weight in the low-selenium group. Mean serum T₃ concentration was slightly depressed in the high-selenium group and slightly increased in the low-selenium group, and the difference was statistically significant (data not shown). Serum TSH was elevated 34% in the high-selenium group (not shown). Serum triglycerides fell slightly in the high-selenium group and rose somewhat in the low-selenium group. These observations are the subjects of separate reports and will not be discussed further here. The other blood chemistry parameters measured were not affected by selenium and remained within normal adult ranges.

Even though blood selenium status was significantly altered, 99 d of dietary selenium treatments did not result in large changes in immune status. There are many deep tissue pools of slowly exchanging selenium in the human body, and it is possible that this study was too short to observe the full effects of dietary selenium on the immune system. For example, sperm selenium (data not shown) did not change at all in either group, reflecting the slow turnover of selenium in testes. Immune cells in lymphoid tissues with slowly equilibrating pools of selenium may not have been affected by this relatively brief dietary intervention. Selenium turnover in human lymphoid tissues appears to be a largely unexplored area, and we did not measure selenium or glutathione peroxidase in white blood cells, so we cannot say to what extent a failure to observe a particular effect of dietary selenium may have been the result of not shifting the selenium status of the immune cells involved. Further studies on the metabolism and kinetics of selenium in human lymphoid tissues and/or longer human nutritional studies with selenium are needed to follow up on these negative observations.

Our observation that PBMNC proliferation in response to pokeweed mitogen was stimulated earlier in the high-selenium group (Figs. 6 and 7) is similar to a previous observation that supplementation of elderly subjects with high-selenium yeast could reverse the age-related decline

Table 8
Effects of Low-Selenium and High-Selenium Diets on DHS Skin Response

Parameter	Low selenium group (n = 6)		High selenium group (n = 5)		Statistical analysis*		
	Baseline value† (mean ± SD)	Final value (mean ± SD)	Baseline value (mean ± SD)	Final value (mean ± SD)	Se (p)	Time (p)	Se × Time (p)
DHS total induration at 48 h, mm	31.5 ± 8.9	28.8 ± 18	33.2 ± 8.3	27.8 ± 4.0	—	—	—
DHS, number of indurations at 48 h	3.2 ± 0.8	2.7 ± 1.0	3 ± 1	2.4 ± 0.5	—	—	—
DHS total induration at 72 h, mm	35.8 ± 11	26.0 ± 12.5	38.6 ± 9.8	24.8 ± 3.7	—	0.032	—
DHS, number of indurations at 72 h	3 ± 0.6	2.7 ± 0.8	3 ± 1	2.4 ± 0.5	—	—	—

*Two-way repeated measures analysis of variance, SigmaStat 2.0.

†Average value during 21-d baseline period.

in lymphocyte proliferative capacity in response to pokeweed mitogen (2). On the other hand, we did not observe any effect of dietary selenium on proliferation in response to phytohaemagglutinin, as was reported for sodium selenite supplements (3). The forms of selenium in our study were more similar to the forms in high-selenium yeast than they were to sodium selenite, which is relatively more reactive and more rapidly metabolized. The lack of a proliferative response with phytohemagglutinin in our study may be related to our use of food selenium instead of a pure selenium salt. Indeed, this study was designed to isolate only the effects of food-borne selenium and to exclude any chemical or pharmacological effects of the pure selenium chemicals most often used, but which typically do not occur in the human diet. Stimulation by selenium of lymphocyte proliferation in response to phytohemagglutinin may be an effect that depends on the chemical form of the selenium.

The apparent increases in circulating cytotoxic lymphocytes and activated lymphocytes expressing the HLA-DR antigen, although only approaching statistical significance, would tend to support an earlier report that lymphocyte-mediated tumor cytotoxicity was increased in sodium-selenite-supplemented subjects and to support those authors' interpretation that the increased tumor cytotoxicity was caused by selenium's increasing the activation of lymphocytes (5). This earlier study also reported that sodium-selenite-supplemented subjects had 82% higher levels of NK cell activity. We did not observe any change in NK cell activity. However, the number of circulating lymphocytes carrying surface markers for NK cells (CD3⁻,16⁺,56⁺) appeared to be higher in the high-selenium group in our study, but the trend was not statistically significant. The different forms of selenium, food-borne versus sodium selenite, may explain these differing observations on NK cells. Indeed, a recent study in mice found that sodium selenite had many more and dramatically larger effects on the immune system than did selenomethionine (37), the major form of selenium reported in yeast and other foods.

Our observation of enhanced secondary immune responses of specific serum antibodies to reinoculation with diphtheria vaccine appears to be the first report of increased production of specific antibodies resulting from dietary selenium in humans. Increased antibody responses to influenza vaccinations have been reported in elderly subjects supplemented with zinc and selenium sulfide (38), but the effect could not be attributed solely to selenium. The increased secondary antibody response to diphtheria vaccine and the earlier increase of B-lymphocyte proliferation observed in the high-selenium group may be related to reports from China that selenium supplementation decreases the incidence of hepatitis B infections (4). Most animal studies have failed to observe an effect of selenium on antibody production, but there have been a few reports in which selenium improved primary humoral immune responses to foreign antigens in sheep, calves, and ponies (39–42). However, we could

find no previous reports of selenium affecting the secondary immune response in animals or humans.

The lack of an effect of dietary selenium on DHS skin responses in the current study is consistent with at least one previous human study that failed to observe improved DHS with selenium supplementation (38), but stands in contrast to other human studies where significant improvements in DHS have been associated with selenium supplementation (43,44). Animal studies have reported stimulatory, inhibitory, or no effects of selenium on DHS (45–49). Differences in experimental designs, forms of selenium, initial selenium status, and the recall antigens used may explain some of these disparate results. Our results imply that increased intake of food selenium over that supplied by a typical American diet may improve the effectiveness of vaccinations and resistance to subsequent infections, but may not improve cellular immune status.

Many of the effects of dietary selenium observed in the current study—enhanced secondary humoral immune response, increased lymphocyte counts, and apparent increases in cytotoxic lymphocytes, NK cells, and activated lymphocytes—are consistent with a generalized role of selenium in supporting the cellular immune system. The decrease of granulocyte counts with high selenium and the increase in granulocytes with low selenium may be related to the cell growth-regulatory properties of selenium or they might reflect selenium's pro-apoptotic functions. The decreased granulocyte counts in the high-selenium group might also reflect the beneficial effects of selenium on other components of the immune system, which lead to fewer infections and less granulocyte production, or they might indicate a compensatory decrease in nonspecific immunity secondary to the observed increases in specific immune responses. More studies in humans and animals will be needed to clarify the effects of dietary selenium on the immune system and to fully understand the underlying mechanisms. The apparent efficacy of selenium supplementation for cancer prevention (50), the increased pathogenicity of viruses from selenium-deficient hosts (51), and the decreased survival of selenium-deficient AIDS patients (16) highlight the need to more fully understand the functions of selenium, especially individual selenoproteins, in each of the cell types of the immune system.

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