



Memorandum

Rec'd 8/30/02 j6

Date: **AUG 21 2002**

From: Director, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

New Dietary Ingredient: L-Se-methylselenocysteine (SeMC)

Firm: PharmaSe, Inc.

Date Received by FDA: October 10, 2001

90-Day Date: January 8, 2002

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Felicia B. Satchell
Felicia B. Satchell

Attachments

95S-0316

RPT 102



NOV 28 2001

Julian Spallholz, Ph.D.
President and CEO
PharmaSe, Inc.
3416 Knoxville Avenue
Lubbock, Texas 79413

Dear Dr. Spallholz:

This is to inform you that the notification dated October 8, 2001, you submitted pursuant to 21 U.S.C. 350b(a)(2) was received and filed by the Food and Drug Administration (FDA) on October 10, 2001. Your notification, which represents a resubmission, concerns your intent to market for adults only the new dietary ingredient called "L-Se-methylselenocysteine (SeMC)" in a dietary supplement providing 100 mcg of selenium that you suggest should be taken up to twice a day.

In May 2001, you sent FDA an earlier notification about this same level and use of selenium in a SeMC dietary supplement. FDA responded in a letter dated July 27, 2001, stating that your May 2001 notification provided an inadequate basis for concluding that up to 200 mcg of additional selenium provided in a SeMC dietary supplement was reasonably expected to be safe for adults already consuming a high amount of dietary selenium.

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit certain information to FDA at least 75 days before the dietary ingredient is introduced or delivered for introduction into commerce. This information must include the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the new dietary ingredient is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B), because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness and injury.

21 CFR §190.6 specifies the requirements for a premarket notification on a new dietary ingredient. These Federal regulations state that an original and two copies of all documentation pertaining to a notification must be submitted to FDA. Your notification does not comply with this requirement, because you submitted only a single original copy of your notification to us. In addition, you included three PubMed abstracts that cannot be completely read since they were printed using the portrait versus landscape view, which resulted in missing text from the right-hand side of the paper.

You must provide two more copies of all pages of your current notification to meet the minimum requirements of a new dietary ingredient premarket notification. Please also be advised, that your notification must include legible copies of all information that you want FDA to consider as your basis for concluding that 100 mcg of SeMC in a dietary supplement is reasonably expected to be safe for adults when taken up to twice a day. Therefore, if you want FDA to consider the cited PubMed abstracts as part of the basis for your safety determination of SeMC, please provide us legible copies. You also are welcome to send us in triplicate any additional scientific references that support your conclusion of safety.

Because your current notification does not meet the minimum requirements of 21 CFR §190.6, FDA did not review the evidence of safety information you submitted on the level and use of your SeMC dietary supplement. You can correct this deficiency in your notification by sending us the additional two copies of your notification as discussed above. If you prefer, you instead can elect to send us a new notification that is complete and fully complies with 21 CFR §190.6. We will review the safety information for consuming a SeMC dietary supplement providing up to 200 mcg of additional selenium a day upon receipt of the missing information or a new complete notification. If you opt to amend your current notification or send us a new one, we will revise the notification's filing date, which will be the date FDA receives this information.

For the reasons discussed above, the information in your notification does not provide an adequate basis to conclude that a SeMC dietary supplement for adults, when used under the conditions recommended or suggested in the labeling of your product (i.e., taken up to twice a day), will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient at a level for which there is inadequate information to provide reasonable assurance that it will not present a significant or unreasonable risk of illness or injury. Introduction of such products into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

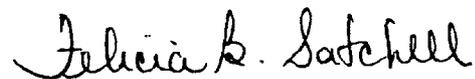
Your notification will be kept confidential for 90 days from the date of its receipt. After January 8, 2002, your notification will be placed on public display at FDA's Dockets Management Branch in docket number 95S-0316. However, any trade secret or otherwise confidential commercial information in the notification will not be disclosed to the public.

Page 3 - Julian Spallholz, Ph.D.

For FDA's consideration, you may wish to identify in writing specifically what information you believe is proprietary in your current notification or in any amended or new notification that you may send us. Nevertheless, our Center's Freedom of Information Officer has the authority to make the final decision about what information in the notification should be redacted before it is posted at Dockets.

Should you have any questions concerning this matter, please contact me at (202) 205-4168.

Sincerely yours,

A handwritten signature in cursive script that reads "Felicia B. Satchell".

Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

PharmaSe, Inc.

Selenium Nutraceuticals for the Future

Julian E. Spallholz, PhD
Cell Ph. (806) 786-8349

3416 Knoxville Ave.
Lubbock, TX
79413
Ph/fax (806) 784-0104
Email: sestech@door.net



Felicia B. Satchell

October 8, 2001

Director

Division of Standards and Labeling Regulations

Office of Nutritional Products, Labeling and Dietary

Supplements

Center for Food Safety and Applied Nutrition

US Food and Drug Administration

Washington, DC

Dear Director Satchell:

On May 10, 2001 we made a request to your office for permission to change formulation for adults of our dietary ingredient Se-methylselenocysteine from 2 - 50 ug Se/day tablets (100 ug Se/day) to two 100 ug Se/day tablets for an adult dose total of 200 ug Se/day. Our request was rejected for safety considerations by your office in your letter to PharmaSe, Inc of July 27, 2001. We would like to request a reappraisal of your rejection of our May 10, 2001 request to increase our supplements to 200 ug Se/day of Se-

methylselenocysteine based upon the following documentation which is enclosed and Commentary over 3 points for review consideration.

1) Your Letter of July 27th To Us Cites Rejection Based upon Supplementation of Selenocysteine and the Accumulation of Organic Selenium in Tissues that pose a Health Hazard, page 2 paragraph 4.

Commentary. The supplement is not selenocysteine that we are requesting permission to increase to 200 ug Se/day page 2, paragraph 4. Our request is for a dietary supplementation of Se-methylselenocysteine, a natural product produced by plants including garlic, broccoli, onions, leeks, other allium plants and also to a lesser degree the widely used selenium supplement selenium-yeast. You are absolutely correct that L-selenomethionine accumulates in protein in place of methionine but Se-methylselenocysteine is a non-protein amino acid and therefore does not accumulate in body tissues as you so stated and implied. Attached are references that experimentally show this to be correct. Thus there should be no basis for your rejection on the basis of tissue accumulation and toxicity of Se-methylselenocysteine.

2) Presently Marketed Selenium Supplements Contain up to 250 ug Se/day

Commentary. Your letter of July 27th to PharmaSe, Inc sites concern of toxicity of a dosage of 200 ug Se/day of Se-methylselenocysteine based upon dietary intake, page 3 paragraph 1. Attached is a list of different selenium supplements marketed by nineteen different manufacturers (names, address and in some cases websites are included). These supplements include L-selenomethionine, selenium-yeast and/or sodium selenite. Many of these supplements are marketed at 200 ug Se/day and most all contain or are exclusively L-selenomethionine. All supplements contain selenomethionine with the exception of the sodium selenite supplement marketed at 250 ug Se/day from Twin Labs, All of these selenium supplements except sodium selenite would be expected to accumulate and raise tissue levels of selenium for which you expressed concern over toxicity. Since sodium selenite is potentially much more toxic than L-selenomethionine or L-Se-methylselenocysteine (by at least an order of magnitude) selenite appears to be marketed without FDA objection or reports of toxicity. Additionally, we have attached a copy of the paper by Clark et al published in JAMA in 1996 whereby adults consumed 200 ug Se/day selenium supplement over a

period of several years without any reported adverse effects. There is presently a very large human prostate cancer trial under way where adults are consuming supplemental levels of selenium at 200 ug Se/day as L-selenomethionine with no adverse effects anticipated or having been reported.

3). Toxicity of Selenium and its Compounds

Commentary. Your letter to PharmaSe, Inc of July 27th cited supplemental toxicity concerns based upon dietary intake page 2, paragraph 6 at which a selenium supplement when added to normal dietary intake would reasonably be expected to produce chronic selenium toxicity.

Your data is correct and your conclusions would equally be appropriate to apply to most of the selenium supplements and their manufactures listed in the attachment where 200 ug Se/day or more supplementation is supplied per single dosage. The reality is that the chronic level of selenium toxicity that is suggested in your letter for the upper percentile consumer would not be met until a dietary plus supplemental level of selenium intake of nearly 819 ug Se/day was reached. (See enclosed Table). Additionally,

the toxicity data you cite may not be applicable the natural forms of selenium found in foods, L-selenomethionine and L-Se-methylselenocysteine. These natural selenoamino acids from food are not very toxic (see enclosue) and to our knowledge no adverse effects of any selenium supplement has been reported at 200 ug Se/day a level of supplementation that has been commercially available to the public since the early 1980's. A recent publication (copy enclosed) reported that supplementation of humans to 296 ug Se/day with selenium actually improve immune status. Our own experience in evaluating the dietary toxicity of L-selenomethionine and L-Se-methylselenocysteine reveals that if there is indeed any dietary difference in toxicity between these two amino acids, L-selenomethionine is slightly more toxic than Se-methylselenocysteine.

It is our hope that given the levels of selenium supplementation presently available to the public within the market place for which no adverse selenium effects have been reported, the level of supplementation to adults published without any reported adverse effects in the past, the on going supplemental trials at 200 Se/day by the National Cancer Institute's supported research and our assurance to you that there is no significant experimental

differences between the toxicity of L-selenomethionine and Se-methylselenocysteine that the FDA can grant to us with confidence permission to increase the optional adult supplementation recommendation on a label to 200ug Se/day as Se-methylselenocysteine. Were there any concerns or data from the literature to suggest to us complete safety of Se-methylselenocysteine we certainly would not make this request for increasing supplemental levels.

We appreciated your time spent in the past review of our request and we will be happy to submit any additional information, which shows Se-methylselenocysteine to be non-toxic under any reasonable level of supplementation at or less than 200 ug Se/day. Thank you.

Sincerely,

A handwritten signature in black ink, appearing to read "Julian Spallholz". The signature is fluid and cursive, with the first name "Julian" written in a larger, more prominent script than the last name "Spallholz".

Julian Spallholz, PhD
President and CEO
PharmaSe, Inc

enclosures

Companies that Retail Selenium Supplements

1. **Country Life**
Hauppauge NY 11788
200 ug Se as yeast selenomethionine
www.country-life.com
2. **Twin Labs**
Ronkonkoma, NY 11779
250 ug Se as sodium selenite
www.veromaxxx.com/TwinLab/
3. **Solaray Made They are a label for Nutraceutical Corp**
200 ug Se as yeast selenomethionine
4. **Natural Factors**
3686 Bonneville Place,
Burnaby BC
Canada V3N 4T6
Tel: (604) 415-4187 Fax: (604) 420-0743
100 ug Se as yeast selenomethionine
www.naturalfactors.com
5. **Now Foods**
Bloomington IL 60108
www.Nowfoods.com
200 ug Se as selenomethionine advertised as yeast free do not take more than one capsule daily
sales@nowfoods.com
internationalsales@nowfoods.com
888-NOW-FOODS
6. **BlueBonnet**
Sugarland, TX 77478
200 ug Se as selenomethionine

7. Solgar
500 Willow Tree Road
Leonia, N.J. 07605
200 ug Se as selenomethionine they also sell a 200ug yeast product (from Cypress) and a
150 ug Se as selenomethionine plus vitamin E (500IU)
www.solgar.com
1-877-765-4274 (for Consumers)
1-800-645-2246 (for Retail Stores)

8. Natures Plus.com
Natural Organics Headquarters
548 Broadhollow Rd.
Melville, NY 11747-3708
Email - Info@naturesplus.com
Telephone - (631) 293-0030
Fax - (631) 293-0349
<http://www.naturesplus.com>
200 ug Se as selenomethionine yeast plus 100 IU Vit E

9. Jarrow Formulas™
1824 S. Robertson Blvd
Los Angeles, CA 90035
(800)726-0886
www.jarrow.com
200 Se as ug selenomethionine

10. Nutraceutical Corp.
1400 Kearns Blvd., Second Floor
Park City, UT 84060. Call 800.669.8877
200 ug Se as selenomethionine yeast they also have a 100 ug yeast free capsule
www.nutraceutical.com
their brands are Solaray, KAL, NaturalMax, VegLife, Premier One, Solar Green and
Natural Sport

11. Whole Foods Inc.
601 N. Lamar
Austin, TX 78703
200 ug Se as selenomethionine
512-477-5566
www.wholefoods.com

12. New Chapter
22 High Street
Brattleboro, VT 05301
(800) 543-7279 Fax: (800) 470-0247 info@new-chapter.com
200 ug Se as selenomethionine yeast
www.Newchapter.com
800-543-7279

13. Wild Oats
3375 Mitchell Lane
Boulder, CO 80301
www.wildoats.com
800-494-WILD
200 ug Se as selenomethionine yeast

14. Natures Life
7180 Lampson Ave.
Garden Grove, CA 92841
<http://www.natlife.com/>
(714) 379-6500 FAX (714) 379-6501
200 ug Se as selenomethionine yeast

15. Megafood
P.O. Box 325
Derry, NH 03038
www.megafood.com
800-848-2542 FAX 603-432-2111
100 ug Se as foodstate ?? selenium

16. Walgreens
200 Wilmot Road
Deerfield, IL 60015
(847) 914-2500
<http://www.walgreens.com>
200 ug Se as selenomethionine yeast made by Nutrition 21

17. **Nature Made**
Mission Hills, CA 91346
800-276-2878
www.naturemade.com
200 ug Se as selenomethionine yeast

18. **Sundown**
6111 Broken Sound Pkwy. N.W.
Boca Raton, FL 33487
<http://www.rexallsundown.com>
(800) 327-0908
200 ug Se as selenomethionine yeast

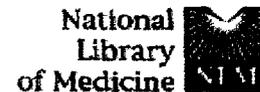
19. **Linear Health Products**
Carson CA 90745
selenium

Essentiality of Selenium in Humans

<u>Se ug/day</u>	<u>ug Se/kg Body Weight</u>	<u>Chemical Form</u>	<u>Effect(s)</u>
<11	<0.20	Dietary	Keshan Disease Kashin-Beck Disease
16	0.31	Dietary	Minimum Dietary Requirement
41	0.67	Dietary	Adequate dietary Requirement
55	-----	Dietary	1989 US RDA for Women
55	-----		1999 US RDA for Women
73	-----		2000 a Calculated Required US RDA for Women (Rayman, The Lancet, 2000)
70	----	Dietary	1989 US RDA for Men
55	----		1999 US RDA for Men
80-165	----	Dietary	US Dietary Intake Range
100		Dietary	Glutathione Peroxidase Saturation of Platelets
300+	----	200 ug Se/day Supplements, Selenomethionine	Immune Enhancement, Cancer Reductions in Humans, NCI SELECT Trial
350	5	Diet and Supplements	RfD 70 kg Adult
400	-----	Diet and Supplements	Suggested Maximum Safe Limit
600	11	Diet and Supplements	Individual Maximum Safe Limit
724	----	Diet and Supplements	Level Identified as Safe in Adult Americans
819	15	Diet and Supplements	Maximum Safe Limit (NOAEL)

Toxicity of Selenium in Humans

<u>Se ug/day</u>	<u>ug Se/kg Body Weight</u>	<u>Chemical Form</u>	<u>Effect(s)</u>
900	17	Diet and Supplements	Low Level Toxicity (Individual LOAEL)
1000	- ----	Diet and Na ₂ SeO ₃ Supplement	Personally Known Intake Daily for Years with NOAEL
1,540	28	Diet and Supplements	Low Level Toxicity (Mean LOAEL)
1,600	30	Diet and Supplements	Adverse Effective Level
5,000	90	Diet and Supplements	Selenosis, Hair and Nail Loss
15,000	270	Diet and Supplements	Overt Selenosis



PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Bc

Search PubMed for

Limits Preview/Index History Clipboard Details

About Entrez

Display Abstract Sort Save Text Clip Add Order

Entrez PubMed

Overview
Help | FAQ
Tutorial
New/Noteworthy

PubMed Services

Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Cubby

Related Resources

Order Documents
NLM Gateway
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: JAMA 1996 Dec 25;276(24):1957-63

[Related Articles, Books, LinkC](#)

Erratum in:

- JAMA 1997 May 21;277(19):1520

Comment in:

- JAMA. 1996 Dec 25;276(24):1984-5
- JAMA. 1997 Mar 19;277(11):880-1; discussion 881
- JAMA. 1997 Mar 19;277(11):880; discussion 881

Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group.

Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Leshner JL, Park HK, Sanders BB Jr, Smith CL, Taylor JR.

Arizona Cancer Center, College of Medicine, University of Arizona, Tucson, USA.

OBJECTIVE: To determine whether a nutritional supplement of selenium will decrease the incidence of cancer. **DESIGN:** A multicenter, double-blind, randomized, placebo-controlled cancer prevention trial. **SETTING:** Seven dermatology clinics in the eastern United States. **PATIENTS:** A total of 1312 patients (mean age, 63 years; range, 18-80 years) with a history of basal cell squamous cell carcinomas of the skin were randomized from 1983 through 1991. Patients were treated for a mean (SD) of 4.5 (2.8) years and had a total follow-up of 6.4 (2.0) years. **INTERVENTIONS:** Oral administration of 200 microg of selenium per day or placebo. **MAIN OUTCOME MEASURES:** The primary end points for the trial were the incidences of basal and squamous cell carcinomas of the skin. The secondary end points, established in 1990, were all-cause mortality and total cancer mortality, total cancer incidence, and the incidences of lung, prostate, and colorectal cancers. **RESULTS:** After a total follow-up of 8271 person-years, selenium treatment did not significantly affect the incidence of basal cell or squamous cell skin cancer. There were 377 new cases of basal cell skin cancer among patients in the selenium group and 350 cases among the control group (relative risk [RR], 1.10; 95% confidence interval [CI], 0.95-1.28), and 218 new squamous cell skin cancers in the

selenium group and 190 cases among the controls (RR, 1.14; 95% CI, 0.93-1.39). Analysis of secondary end points revealed that, compared with control patients treated with selenium had a nonsignificant reduction in all-cause mortality (108 deaths in the selenium group and 129 deaths in the control group [RR; 0.83; 95% CI, 0.63-1.08]) and significant reductions in total cancer mortality (29 deaths in the selenium treatment group and 57 deaths in control [RR, 0.50; 95% CI, 0.31-0.80]), total cancer incidence (77 cancers in the selenium group and 119 in controls [RR, 0.63; 95% CI, 0.47-0.85]), and incidences of lung, colorectal, and prostate cancers. Primarily because of the apparent reductions in total cancer mortality and total cancer incidence in the selenium group, the blinded phase of the trial was stopped early. No cases of selenium toxicity occurred. CONCLUSIONS: Selenium treatment did not protect against development of basal or squamous cell carcinomas of the skin. However, results from secondary end-point analyses support the hypothesis that supplemental selenium may reduce the incidence of, and mortality from, carcinomas of several sites. These effects of selenium require confirmation in an independent trial of appropriate design before new public health recommendations regarding selenium supplementation can be made.

Publication Types:

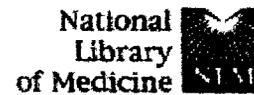
- Clinical trial
- Multicenter study
- Randomized controlled trial

PMID: 8971064 [PubMed - indexed for MEDLINE]

Display	Abstract	Sort	Save	Text	Clip Add	Order
---------	----------	------	------	------	----------	-------

[Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)
[Department of Health & Human Services](#)
[Freedom of Information Act](#) | [Disclaimer](#)

sparc-sun-solaris2.8 Oct 4 2001 12:24



PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Bc

Search PubMed for [] Go Clear

Limits Preview/Index History Clipboard Details

About Entrez

Display Abstract Sort Save Text Clip Add Order

Entrez PubMed

- Overview
- Help | FAQ
- Tutorial
- New/Noteworthy

PubMed Services

- Journal Browser
- MeSH Browser
- Single Citation Matcher
- Batch Citation Matcher
- Clinical Queries
- Cubby

Related Resources

- Order Documents
- NLM Gateway
- Consumer Health
- Clinical Alerts
- ClinicalTrials.gov
- PubMed Central

Privacy Policy

1: Br J Urol 1998 May;81(5):730-4

Related Articles, Books, LinkC

Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial.

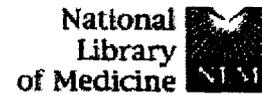
Clark LC, Dalkin B, Krongrad A, Combs GF Jr, Turnbull BW, Slate EF, Witherington R, Herlong JH, Janosko E, Carpenter D, Borosso C, Falk R, Rounder J.

Arizona Cancer Center, College of Medicine, University of Arizona, Tucson 85716, USA.

OBJECTIVE: To test if supplemental dietary selenium is associated with changes in the incidence of prostate cancer. **PATIENTS AND METHOD:** A total of 974 men with a history of either a basal cell or squamous cell carcinoma were randomized to either a daily supplement of 200 microg of selenium or a placebo. Patients were treated for a mean of 4.5 years and followed for a mean of 6.5 years. **RESULTS:** Selenium treatment was associated with a significant (63%) reduction in the secondary endpoint of prostate cancer incidence during 1983-93. There were 13 prostate cancer cases in the selenium-treated group and 35 cases in the placebo group (relative risk RR=0.37, P=0.002). Restricting the analysis to the 843 patients with initially normal levels of prostate-specific antigen (< or = 4 ng/mL), only four cases were diagnosed in the selenium-treated group and 16 cases were diagnosed in the placebo group after a 2 year treatment lag, (RR=0.26 P=0.009). There were significant health benefits also for the other secondary endpoints of total cancer mortality, and the incidence of total, lung and colorectal cancer. There was no significant change in incidence for the primary endpoints of basal and squamous cell carcinoma of the skin. In light of these results, the 'blinded' phase of this trial was stopped early. **CONCLUSIONS:** Although selenium shows no protective effects against the primary endpoint of squamous and basal cell carcinomas of the skin, the selenium-treated group had substantial reductions in the incidence of prostate cancer, and total cancer incidence and mortality that demand further evaluation in well-controlled prevention trials.

Publication Types:

- Clinical trial
- Randomized controlled trial



PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Br

Search PubMed

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

About Entrez

Display

Abstract

Sort

Save

Text

Clip Add

Order

Entrez PubMed

Overview
Help | FAQ
Tutorial
New/Noteworthy

PubMed Services

Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Cubby

Related Resources

Order Documents
NLM Gateway
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: Med Klin 1997 Sep 15;92 Suppl 3:42-5

Related Articles, Books, Links

Reduction of cancer mortality and incidence by selenium supplementation.

Combs GF Jr, Clark LC, Turnbull BW.

Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA.
gfc2@cornell.edu

PATIENTS AND METHOD: In order to test the hypothesis that a dietary supplement of selenium (Se) may reduce cancer risk, 1312 patients with histories of basa/squamous cell carcinomas of the skin were assigned in random, double-blind fashion to daily oral supplements of either Se-enriched yeast (200 micrograms Se/day), or a low-Se yeast placebo. Patients were recruited in 1983 to 1990 and were followed with regular dermatologic examinations through, 1993 for a total of 8269 person-years of observation. Skin cancer diagnoses were confirmed histologically and plasma Se concentration was determined at 6 to 12 months intervals. All deaths and patient-reported illnesses were confirmed and documented by consultation with the patient medical care providers. **RESULTS:** Results showed that Se-supplementation did not significantly affect the incidences of recurrent basal/squamous cell carcinomas of the skin. However, Se-treatment was associated with reductions in total cancer mortality and in the incidences of lung, colorectal, prostate and total cancers. These effects were consistent over time and between study clinics. **CONCLUSION:** The results strongly suggest benefits of Se-supplementation for this cohort of patients and support the hypothesis that supplemental Se can reduce risks to at least some types of cancer.

Publication Types:

- Clinical trial
- Randomized controlled trial

PMID: 9342915 [PubMed - indexed for MEDLINE]

Display

Abstract

Sort

Save

Text

Clip Add

Order

Commentary

Nutritional Selenium Supplements: Product Types, Quality, and Safety

Key words: selenium, selenomethionine, selenium yeast, sodium selenite, sodium selenate, dietary supplements

Selenium supplements contain selenium in different chemical forms. In the majority of supplements, the selenium is present as selenomethionine. However, in multivitamin preparations, infant formulas, protein mixes, weight-loss products and animal feed, sodium selenite and sodium selenate are predominantly used. In some products, selenium is present in protein- or amino acid chelated forms; in still others, the form of selenium is not disclosed. Current evidence favors selenomethionine over the other forms of selenium. Extradietary supplementation of selenium at the dosage of 200 micrograms per day is generally considered safe and adequate for an adult of average weight subsisting on the typical American diet.

The typical American diet provides the average adult with about 80 to 150 micrograms of selenium per day, which is more than the newly revised RDA for selenium of 55 μg [1], but less than one half of the amount considered optimal for utilization of the protective potential of selenium, especially for cancer prevention [2,3]. Accordingly, extradietary selenium supplementation is increasingly recommended by health professionals. Pending the outcome of ongoing human cancer prevention trials, selenium supplementation is likely to be officially recognized as a means of lowering cancer risk. These developments raise the question as to which form of selenium is the most desirable for supplementation. In addition, the quality and safety of the selenium supplements become matters of concern.

Nutritional Forms of Selenium

Ideally, selenium should be supplemented in the form or forms in which it occurs in major staple foods. Since more than 80% of the total selenium in seleniferous corn, wheat and soybeans consists of L(+)-selenomethionine [4], this amino acid is the most appropriate supplemental form of selenium. Some other compounds, namely Se-methylselenocysteine and selenocystathionine are present primarily in selenium accumulator plants [5], but also in broccoli, garlic and onions if these are grown in Se-rich media [6]. Se-methylselenocysteine has recently been suggested as a possible form of selenium for cancer prevention [7], but its value relative to selenomethionine

as a supplemental source of selenium still remains to be demonstrated. Moreover, Se-methylselenocysteine, as well as selenocysteine (or -cystine), selenohomocysteine (or homocystine), selenocystathionine and γ -glutamyl-Se-methylcysteine are normally found in edible plants only in nutritionally insignificant amounts. Selenomethionine, however, replaces methionine in plant proteins and thus is the major form of selenium for higher animals and humans. Selenomethionine is well absorbed and is either metabolized directly or is incorporated into body proteins in place of methionine. The extent of selenomethionine incorporation into proteins depends on the dosage and methionine status and diminishes at high methionine intakes [8,9]. Selenomethionine is incorporated primarily into the proteins of the skeletal muscles, erythrocytes, pancreas, the liver, stomach, the kidneys and the gastrointestinal mucosa [10]; its release from body proteins is linked to protein turnover and occurs continuously. At constant intakes of selenomethionine, a steady state is established which is maintained indefinitely and over a large range of intakes.

Blood Se levels and dietary Se intakes thus primarily reflect the selenomethionine content of foods [11]. Selenomethionine not used for protein synthesis is degraded by the transsulfuration pathway to selenocysteine and subsequently, in the liver, to serine and selenide [3]. In the liver of the rat, selenomethionine is in part also degraded by a γ -lyase to methylselenol and homoserine [12]. Just as methionine can serve as the sole source of sulfur, selenomethionine provides all forms of bioactive selenium needed for selenoprotein biosynthesis. However, since selenomethionine belongs to the group of amino acids

Address reprint requests to: Gerhard N. Schrauzer, PhD, CNS, FACN, Biological Trace Element Research Institute, 11526 Sorrento Valley Rd., Ste. A., San Diego, CA 92121

Journal of the American College of Nutrition, Vol. 20, No. 1, 1-4 (2001)
Published by the American College of Nutrition

which higher animals and humans cannot synthesize, selenomethionine may also be needed for some specific functions in the organism. For example, selenomethionine has been suggested to act as a cellular antioxidant; on reaction with peroxynitrite, selenomethionine oxide formed which is reduced back to selenomethionine by ascorbic acid [13].

Supplemental Forms of Selenium

Unfortunately, not much was known about selenomethionine in the early 1970s, when regulatory agencies had to decide which selenium compounds to allow for use in animal feed. The approval in 1974 of sodium selenite and sodium selenate as feed additives created an unsatisfactory situation. First, the approval suggested that these inorganic selenium salts are nutritional forms of selenium, which they are not. Secondly, the approval diverted attention from selenomethionine, which was soon recognized to be superior to the inorganic selenium salts [14]. However, at the time the regulatory action was taken, only the inorganic selenium salts were available at a cost permitting their use in animal feed.

In the search for an economical source of organic nutritional forms of selenium, attempts were made to increase the normally low selenium content of yeast by growing it in selenium-enriched media. Yeast was chosen because it can be produced in quantity under controlled conditions and was known to contain a highly bioactive organic form of selenium. Yeast had also played a major role in the discovery of the nutritional essentiality of selenium, when it was shown to contain 'Factor 3,' the naturally occurring selenium compound most effective in preventing dietary liver necrosis in the rat [15]. Although Factor 3 was as such not identified, from what is known today, it must have consisted primarily of selenomethionine. By the mid 1970s, the first 'high selenium yeasts' became commercially available. Today's commercial products typically contain from 1,000 to 2,000 micrograms of selenium per gram, with 90+% of the selenium in the form of L(+)-selenomethionine [3,16]. In 1983, this selenomethionine-rich yeast was chosen as the source of selenium for a large-scale cancer prevention trial [17]. This trial showed that taking an extra 200 micrograms of selenium per day significantly lowered the risks of developing prostate, lung and colorectal cancer.

In 1984, synthetic selenomethionine became available. It is used in supplements specifically formulated to be yeast-free or when a concentrated, compact source of selenium is required. Since selenomethionine, like all amino acids, can exist in the L- and in the D-form and since only the L-isomer occurs naturally in foods, this form is preferable for use in supplements intended for humans; for use in animal feed, the D,L-mixture of the isomers is deemed acceptable [3].

Yeast-selenium at the level of 0.3 ppm in feed dry matter was twice as effective as selenite in increasing the selenium content of the sirloin muscles in pigs [18]; it also raised the selenium levels in serum and the liver significantly more than

selenite [19]. Selenium yeast accordingly has been recommended for general use in animal nutrition [20]. In June, 2000, the use of selenium yeast in poultry broiler and layer diets was FDA approved. This is only the beginning of a development which will eventually result in the complete replacement of the inorganic selenium compounds as feed additives by selenomethionine or nutritional sources thereof.

Quality Concerns

Popular demand for selenium supplements requires the cautionary note that the quality of some of the presently marketed supplements is questionable. Some products, for example, are made with yeasts containing inorganic selenium (selenite or selenate) instead of selenomethionine [21,22]. Since only selenomethionine-containing yeast was used in the cancer prevention trial, the form of selenium actually present should be indicated, but this is often not done. In other supplements, the form of selenium is stated but is ill-defined. Supplements containing 'selenium proteinates' or 'selenium amino acid chelates' belong to this category. In some multivitamin preparations, both sodium selenite and vitamin C are present. In such supplements, elemental selenium may gradually form by the reaction of selenite with vitamin C [23]. Although the quality of selenium supplements is steadily improving, the supplement industry has still a ways to go before its selenium products can be generally recommended.

Selenium in Infant Formulas, Protein Mixes and Weight-Loss Products

Infant formulas, protein mixes and weight loss products still use almost exclusively sodium selenite or sodium selenate. The continuing use of the inorganic selenium compounds is difficult to justify. This is especially true for infant formulas, which through the use of the inorganic selenium salts deprive the growing infant of the benefits which only selenomethionine can provide. Studies with preterm infants [24,25] have already demonstrated that selenium yeast is safe and effective for enteral selenium supplementation. Selenium yeast and selenomethionine were furthermore shown to be superior to selenite in studies with nursing mothers; specifically, more selenium appeared in the milk of mothers obtaining selenium from selenomethionine than from selenite [26].

Safety of Selenium Supplements

As to the safety of selenium, a supplemental dose of 200 micrograms per day would cause the total daily selenium intake of an average adult to increase to 280 to 350 micrograms. This is a safe amount since it is below or equal to the Reference Dose (RfD) for selenium, which, for an adult of 70 kg, was set by the EPA at 350 micrograms [27]. The RfD is defined as 'an

estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.' In line with this definition, studies have shown that prolonged daily selenium intakes of 750 to 850 micrograms do not produce adverse effects. Intakes of selenium of this magnitude were provisionally suggested to represent the 'No Adverse Effect Level' (NOAEL). The 'Lowest Adverse Effect Level' (LOAEL), defined as the 'average daily selenium intake causing individuals within a population to develop overt signs of toxicity,' is believed to be in the order of 1540 ± 653 micrograms/day [28]. 'Low Adverse Effects' of selenium usually do not develop after a single dose of this magnitude, but only after weeks or months of exposure. Moreover, the early warning signs of selenium overload are easily recognized. Accordingly, a wide margin of safety exists when 200 micrograms of selenium are taken daily, and even if this amount is temporarily exceeded, no adverse effects need to be feared. Indeed, selenium has an excellent safety record, and the only cases of selenium toxicity, which occurred several decades ago, were due to inadvertent dosage errors by inexperienced supplement manufacturers which were not using selenium yeast or selenomethionine in their products.

Gerhard N. Schrauzer, PhD, CNS, FACN

Department of Chemistry and Biochemistry
University of California San Diego
and
Biological Trace Element Research Institute
San Diego, California

REFERENCES

1. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine: 'Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.' Washington, DC: National Academy Press, 2000.
2. Schrauzer GN, White DA: Selenium in human nutrition: Dietary intakes and effects of supplementation. *Bioinorg Chem* 8:303-318, 1978.
3. Schrauzer, GN: Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *J Nutr* 130:1653-1656, 2000.
4. Yang X, Tian Y, Ha P, Gu L: Determination of the selenomethionine content in grain and in human blood. *Wei Sheng Yen Chiku (J Hyg Res)* 26:113-116, 1997.
5. Shrift A: Metabolism of selenium in plants and microorganisms. In Klayman DE, Günther WHH (eds): 'Organic Selenium Compounds in Chemistry and Biology.' New York: Wiley-Interscience, pp 763-814, 1973.
6. Cai X-J, Block E, Uden PC, Zhang X, Quimby BD, Sullivan JJ: Allium chemistry: identification of selenoamino acids in ordinary and selenium-enriched garlic, onion and broccoli using gas chromatography with atomic emission detection. *J Agric Food Chem* 43:1754-1757, 1995.
7. Finley JW, Davis CD, Feng Y: Selenium from high selenium broccoli protects rats from colon cancer. *J Nutr* 130:2384-2389, 2000.
8. Butler JA, Beilstein MA, Whanger PD: Influence of dietary methionine on the metabolism of selenomethionine in rats. *J Nutr* 119:1001-1009, 1989.
9. Salbe AD, Levander OA: Comparative toxicity and tissue retention of selenium in methionine-deficient rats fed sodium selenate or L-selenomethionine. *J Nutr* 120: 207-212, 1990.
10. Hansson E, Jacobsson SO: Uptake of [⁷⁵Se] selenomethionine in the tissues of the mouse studied by whole-body autoradiography. *Biochim Biophys Acta* 115:285-293, 1966.
11. Yang X, Tian Y, Ha P, Gu L: Determination of the selenomethionine content in grain and human blood. *Wei Sheng Yen Chiku (J Hyg Res)* 26:113-116, 1997.
12. Nakamuro K, Nakanishi K, Okuno T, Hasegawa T, Sayato Y: Comparison of methylated selenium metabolites in rats after oral administration of various selenium compounds. *Jpn J Toxicol Environ Health* 43:1482-1489, 1997.
13. Artee GE, Gavin E, Bribiva K, Sies H: Protection against peroxinitrite. *FEBS Lett* 445: 226-230, 1999.
14. Griffiths NM, Stewart RDH, Robinson MF: The metabolism of ⁷⁵Se selenomethionine in four women. *Br J Nutr* 35:373-382, 1976.
15. Schwarz K, Foltz CM: Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *J Am Chem Soc* 79: 3292-3293, 1957.
16. Schrauzer GN: Characterization of selenium yeasts for nutritional selenium supplementation. In Palmieri Y (ed): 'Proceedings of the 6th International Symposium on the Uses of Selenium and Tellurium.' Scottsdale, AZ: 6th International Symposium on the Uses of Selenium and Tellurium, pp 77-79, 1998.
17. Clark LC, Combs Jr GF, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Leshner JL, Park HK, Sanders BB, Smith CL, Taylor JR: Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. *JAMA* 276: 1957-1963, 1996.
18. Mahan DC: Selenium metabolism in animals: What role does selenium yeast have? In Lyons TP, Jacques KA (eds): 'Biotechnology in the Feed Industry.' Loughborough, England: Nottingham University Press, pp 257-267, 1994.
19. Suomi K, Alaviuhkola T: Response to organic and inorganic selenium in the performance and blood selenium content of growing pigs. *Agric Sci Finn* 1:211-215, 1992.
20. Lyons TP, Oldfield JE: The case for organic selenium. *Bull Selenium-Tellurium Dev Assoc*, pp 1-3, June 1995.
21. Schrauzer GN, McGinness JE: Observations on human selenium supplementation. In 'Trace Substances in Environmental Health XIII.' Columbia: ESD Publications, University of Missouri, pp 64-67, 1979.
22. Schrauzer GN: Selenomethionine and selenium yeast: appropriate forms of selenium for use in infant formulas and nutritional supplements. *J Med Foods* 1:201-206, 1998.
23. Schrauzer GN, unpublished observations.

Commentary

24. Bogye G, Alfthan G, Machay T: Bioavailability of enteral yeast selenium in preterm infants. *Biol Trace Elements Res* 65:143-151, 1998.
25. Kumpulainen J, Salmenperä L, Siimes MA, Koivistoinen P, Perheentupa J: Selenium status of exclusively breast-fed infants as influenced by maternal organic or inorganic selenium supplementation. *Am J Clin Nutr* 42:829-835, 1985.
26. McGuire MK, Burgert SL, Milner JA, Glass L, Kummer R, Deering R, Boucek R, Picciano MF: Selenium status of lactating women is affected by the form of selenium consumed. *Am J Clin Nutr* 58:649-652, 1993.
27. Patterson BH, Levander OA: Naturally occurring selenium compounds in cancer chemoprevention trials: A workshop summary. *Cancer Epidemiol Biomarker Prev* 6: 63-69, 1997.
28. Yang G, Yin S, Zhou R, Gu L, Yan B, Liu Y, Liu Y: Studies of safe maximal daily dietary Se-intake in a seleniferous area in China. *J Trace Elem Electrolytes Health Dis* 3:123-130, 1989.

Received August 8, 2000.

de Tijuana, Mexico
• GfS, Ingolstädter

cupational Health •

ichallon, BP 217,

versity, Manhattan, KS
Forks, ND
of Agricultural

of Medicine.

xas, Galveston, TX
ren, Toronto, Canada
rsity, Lubbock, TX
y, VT
ty.

v., Corvallis, OR
es, Cardiff, Wales, UK
y, Kumamoto, Japan
ty of Padova, Italy
land

nted Library Materials.

/ means, electronic or
t, without permission in
inions, conclusions, or
publisher.

n *Chemical Abstracts*,
e *Citation Index*, and

lished 18 times per year
sign); please add \$40 (US)

999 Riverview Dr., Suite
oth old and new addresses
eriodicals postage is paid

or the internal or personal
plus US \$00.25 per page,
r those organizations that
nged and is acceptable to
0.00 + \$00.25.

©Copyright 2001 by Humana Press Inc.
All rights of any nature, whatsoever, reserved.
0163-4984/01/8103-0189 \$16.25

Accelerated Article

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

WAYNE CHRIS HAWKES,* DARSHAN S. KELLEY,
AND PETER C. TAYLOR

*U.S. Department of Agriculture, Agricultural Research Service,
Western Human Nutrition Research Center, University of
California at Davis, One Shields Avenue, Davis, CA 95616*

Received October 7, 2000; Accepted November 15, 2000

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.

*Author to whom all correspondence and reprint requests should be addressed.

of the immune
 tion in experi-
 mental-killer (NK)
 ceptor expres-
 sivity, delayed-
 uced immunity
 have observed
 activation, de-
 (TNF) mRNA
 in tumor cells,
 creased killing
 -cells (1). Sele-
 -based antibody
 -like virus, de-
 creased CD4⁺
).

role in human
 elenium (usu-
 oliferation of
 togen (2) and
 high-affinity
 tion and pri-
 diated tumor
 humans has
 ropathy (7),
 cretinism (9).
 ale infertility
 order of un-
 eart (14) and
 n associated
), $p < 0.0001$)

nent of sev-
 enoenzymes
 are effective
 amage from
 18). Thiore-
 an essential
 ne cell acti-
 ffects of the
 T pathway
 at the virus
 selenium-
 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)		High selenium group (n = 5)	
	mean \pm SD	range	mean \pm SD	range
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

Protein
Carbohydrate
Fat
saturated
monounsaturated
polyunsaturated
Fiber*
Cholesterol*
Selenium (st)
Selenium (lc)
Selenium (h)
Iodine*
Calcium
Iron
Magnesium
Phosphorus
Zinc
Copper
Manganese
Potassium

Note: Unl
from each exj
supplement a
*Dietary c
†Estimate.

diets was th
obtained fr
all other co
analysts we
A meta
using a stab
the low sele
(10 μ g seleni
selenium gr
cal form of s
selenium in
and serum a
vaccines) me

Selenium

nium = 5) SD	range
7	20 - 44
18	170 - 184
2.6	60 - 94
.0	10.0 - 14.0
.4	18 - 29
1.7	2.8 - 31
24	1.15 - 1.67
3	1.6 - 2.2
36	1.5 - 2.3
31	1.5 - 2.6

ram protein).

o., Kalamazoo, diet (food plus ended dietary (56%), calcium ee daily meals menus, using day. Foods for m. All meals of staff mem- glasses were ed.

ided 47 µg/d dapt the sub- ts. The initial i the Harris- t was subse- ges in body ts of the diet sition of the

elenium con- elenium diet it 11.7 MJ/d) xperimental

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 Na₂⁷⁴SeO₃ (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 diphtheria 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 Sk

Durin
(d 113-116)
intraderm
The antig
five inter
tetanus to
tion units
[v/v] dilu
iodin (bic
Office of
diluted w
and 9 g sc
and tetan
water, PA
diluent w
streptase a
burg/Lahu
tively. The
mean indu
injections.
itive respo
to the seve

Humoral

A dip
and again
immune r
influenza
effect on t
body titer:
inoculation
assay (36).

Statistica

For m
subtracted
changes, a
significant
effect or t
Newman-
differences
ments obta
within-sub

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

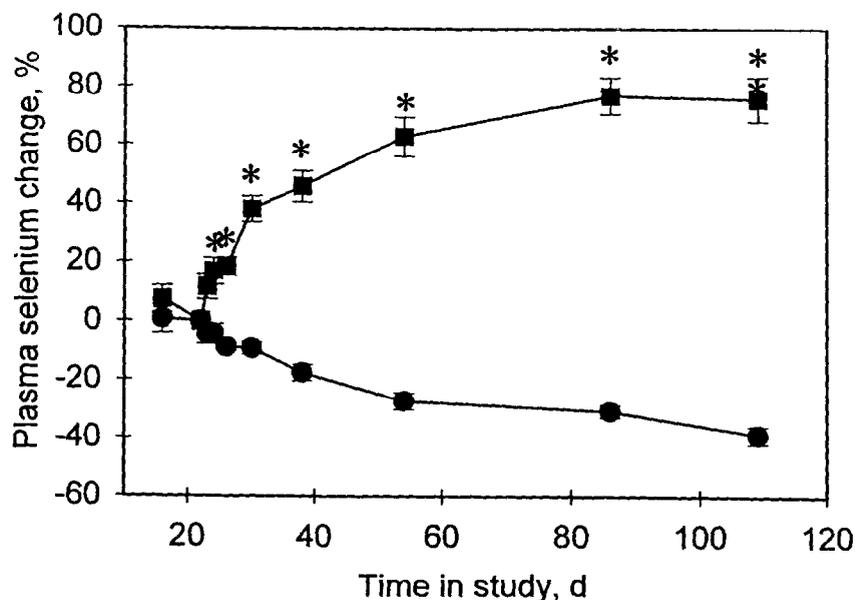


Fig. 1. Changes in blood plasma selenium. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

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

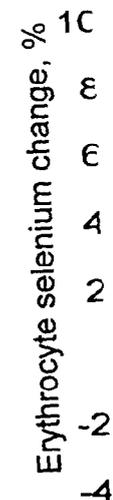


Fig. 2. Changes in erythrocyte selenium. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

clinically r
primary imm
lence with
between g
the second
lenged at
sponse fro
analysis of
when the c
specific an
the initial
t-test of log
vs $0.9 \pm 0.$
range of th

The m
selenium g
4 and Fig. 2
group, wit
ended wit
Granulocyt
counts, dec
12% in the



present the mean
the high-selenium
the time-points at

udy were com-
any correction.
(SPSS, Chicago,
nt.

changes in cir-
and decreased
eased by 27%
trations were
.4 ± 9.5 µg/L
igh-selenium
oncentrations
n glutathione
thione perox-

e experimen-
ps (Table 3).
but C3 was
ined within

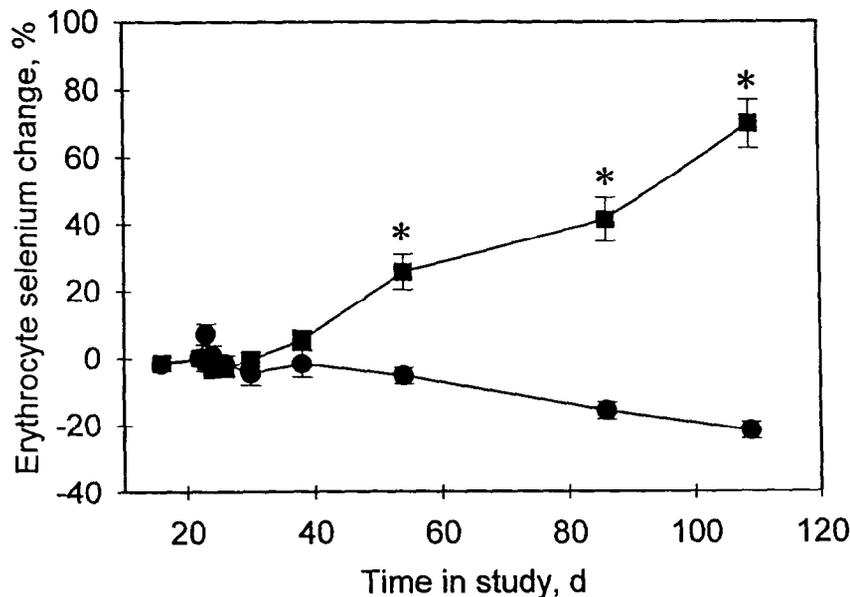


Fig. 2. Changes in erythrocyte selenium. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

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).

12,400 ± 16,400 — — —
 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).

Vol. 81, 2001

Biological Trace Element Research

Vol. 81, 2001

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)	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	6.1 ± 1.3	5.8 ± 1.4	—	0.017	0.021
Lymphocytes, thou/cu mm	1.66 ± 0.30	1.78 ± 0.16	2.04 ± 0.37	2.14 ± 0.53	—	0.019	0.007
Granulocytes, thou/cu mm	2.08 ± 0.64	2.3 ± 0.71	3.61 ± 0.97	3.3 ± 1.05	—	—	0.001
Platelets, thou/cu mm	253 ± 48	245 ± 52	281 ± 76	274 ± 69	—	—	—
Erythrocytes, million/cu mm	5.1 ± 0.52	4.9 ± 0.45	4.9 ± 0.62	4.7 ± 0.68	—	—	—
Hemoglobin, g/dL	14.9 ± 1.3	14.2 ± 1.00	14.2 ± 0.59	13.8 ± 1.07	—	<0.001	—
Hematocrit, %	44.5 ± 4.0	41.8 ± 3.3	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.

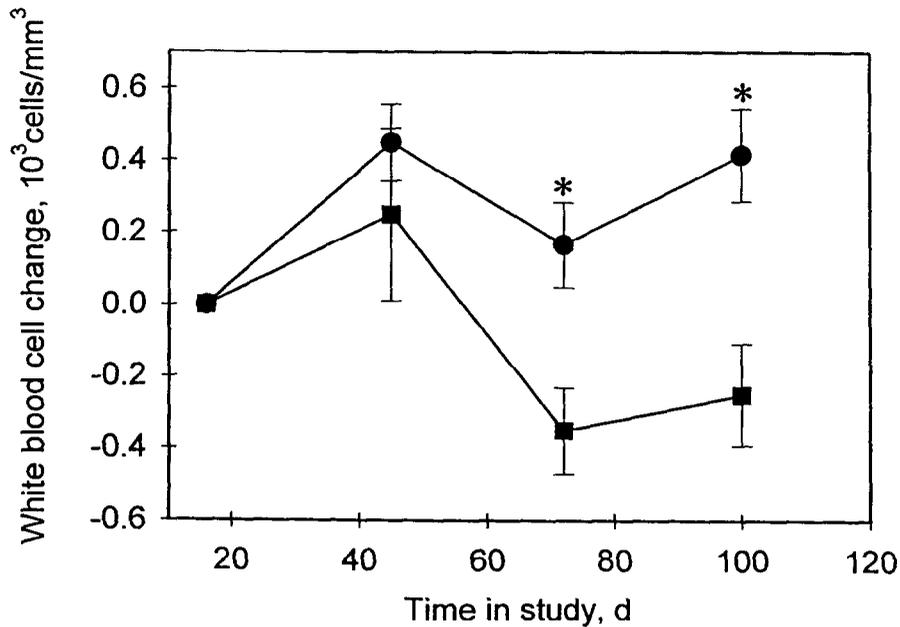


Fig. 3. Changes in white blood cell count. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

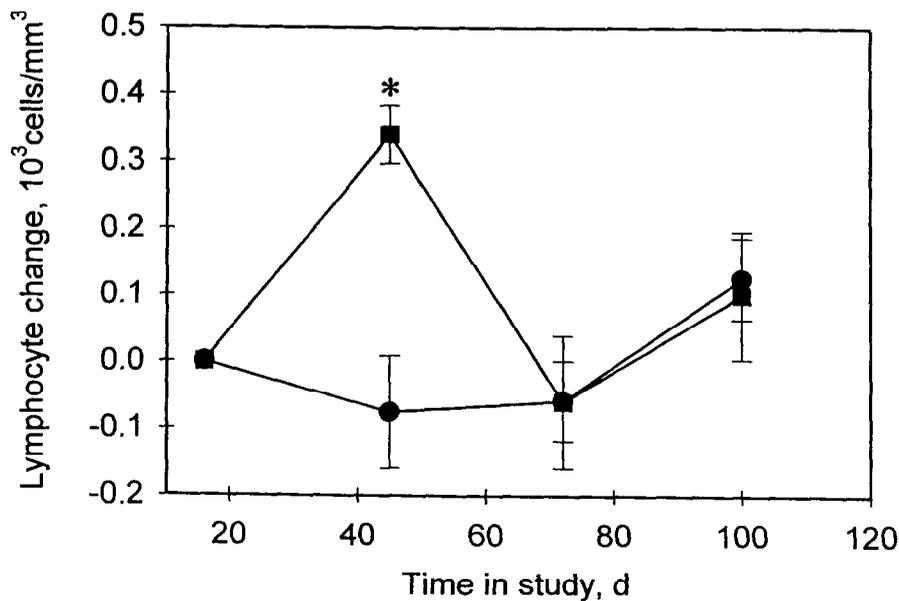


Fig. 4. Changes in lymphocyte count. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

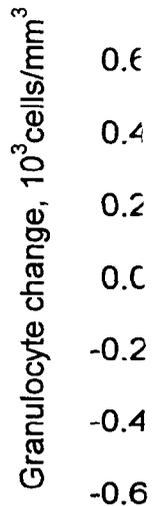
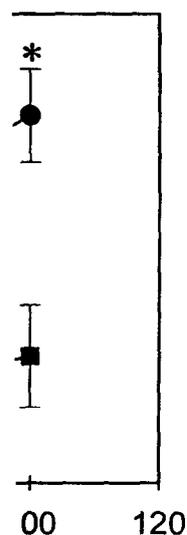


Fig. 5. Changes in granulocyte count. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were significantly different.

hemoglobin groups during plings (appreciable abundance by dietary selenium tended to include the difference toxic T-lymphocytes (7), but the treatment

In vitro, selenium affected granulocyte serum from a group (6). However, in serum from proliferated monocytes (Table 7). By ended with significant to pokeweed in the high-selenium concentrations logarithmic serum.



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.

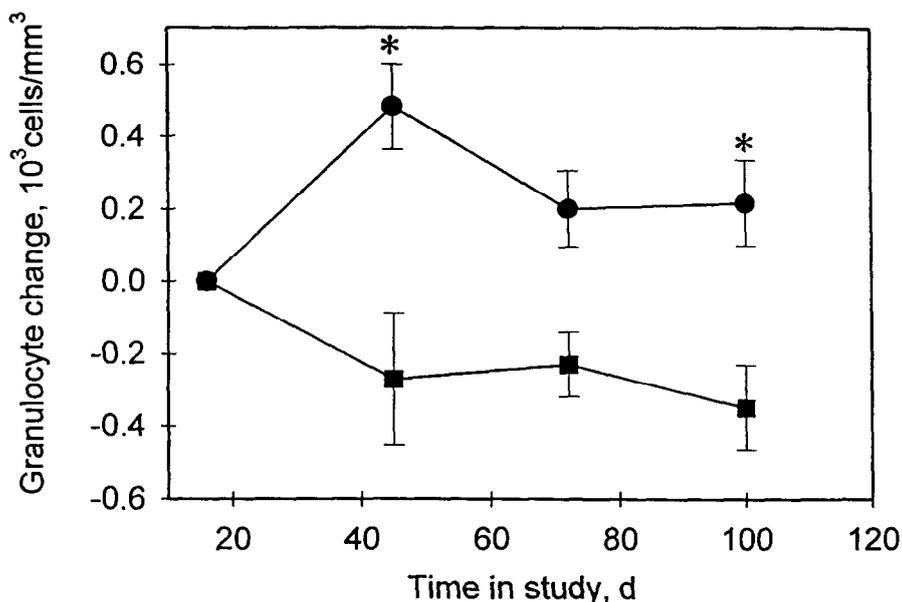
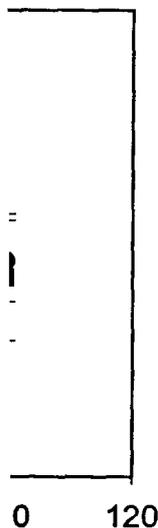


Fig. 5. Changes in granulocyte counts. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (●) or the low-selenium diet (■). Asterisks designate the time-points at which the group means were significantly different.

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



the mean within-subject changes from baseline for subjects consuming the low-selenium diet (■) or the high-selenium diet (●) at the time-points at which the group means were significantly different.

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.

Activated (HLA-DR) T cell change, 10⁶ cells/L

-2
-1
0
1
2

Fig. 6.
measured b
within-subje
diet (■) or †

CTL cell change, 10⁶ cells/L

-10
-5
0
5
10
15
20
25

Fig. 7.
by fluorescen
changes from
low-selenium

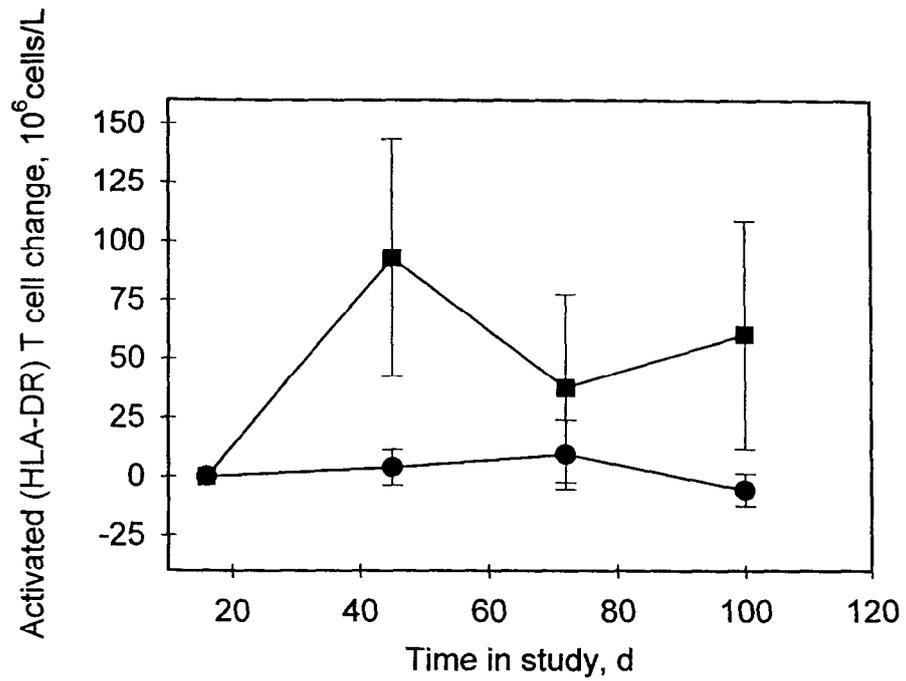


Fig. 6. Changes in HLA-DR antigen on peripheral blood lymphocytes measured by fluorescence-activated cell sorting. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●).

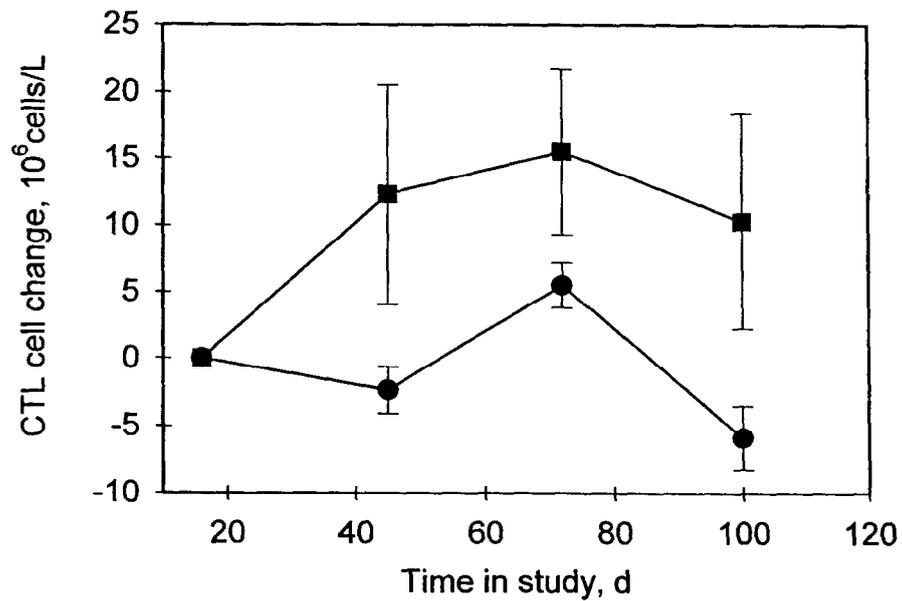


Fig. 7. Changes in cytotoxic T-lymphocytes. $CD3^+, 56^+$ cells were measured by fluorescence-activated cell sorting. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●).

Table 6
Effects of Low-Selenium and High-Selenium Diets on Mitogen-Stimulated In Vitro Proliferation of PBMNCs 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.

*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.

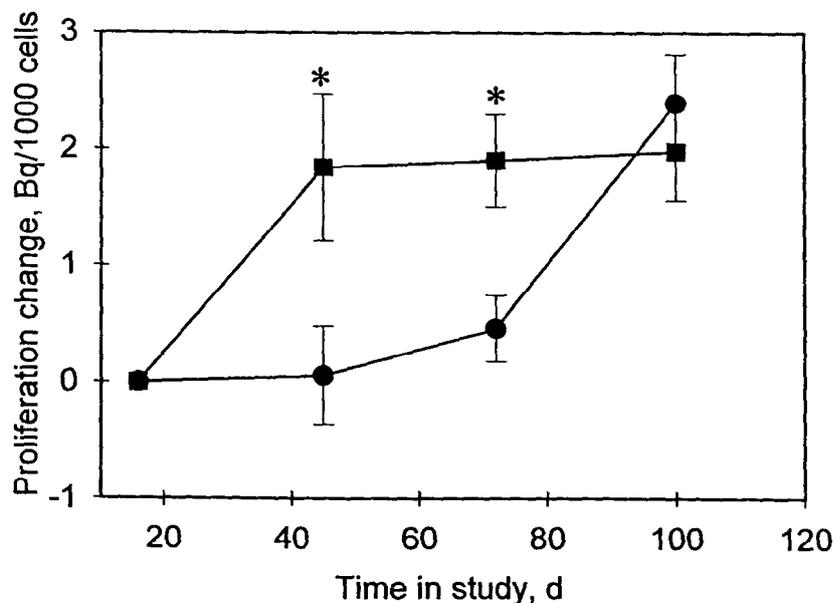


Fig. 8. Changes in lymphocyte proliferation with 1 mg/L pokeweed mitogen in autologous serum. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were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

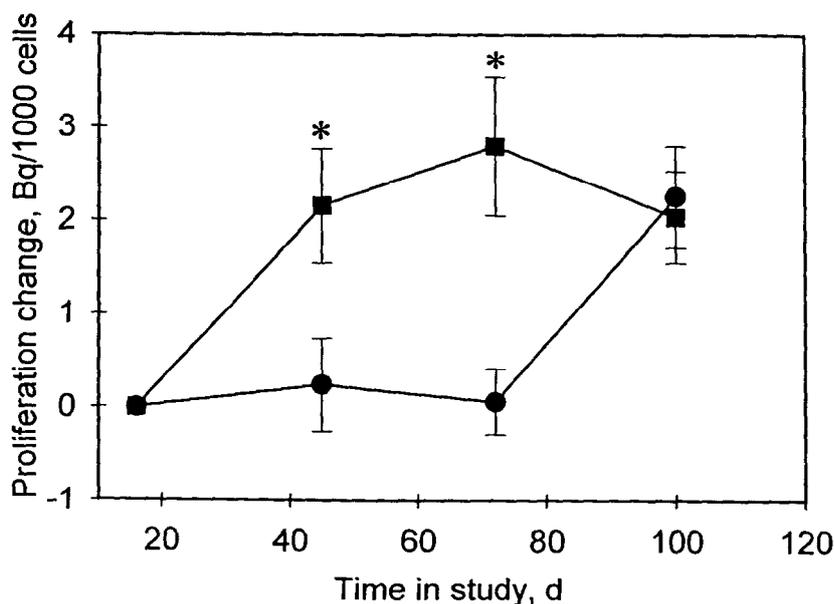


Fig. 9. Changes in lymphocyte proliferation with 2 mg/L pokeweed mitogen in autologous serum. Points represent the mean within-subject changes from baseline for subjects consuming the high-selenium diet (■) or the low-selenium diet (●). Asterisks designate the time-points at which the group means were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

Selenium a

displayed th
in the high-
nificant in t

Dietary
study (Table
responses in
after injectio
cantly decre
baseline sco

DISCUSS

The ov
There was a
body weigh
decrease in
T₃ concentra
slightly incre
tically signif
high-seleniu
the high-sel
These obser
discussed fu
were not aff

Even th
dietary sele
status. Ther
in the hum
observe the
example, sp
group, refle
lymphoid ti
have been a
turnover in
area, and w
white blood
particular e
shifting the
ies on the n
sues and/or
to follow up

Our obs
mitogen wa
7) is similar
subjects wit

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).

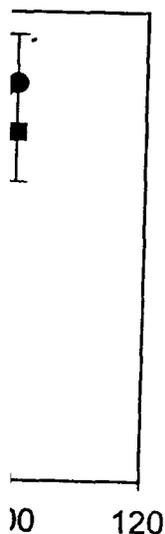
Dietary selenium did not appear to affect DHS skin responses in this study (Table 8). However, there was a general trend toward decreasing responses in both groups during the study. The induration scores at 72 h after injection of the recall antigens at the end of the study were significantly decreased 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 T_3 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



pokeweed mitogen changes from the low-selenium means were sig-



pokeweed mitogen changes from the low selenium means were sig-

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	Time	Sex × Time
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.

Selenium a.

in lymphocy (2). On the c on proliferat sodium sele were more s sodium sele metabolized tinin in our a pure selen effects of fo cological eff which typica of lymphocy an effect tha

The app vated lymph approaching report that sodium-selen interpretatio nium's incre also reporte higher levels cell activity. surface marl high-seleniu nificant. The selenite, may recent study matically lar ine (37), the

Our obs cific serum a to be the firs ing from die influenza va nmented with attributed so to diphtheria tion observe China that s tis B infectio of selenium in which sele eign antigen.

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.

ACKNOWLEDGMENTS

The work described in this article was supported by intramural funding of the US Department of Agriculture and the Agricultural Research Service. The authors gratefully acknowledge the excellent technical assistance of Ms. Linda Wong for CBCs and clinical chemistry assays. We thank the recruiting, metabolic kitchen, nursing, and administrative staff of Bionetics Corporation and the Bioanalytical Support Laboratory staff of

Selenium

WHNRC f
cially than
Medicine f
from China

Mentic
does not c
Agriculture
ucts that m
of the auth
ment of Ag

REFERE

1. R. C. McI
immune f
2. A. Peretz
phocyte r
enriched
3. M. Roy, I
plementat
cyte proli
103-114 (
4. S. Y. Yu,)
and prim.
5. L. Kiremi
plementat
lymphocy
6. X. Chen,
selenium
7. D. Ding,)
nium and
8. P. I. Mans
nium sup,
ety Of Pa.
9. J. B. Vand
Iodine an
Clin. Nutr
10. H. Krsnjav
tility in m
11. J. M. Brag
pancreatit
(1988).
12. E. Delilba
Behcet's d
13. J. R. O'de
L. W. Klas
Dis. 50, 37
14. K. Samma
in acute ir
15. M. P. Lool
Serum sele
(GSH-Px)-
virus-1 (H

condary immune

skin responses in
ous human study
plementation (38),
where significant
um supplementa-
inhibitory, or no
rimental designs,
all antigens used
sults imply that
a typical Amer-
and resistance to
mune status.

d in the current
; increased lym-
mphocytes, NK
generalized role
The decrease of
in granulocytes
rowth-regulatory
's pro-apoptotic
high-selenium
n on other com-
ections and less
mpensatory de-
ved increases in
rd animals will
re immune sys-
s. The apparent
ention (50), the
ient hosts (51),
S patients (16)
ns of selenium,
ll types of the

intramural fund-
ltural Research
technical assis-
try assays. We
ministrative staff
oratory staff of

WHNRC for their assistance with the conduct of this study. We are especially thankful to Dr. Yiming Xia of the Chinese Academy of Preventive Medicine for her assistance in obtaining high- and low-selenium rice from China.

Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture, nor does it imply approval to the exclusion of other products that may be suitable. The opinions expressed herein represent those of the authors and do not necessarily represent those of the US Department of Agriculture.

REFERENCES

1. R. C. McKenzie, T. S. Rafferty, and G. J. Beckett, Selenium: an essential element for immune function, *Immunol. Today* **19**, 342-345 (1998).
2. A. Peretz, J. Neve, J. Desmedt, J. Duchateau, M. Dramaix, and J. P. Famaey, Lymphocyte response is enhanced by supplementation of elderly subjects with selenium-enriched yeast, *Am. J. Clin. Nutr.* **53**, 1323-1328 (1991).
3. M. Roy, L. Kiremidjianschumacher, H. I. Wishe, M. W. Cohen, and G. Stotzky, Supplementation with selenium and human immune cell functions. 1. Effect on lymphocyte proliferation and interleukin 2 receptor expression, *Biol. Trace Element Res.* **41**, 103-114 (1994).
4. S. Y. Yu, Y. J. Zhu, and W. G. Li, Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong, *Biol. Trace Element Res.* **56**, 117-124 (1997).
5. L. Kiremidjianschumacher, M. Roy, H. I. Wishe, M. W. Cohen, and G. Stotzky, Supplementation with selenium and human immune cell functions. 2. Effect on cytotoxic lymphocytes and natural killer cells, *Biol. Trace Element Res.* **41**, 115-127 (1994).
6. X. Chen, G. Yang, J. Chen, X. Chen, Z. Wen, and K. Ge, Studies on the relations of selenium and Keshan disease, *Biol. Trace Element Res.* **2**, 91-107 (1980).
7. D. Ding, S. Zhang, C. Bai, and A. L. Et, The study of the relationship between selenium and kashin-beck disease, *J. Xi'an Med. Univ.* **12**, 14-18 (1991).
8. P. I. Mansell, S. P. Allison, and A. Shenkin, Reversal of a skeletal myopathy with selenium supplementation in a patient on home parenteral nutrition, 7th European Society Of Parenteral And Enteral Nutrition Congress, Paris, p. 85 (1986).
9. J. B. Vanderpas, B. Contempre, N. L. Duale, W. Goossens, N. Bebe, R. Thorpe, et al., Iodine and selenium deficiency associated with cretinism in northern zaire, *Am. J. Clin. Nutr.* **52**, 1087-1093 (1990).
10. H. Krsnjavi, B. A. Grgurevic, D. Beker, Z. Romc, and A. Krsnjavi, Selenium and fertility in men, *Trace Elements Med.* **9**, 107-108 (1992).
11. J. M. Braganza, C. D. Hewitt, and J. P. Day, Serum selenium in patients with chronic pancreatitis lowest values during painful exacerbations, *Trace Elements Med.* **5**, 79-84 (1988).
12. E. Delilbasi, B. Turan, E. Yucel, R. Sasmaz, A. Isimer, and A. Sayal, Selenium and Behcet's disease, *Biol. Trace Element Res.* **28**, 21-26 (1991).
13. J. R. O'dell, S. Lemley-Gillespie, W. R. Palmer, A. L. Weaver, G. F. Moore, and L. W. Klas Sen, Serum selenium concentrations in rheumatoid arthritis, *Ann. Rheum. Dis.* **50**, 376-378 (1991).
14. K. Sammalkorpi, V. Valtonen, G. Alfthan, A. Aro, and J. Huttunen, Serum selenium in acute infections, *Infection* **16**, 222-224 (1988).
15. M. P. Look, J. K. Rockstroh, G. S. Rao, K. A. Kreuzer, S. Barton, H. Lemoch, et al., Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection, *Eur. J. Clin. Nutr.* **51**, 266-272 (1997).

16. M. K. Baum, G. Shor-Posner, S. Lai, G. Zhang, H. Lai, M. A. Fletcher, et al., High risk of HIV-related mortality is associated with selenium deficiency, *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **15**, 370–374 (1997).
17. A. Campa, G. Shor-Posner, F. Indacochea, G. Y. Zhang, H. Lai, D. Asthana, et al., Mortality risk in selenium-deficient HIV-positive children, *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **20**, 508–513 (1999).
18. R. Ebert-Dumig, J. Seufert, D. Schneider, J. Kohrle, N. Schutze, and F. Jakob, Expression of selenoproteins in monocytes and macrophages—Implications for the immune system, *Med. Klin.* **94**, 29–34 (1999).
19. C. K. Sen, Cellular thiols and redox-regulated signal transduction, *Curr. Top. Cell Regul.* **36**, 1–30 (2000).
20. E. R. Hofman, M. Boyanapalli, D. J. Lindner, W. H. Xiao, B. A. Hassel, R. Jagus, et al., Thioredoxin reductase mediates cell death effects of the combination of beta interferon and retinoic acid, *Mol. Cell. Biol.* **18**, 6493–6504 (1998).
21. E. W. Taylor, C. S. Ramanathan, R. K. Jalluri, and R. G. Nadimpalli, A basis for new approaches to the chemotherapy of AIDS: novel genes in HIV-1 potentially encode selenoproteins expressed by ribosomal frameshifting and termination suppression, *J. Med. Chem.* **37**, 2637–2654 (1994).
22. E. W. Taylor, A. Bhat, R. G. Nadimpalli, W. Zhang, and J. Kececioglu, HIV-1 encodes a sequence overlapping env gp41 with highly significant similarity to selenium-dependent glutathione peroxidases, *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **15**, 393–394 (1997).
23. E. W. Taylor, Selenium and cellular immunity—Evidence that selenoproteins may be encoded in the + 1 reading frame overlapping the human CD4, CD8, and HLA-DR genes, *Biol. Trace Element Res.* **49**, 85–95 (1995).
24. W. C. Hawkes, E. C. Wilhelmsen, and A. L. Tappel, Abundance and tissue distribution of selenocysteine-containing proteins in the rat, *J. Inorg. Biochem.* **23**, 77–92 (1985).
25. Metropolitan Life Insurance Co., *New Height and Weight Tables. 1979 Build Study*, Society of Actuaries and Association of Life Insurance Medical Directors of America, Chicago, IL (1980).
26. National Research Council (NRC) Committee on Dietary Allowances. *Recommended Dietary Allowances*, National Academy Press, Washington, DC (1989).
27. Panel on Dietary Antioxidants and Related Compounds, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, and Food and Nutrition Board, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington, DC (2000).
28. US Department of Agriculture, *Composition of Foods*, Handbook 8, Government Printing Office, Washington, DC (1991).
29. National Research Council (NRC) Committee on Dietary Allowances, *Recommended Dietary Allowances*, National Academy Press, Washington, DC (1980).
30. D. S. Kelley, P. C. Taylor, G. J. Nelson, and B. E. Mackey, Dietary docosahexaenoic acid and immunocompetence in young healthy men, *Lipids* **33**, 559–566 (1998).
31. W. C. Hawkes, and M. A. Kutnink, High-performance liquid chromatographic-fluorescence determination of traces of selenium in biological materials, *Anal. Biochem.* **241**, 206–211 (1996).
32. W. C. Hawkes, and K. A. Craig, Automated continuous-flow colorimetric determination of glutathione peroxidase with dichloroindophenol, *Anal. Biochem.* **186**, 46–52 (1990).
33. W. C. Hawkes, and K. A. Craig, Adaptation of the bicinchoninic acid protein assay to a continuous-flow autoanalyzer, *Lab. Robot. Autom.* **3**, 13–17 (1990).
34. D. S. Kelley, L. B. Branch, and J. M. Iacono, Nutritional modulation of human immune status, *Nutr. Res.* **9**, 965–975 (1989).
35. D. S. Kelley, P. C. Taylor, G. J. Nelson, P. C. Schmidt, B. E. Mackey, and D. Kyle, Effects of dietary arachidonic acid on human immune response, *Lipids* **32**, 449–456 (1997).

36. J. C. Hier and hemato
- 18, 824–83
37. V. J. John tory cytol
- but not to
38. F. Girodc P. Prezios
- nity and i
- MIN. VII
39. J. K. Reffe min e on
- parainflue
40. P. D. Jelin son, The e
- experimere
- selenium
41. W. S. Swe J. B. Meld
- weaned by
42. D. A. Knig competenc
43. M. Bonom selenium :
- haemodial
44. M. Weide, cer patien
- Trace Eleme*
45. L. D. Kolle responses
46. D. J. Blodg amin E sel
- 38, 37–44 (
47. A. Fairbro methionine
- 19, 836–84
48. M. Zhu an with esopl
- Zhongliu Zi*
49. N. Laceter. sodium sel
- ing adequa
50. L. C. Clark al., Effects o
- noma of th
- (1996).
51. M. A. Beck Increased v
- mice, *J. Infe*

cher, et al., High risk
y, *J. Acquired Immune*

si, D. Asthana, et al.,
Acquired Immune Defic.

and F. Jakob, Expres-
sions for the immune

ction, *Curr. Top. Cell*

u. Hassel, R. Jagus, et
bination of beta inter-

calli, A basis for new
-1 potentially encode
nation suppression, *J.*

ioглу, HIV-1 encodes
nilarity to selenium-
Yndr. Hum. Retrovirol.

selenoproteins may be
l, CD8, and HLA-DR

e and tissue distribu-
hem. 23, 77-92 (1985).
979 Build Study, Soci-
Directors of America,

vances. *Recommended*
1989).

ommittee on Upper
1 and Uses of DRIs,
ference Intakes, and
, *Vitamin E, Selenium,*
)).

3, Government Print-

vances, *Recommended*
1980).

ary docosaheanoic
559-566 (1998).

d chromatographic-
cal materials, *Anal.*

orimetric determina-
Biochem. 186, 46-52

ic acid protein assay
1990).

ulation of human

lackey, and D. Kyle,
e, *Lipids 32, 449-456*

36. J. C. Hierholzer, M. T. Suggs, and E. C. Hall, Standardized viral hemagglutination and hemagglutination tests. II. Description and statistical evaluation, *Appl. Microbiol.* **18**, 824-833 (1969).
37. V. J. Johnson, M. Tsunoda, and R. P. Sharma, Increased production of proinflammatory cytokines by murine macrophages following oral exposure to sodium selenite but not to seleno-L-methionine, *Arch. Environ. Contam. Toxicol.* **39**, 243-250 (2000).
38. F. Girodon, P. Galan, A. L. Monget, M. C. Boutron-Ruault, P. Brunet-Lecomte, P. Preziosi, et al., Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: a randomized controlled trial. MIN. VII. AOX. geriatric network, *Arch. Intern. Med.* **159**, 748-754 (1999).
39. J. K. Reffett, J. W. Spears, and T. T. Brown, Jr., Effect of dietary selenium and vitamin E on the primary and secondary immune response in lambs challenged with parainfluenza-3 virus, *J. Anim. Sci.* **66**, 1520-1528 (1988).
40. P. D. Jelinek, T. Ellis, R. H. Wroth, S. S. Sutherland, H. G. Masters, and D. S. Petter-son, The effect of selenium supplementation on immunity and the establishment of experimental haemonchus-contortus infection in weaner merino sheep fed a low selenium diet, *Aust. Vet. J* **65**, 214-217 (1988).
41. W. S. Swecker, Jr., D. E. Eversole, C. D. Thatcher, D. J. Blodgett, G. G. Schurig, and J. B. Meldrum, Influence of supplemental selenium on humoral immune responses in weaned beef calves, *Am. J. Vet. Res.* **50**, 1760-1763 (1989).
42. D. A. Knight and W. J. Tyznik, The effect of dietary selenium on humoral immuno-competence of ponies, *J. Anim. Sci.* **68**, 1311-1317 (1990).
43. M. Bonomini, S. Forster, F. De Risio, J. Rychly, B. Nebe, V. Manfrini, et al., Effects of selenium supplementation on immune parameters in chronic uraemic patients on haemodialysis, *Nephrol. Dial. Transplant.* **10**, 1654-1661 (1995).
44. M. Weide, D. Zhaoming, L. Baoliang, and X. Huibi, Study of immune function of cancer patients influenced by supplemental zinc or selenium zinc combination, *Biol. Trace Element Res.* **28**, 11-20 (1991).
45. L. D. Koller, J. H. Exon, P. A. Talcott, C. A. Osborne, and G. M. Henningsen, Immune responses in rats supplemented with selenium, *Clin. Exp. Immunol.* **63**, 570-576 (1986).
46. D. J. Blodgett, E. T. Kornegay, G. G. Schurig, J. B. Meldrum, and E. D. Bonnette, Vitamin E selenium and immune response to selected antigens in swine, *Nutr. Rep. Int.* **38**, 37-44 (1988).
47. A. Fairbrother and J. Fowles, Subchronic effects of sodium selenite and seleno-methionine on several immune-functions in mallards, *Arch. Environ. Contam. Toxicol.* **19**, 836-844 (1990).
48. M. Zhu and A. L. Et, Influence of dietary selenium level on immune function of rats with esophageal tumors induced by methylbenzyl nitrosamine nmbza, *Zhonghua Zhongliu Zazhi* **14**, 42-44 (1992).
49. N. Lacetera, U. Bernabucci, B. Ronchi, and A. Nardone, The effects of injectable sodium selenite on immune function and milk production in Sardinian sheep receiving adequate dietary selenium, *Vet. Res.* **30**, 363-370 (1999).
50. L. C. Clark, G. F. Combs, Jr., B. W. Turnbull, E. H. Slate, D. K. Chalker, J. Chow, et al., Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial, *J. Am. Med. Assoc.* **276**, 1957-1963 (1996).
51. M. A. Beck, P. C. Kolbeck, Q. Shi, L. H. Rohr, V. C. Morris, and O. A. Levander, Increased virulence of a human enterovirus (Coxsackievirus B3) in selenium-deficient mice, *J. Infect. Dis.* **170**, 351-357 (1994).