The Journal of Trace Elements in Experimental Medicine 11:11–27 (1998)

Adequacy or Deprivation of Dietary Selenium in Healthy Men: Clinical and Psychological Findings

John W. Finley* and James G. Penland

Agricultural Research Service, Grand Forks Human Nutrition Research Center, United States Department of Agriculture, Grand Forks, North Dakota

Thirty healthy young men were fed diets that provided either 32.6 or 226.5 μg of selenium (Se)/d for 105 d. The high Se diet significantly elevated plasma Se and platelet glutathione peroxidase activity. Selenium balance of subjects consuming the high Se diet was >100 μg/d, whereas subjects on the low Se diet were in approximately zero balance. High dietary Se significantly improved mood; specifically, subjects on the high Se diet improved in the clearheaded/confused, elated/depressed, composed/anxious, and confident/unsure subscores, and total mood disturbance was less in men consuming the high Se diet. These data show that North American men are able to stay in zero Se balance on as little as 24 μg/d. Additionally, they show that psychological function, specifically mood, can be influenced by increasing or decreasing the amount of Se in the diet. J. Trace Elem. Exp. Med. 11:11–27, 1998. © 1998 Wiley-Liss, Inc.†

Key words: selenium; human; assessment; balance; mood

INTRODUCTION

Because of its essentiality for domestic animals [1] and because of its unique metabolic pathways into proteins [2], the trace element selenium [Se] has been the object of considerable research interest in recent years. Although the metabolic pathways whereby Se is incorporated into selenoproteins have been elucidated and several mammalian selenoproteins have been identified and characterized, there is disagreement over the primary biological role of Se in vivo. With the discovery that Se is a component of glutathione peroxidase [3] (GSH-Px), much attention was focused on the possible role of Se in general antioxidant protection for the cell. However, in

The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

*Correspondence to: John W. Finley, USDA, ARS, GFHNRC, P.O. Box 9034, UND Station, Grand Forks, ND 58202–9034.

Received 30 April 1996; Accepted 10 December 1997

TABLE I. Diets Consumed During Study

A. High selenium diet

Day 1	Day 2	Day 3
Orange juice	Grapefruit juice	Orange juice
Pork sausage	Pork sausage	Cheerios
Special K	Golden crisp	Sugar
Sugar	Nondairy creamer	Nondairy creamer
Nondairy creamer	Whole wheat bread	English muffin
Blueberries	Butter	Peanut butter
Whole wheat bread		Strawberry jelly
Butter		
Grape Kool-Aid	Cherry Kool-Aid	
Softshell taco:	Chili w. beans	White hamburger bun
flour tortilla	Cheddar cheese	Baked hamburger
ground beef	Lettuce	Ketchup
cheddar cheese	Ranch dressing	Augratin potatoes
lettuce	White dinner roll	Peas
taco sauce	Butter	Milk chocolate bar
Pound cake	Brownie	
Mandarin oranges		
Cran-raspberry drink	Apple juice	Lemonade Kool-Aid
Pork noodle casserole	Beef macaroni casserole	Spaghetti w. meat sauce
Broccoli	Coleslaw	Green beans
White dinner roll	White dinner roll	Garlic bread
Margarine	Butter	Pineapple chunks
Pears	Sweetened strawberries	
Apple juice		
White wheat bread	Plain bagel	Chex mix:
Peanut butter	Cream cheese	cashews
		Corn Chex
		pretzels

B. Low selenium diet

Day 1	Day 2	Day 3
Orange juice	Grapefruit juice	Orange juice
Pork sausage	Pork sausage	Rice krispies
Corn Chex	Cornflakes	Sugar
Sugar	Sugar	Nondairy creamer
Nondairy creamer	Nondairy creamer	White bread
Blueberries	Strawberries	Peanut butter
		Strawberry jelly
Grape Kool-Aid	Cherry Kool-Aid	Root beer
Taco salad:	Chili w. beans	Baked hamburger
tortilla chips	Lettuce	Ketchup
ground beef	Ranch dressing	Augratin potatoes
cheddar cheese	Saltines	Peas and carrots
lettuce	Butter	Strawberry banana Jello
taco sauce	Apple crisp	Milk chocolate bar
French dressing		
Lemon pie w. Cool Whip		

TABLE I. Continued

B. Low selenium diet

Day 1	Day 2	Day 3
Cran-raspberry drink	Apple juice	Lemonade Kool-Aid
Crispy pork	Beef w. mushrooms	Spaghetti w. meat sauce
Mashed potatoes	Brown gravy	Green beans
Brown gravy	Tater Tots	Pineapple chunks
Broccoli	Ketchup	
Butter	Coleslaw	
Mandarin oranges	Chocolate pudding	
Milk chocolate bar		
Apple juice	Cran-raspberry drink	Grape juice
Ritz crackers	Fritos	Chex mix:
Peanut butter		peanuts
Pears		corn Chex
		pretzels

recent years, the importance of other biological roles of Se has been demonstrated by the discovery of new selenoproteins, including proteins that have priority for Se over GSH-Px when Se is limiting [4], and by reports of supranutritional amounts of Se having a cancer-protective effect [1]. Thus the question of the primary biological role(s) of Se is still unanswered.

Understanding of the biological role of Se in humans has been further hampered by a lack of controlled studies and by the unsuitability of radioactive ^{75}Se as a tracer. The primary purpose of the present study was to investigate the suitability of stable isotopes of Se for studying Se bioavailability in humans fed representative North American diets. However, the design of the study required that diets either low or high in Se be fed for a relatively long period of time. In the course of the feeding study, we did multiple clinical tests and determined Se balance on the subjects; this report gives the results of those tests.

In recent years, there also have been several reports that Se influences psychological function, including sleep behavior in rats [5] and mood in humans [6,7]. However, previous human studies were conducted under relatively uncontrolled conditions and for relatively short periods of time. Thus another objective of the present study was to determine whether feeding diets different in Se for a relatively long period had any effect on mood in healthy young men.

MATERIALS AND METHODS

Volunteers and Diets

Thirty healthy young men (nonsmokers and not taking mineral supplements) between the ages of 18 and 45 years participated in this study. All subjects were informed in detail as to the nature of the research. The study was approved by the Institutional Review Board of the University of North Dakota and the US Department of Agriculture and followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding human subjects.

TABLE II. Nutrient Composition (3 day average) of Diets

	Baseline		Analyzed Values ± SD		Supplement		Total		
	Hi Se	Low Se	Hi Se	Low Se	Hi Se	Low Se	Hi Se	Low Se	RDA males[a]
Energy, Kcals	2,500	2,500							
Protein, g	81	65							
Protein, % kcals	13	10							
Fat, g	99	103							
Fat, % kcals	35	36							
Linoleic/TFSA	0.40	0.49							
Cholesterol, mg	225	156							
CHO, g	333	344							
CHO, % kcals	52	54							
Minerals:									
Calcium, Mg	606	445	582 ± 25[b]	425 ± 19[b]	275	275	857	700	800
Copper, mg	1.7	1.4	1.3 ± 0.05[b]	0.95 ± 0.05[b]	1.1	1.5	2.4	2.45	1.5–3.0
Iron, mg	22	15	24 ± 1.06[b]	16 ± 0.76[b]	11	19	10	35	10
Potassium, mg	2,786	3,073	–	–	–	–	2786	3073	1,600–2,000
Magnesium, mg	294	260	267 ± 6.5[b]	239 ± 9[b]	–	–	267	239	350
Manganese, mg	4.4	3.0	3.6 ± 0.15[b]	2.3 ± 0.13[b]	–	–	3.6	2.3	2.0–5.0
Sodium, ng	3,428	3,425	–	–	–	–	3,428	3,425	500
Phosphorus, mg	1,268	1,026	1,163 ± 41[b]	970 ± 46[b]	–	–	1,163	970	800
Zinc, mg	14	10	12 ± 0.6[b]	7 ± 0.32[b]	–	–	12	7	15
Selenium, mcg	–	–	189 ± 11[c]	21 ± 5.7[c]	–	–	189	21	70

[a]1989 revision, 10th ed., Males 25–50 years old.
[b]ICAP analysis based on triplicate analysis of each day of the 3-day menu cycle.
[c]Selenium Hydride Generation Analysis based on one sample of each day of the 3-day menu cycle that was analyzed in triplicate.

TABLE II. Continued

	Baseline		Analyzed Values ± SD		Supplement		Total		
	Hi Se	Low Se	Hi Se	Low Se	Hi Se	Low Se	Hi Se	Low Se	RDA males[a]
Water-Soluble Vitamins:									
Vitamin C, mg	202	266							60
Thiamin, mg	3.0	2.3							1.5
Riboflavin, mg	2.2	1.6							1.7
Niacin, mg	29	24							19
Vitamin B_6, mg	2.3	2.4							2.0
Folate, mcg	389	382							200
Vitamin B_{12}, mcg	5.2	3.9							2.0
Fat-Soluble Vitamins:									
Vitamin A, RE	1,141	1,034							1,000
Vitamin D, mcg	1.8	1.1			5	5	6.8	6.18	5
Vitamin E, mg (alpha tocopherol)	4.3	4							10

All subjects were given a health screen before the study began. This included determination of plasma Se concentration and blood GSH-Px activity. Given a choice, subjects were selected with values that were nearest to the mean.

All food consumed by the subjects for the duration of the study was provided by the Grand Forks Human Nutrition Research Center (GFHNRC). Subjects consumed one of two mixed Western diets, with a 3-day rotating menu; the diets were not identical and were formulated to be either low or high in Se (Table I). The primary difference between the two diets was that the low Se diet did not contain as many wheat products as the high Se diet and that the low Se diet used meat (beef and pork) with low concentrations of Se. The low Se beef was from animals that came directly from pastures with marginal Se content; the low Se pork was produced by feeding a torula yeast diet to weanling pigs. The low Se beef averaged 6.0 μg Se/100g and the low Se pork averaged 2.6 μg Se/100g. The Se content of all foods was determined before preparation of diets. The Se content of the diet was checked monthly by preparing duplicate daily diets for a 3-day rotation. Diets were formulated to be adequate in all other nutrients (Table II); because of an unforseen error, however, the low Se diet contained more vitamin C and β-carotene.

During the balance period, the diet low in Se provided 24.4 ± 1.8 μg Se/d and the adequate diet provided 167.5 ± 10.0 μg Se/d, based on an intake of 2,500 calories/d; this provided daily Se intakes of 32.6 or 226.5 μg Se/d (3357 average daily caloric intake). Subjects were also allowed limited access to several optional foods (coffee, gum, diet soft drinks, pepper, and salt). A maximum of 0.3 μg of additional Se could have been consumed daily if all of the optional foods had been consumed at their maximal allowable amounts. All water, from the home and work of volunteers, was found to contain nondetectable concentrations of Se (<0.1 ng/mL). Subjects who left their home or work for extended periods took along their home water to consume for the duration of their absence. For logistical purposes, the subjects in the study were divided into two equal groups, which began the study 6 months apart.

Blood Draws

A blood sample was taken the first morning of the study to determine baseline clinical values. Subjects were then assigned to their diets, and further blood samples were taken at 3-week intervals until the end of the study (day 105).

Psychological Measures

Subjects were asked to complete questionnaires at regular intervals in order to assess the influence of Se intake on mood states and sleep. The Profile of Mood States-Bi Polar form (POMS-BI) [8] was filled out after breakfast once each week throughout the study. The POMS-BI is a standardized questionnaire containing 72 adjectives that the respondent rates by using a 4-point scale from "much like this" to "much unlike this" to describe his or her mood state; it requires 5–10 min to complete. The POMS-BI yields six specific measures of mood state, agreeable-hostile, clearheaded-confused, composed-anxious, confident-unsure, elated-depressed, energetic-tired, and a general measure labeled total mood disturbance (TMD). To complement the weekly POMS-BI, the Global Vigor Affect Scale (GVAS) [9] and a sleep behavior inventory (SBI) were completed on Monday, Tuesday, Wednesday, and Thursday of weeks 1, 4, 7, 10, 13, and 15.

Selenium Balance

Selenium balance was determined for all subjects the last 21 days of the study; this period also coincided with the beginning of the stable isotope period. Duplicate diets were prepared and analyzed for Se content. All feces and urine were collected and analyzed for Se content. Balance was computed on pooled 21-day data.

Laboratory Analyses-Selenium Status

Plasma selenium was analyzed by Zeeman graphite furnace atomic absorption spectrometry (ZGFAA) [11]) after dilution with 1% Triton X-100. Quality control was maintained by running standards (UTAK #66816, trace element control and NIST-SRM #1598, bovine serum) and only accepting runs in which the standards were within the reference range. The detection limit was 8 ng/mL; the within run coefficient of variation (CV) averaged 3.5% and the run to run CV averaged 4.5%.

Selenium measures used to calculate balance (diets, urine, and feces) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Because these samples also contained stable isotopes of Se, they were prepared and analyzed as previously described for the preparation of samples containing stable isotopes [12]. Samples were introduced into the argon plasma by a custom-built hydride generator; the total amount of Se present was calculated from ICP-MS intensity data by a computer program developed for use with stable isotopes. Quality control was maintained by running serum trace element standards (UTAK #66816) after every eight samples. If the standard was out of range, the samples from that portion of the run were repeated. Samples were analyzed in duplicate, and the detection limit for this method was ~1 ng/mL; the within run CV was 5.5% and the between run CV was 2.8%.

Foods used to formulate diets, and monthly dietary checks, were analyzed for Se by a previously described procedure [13] using atomic absorption spectrometry coupled to a hydride generator (HG-AAS). All samples were digested in concentrated nitric acid, followed by ashing with MgO as a matrix modifier to prevent Se volatilization in a muffle oven. The detection limit was 0.1 ng/mL.

Glutathione peroxidase activity in plasma, erythrocytes, and platelets was deter-

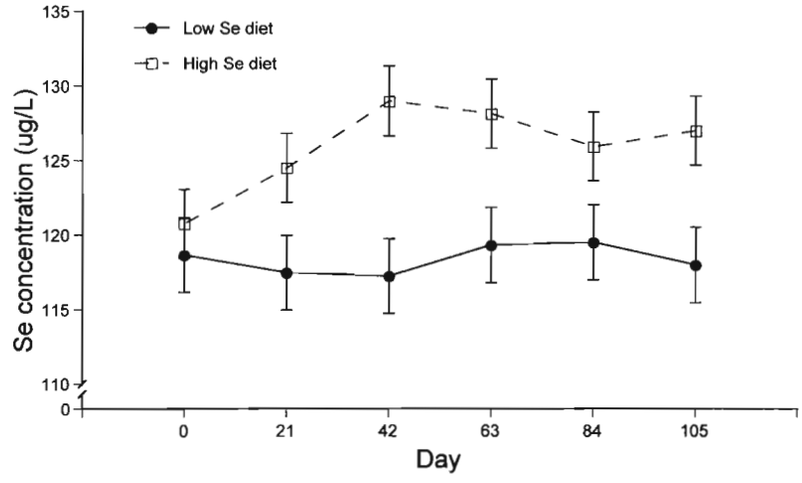

Fig. 1. Influence of dietary selenium status on concentration of selenium in the plasma of healthy men.

TABLE III. Effect of Dietary Se Concentration on Measures of Selenium Status and on Clinical Measures of Health and Metabolism

	Day of study[a]					
	0		21		42	
Variable	Lo Se	Hi Se	Lo Se	Hi Se	Lo Se	Hi Se
Se Status						
RBC Se (ng/g)	154 ± 3	156 ± 3	157 ± 3	156 ± 3	157 ± 3	159 ± 3
RBC GSH-Px (EU/mg Hb)	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0	2.2 ± 0.1	2.2 ± 0.1
Plasma GSH-Px (EU/mg pro)	2.8 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
Platelet GSH-Px	241 ± 22	288 ± 19	280 ± 19	302 ± 18	308 ± 19	328 ± 19
Total T_3 (ng/mL)	1.38 ± 0.05	1.44 ± 0.04	1.18 ± 0.05	1.16 ± 0.04	1.14 ± 0.05	1.06 ± 0.04
Free T_3 (pg/mL)[b]	4.70 ± 0.14	4.85 ± 0.13	5.04 ± 0.14	4.81 ± 0.13	5.29 ± 0.14	4.95 ± 0.14
Total T_4 (μg/dL)	7.13 ± 0.37	7.84 ± 0.35	7.00 ± 0.37	7.21 ± 0.35	6.25 ± 0.37	6.22 ± 0.35
Free T_4 (ng/dL)	1.12 ± 0.05	1.11 ± 0.04	1.16 ± 0.15	1.10 ± 0.04	1.14 ± 0.05	1.14 ± 0.05
Platelet GSH-P[c]	241 ± 22	288 ± 19	280 ± 19	302 ± 18	308 ± 19	328 ± 19
Hematology						
Ferritin (mg/dL)	88 ± 17	87 ± 16	83 ± 17	83 ± 16	79 ± 17	78 ± 16
Hemoglobin (g/dL)	15.2 ± 0.2	15.2 ± 0.2	15.2 ± 0.2	15.1 ± 0.2	15.4 ± 0.2	15.4 ± 0.2
Antioxidant measures						
Superoxide dismutase (EU/g Hb × 10^3)	3.3 ± 0.2	3.0 ± 0.1	3.2 ± 0.2	3.3 ± 0.1	3.3 ± 0.2	3.0 ± 0.1
Catalase (EU/mg Hb)	6.5 ± 0.54	7.0 ± 0.4	6.3 ± 0.3	6.6 ± 0.3	6.2 ± 0.3	6.7 ± 0.3
Retinol (μg/ml)[c,dc]	0.43 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.33 ± 0.02
Vitamin C (mg/dL)[d]	1.17 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.1
β-carotene (μg/mL)[c]	0.25 ± 0.03	0.19 ± 0.03	0.33 ± 0.03	0.22 ± 0.03	0.31 ± 0.03	0.21 ± 0.03
α-tocopherol (μg/mL)	19.3 ± 1.4	17.9 ± 1.3	19.3 ± 1.4	16.2 ± 1.2	18.3 ± 1.4	16.9 ± 1.3

[a]Values are means ± standard error.
[b]Effect of diet, $P = 0.10$.
[c]Effect of diet ($P < 0.05$).
[d]Effect of diet day of sampling ($P \leq 0.05$).

mined by an adaptation of the coupled enzyme method of Paglia and Valentine [14] (0.0048% H_2O_2 used as substrate).

Antioxidant Status Indicators

Catalase [15] and superoxide dismutase (SOD) activity [16,17] were determined by adaptations of published procedures. Ascorbic acid was determined by the procedure of Henry [18]. Vitamins A and E and β-carotene were determined by HPLC; the method and analytical specifications (accuracy, variability and extraction efficiencies, and correction factors) have been published previously [19].

Other Analyses

Complete blood counts were performed with a Coulter counter (Coulter model S+4, Coulter Electronics, Hialeah, FL) and ferritin concentration was determined by using a diagnostic kit (Abbott Laboratories, Diagnostic Division, Abbott Park, IL). Platelet aggregation was determined with a platelet aggregometer (Chrono-Log Corp., Haverton, PA) [20]. Glutathione was determined by a published procedure [21].

TABLE III. Continued

Day of study[a]					
63		84		105	
Lo Se	Hi Se	Lo Se	Hi Se	Lo Se	Hi Se
156 ± 3	161 ± 3	157 ± 3	160 ± 3	157 ± 3	163 ± 3
1.9 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
2.4 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
263 ± 19	295 ± 18	376 ± 20	377 ± 18	265 ± 20	305 ± 18
1.16 ± 0.05	1.18 ± 0.04	1.20 ± 0.05	1.14 ± 0.04	1.25 ± 0.05	1.24 ± 0.04
5.22 ± 0.14	4.94 ± 0.14	5.24 ± 0.15	4.97 ± 0.13	5.30 ± 0.15	4.97 ± 0.13
6.48 ± 0.37	6.79 ± 0.35	6.43 ± 0.37	6.66 ± 0.35	6.47 ± 0.37	6.55 ± 0.35
1.18 ± 0.05	1.15 ± 0.05	1.21 ± 0.05	1.17 ± 0.05	1.19 ± 0.05	1.14 ± 0.05
263 ± 19	295 ± 18	376 ± 20	377 ± 18	265 ± 20	305 ± 18
74 ± 17	74 ± 16	74 ± 17	70 ± 16	54 ± 17	52 ± 16
15.1 ± 0.2	15.3 ± 0.2	15.2 ± 0.2	15.3 ± 0.2	14.7 ± 0.2	14.9 ± 0.2
3.0 ± 0.2	3.0 ± 0.1	3.6 ± 0.2	3.2 ± 0.1	3.6 ± 0.2	3.3 ± 0.1
6.9 ± 0.3	7.2 ± 0.3	6.5 ± 0.4	7.1 ± 0.3	6.2 ± 0.4	6.5 ± 0.3
0.38 ± 0.02	0.33 ± 0.02	0.38 ± 0.02	0.35 ± 0.02	0.36 ± 0.02	0.35 ± 0.02
1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.7 ± 0.1	1.4 ± 0.1
0.33 ± 0.03	0.21 ± 0.03	0.36 ± 0.03	0.21 ± 0.03	0.38 ± 0.03	0.18 ± 0.03
19.6 ± 1.4	17.2 ± 1.3	19.2 ± 1.4	17.0 ± 1.3	19.0 ± 1.4	16.2 ± 1.2

Thyroid hormones and metabolites (TSH, Free T3, T4, total T3, and total T4) were determined with diagnostic kits (Abbott Laboratories).

Statistical Analyses

Clinical measures, selenium status measures, and balance data were analyzed by the appropriate analysis of variance.

Preliminary analysis of POMS-BI measures showed that the two groups of men were not equivalent in many mood states at the beginning of the study; therefore, further analyses utilized changes from initial mood states to determine effects of Se intakes. Data from the first and last weeks of the study were excluded and for each of

TABLE IV. Effect of Dietary Se Concentration on Se Balance*

	High Se diet[a] (μg/d)	Low Se diet[a] (μg/d)
Se intake	226.5 ± 4.6	32.6 ± 0.9
Se urinary excretion	88.7 ± 5.2	23.8 ± 1.6
Se fecal excretion	33.6 ± 2.9	14.7 ± 2.5
Se balance	104.4 ± 2.8	−6.6 ± 1.7

*All measures significantly different ($P < 0.0001$).
[a]Values are means ± standard error.

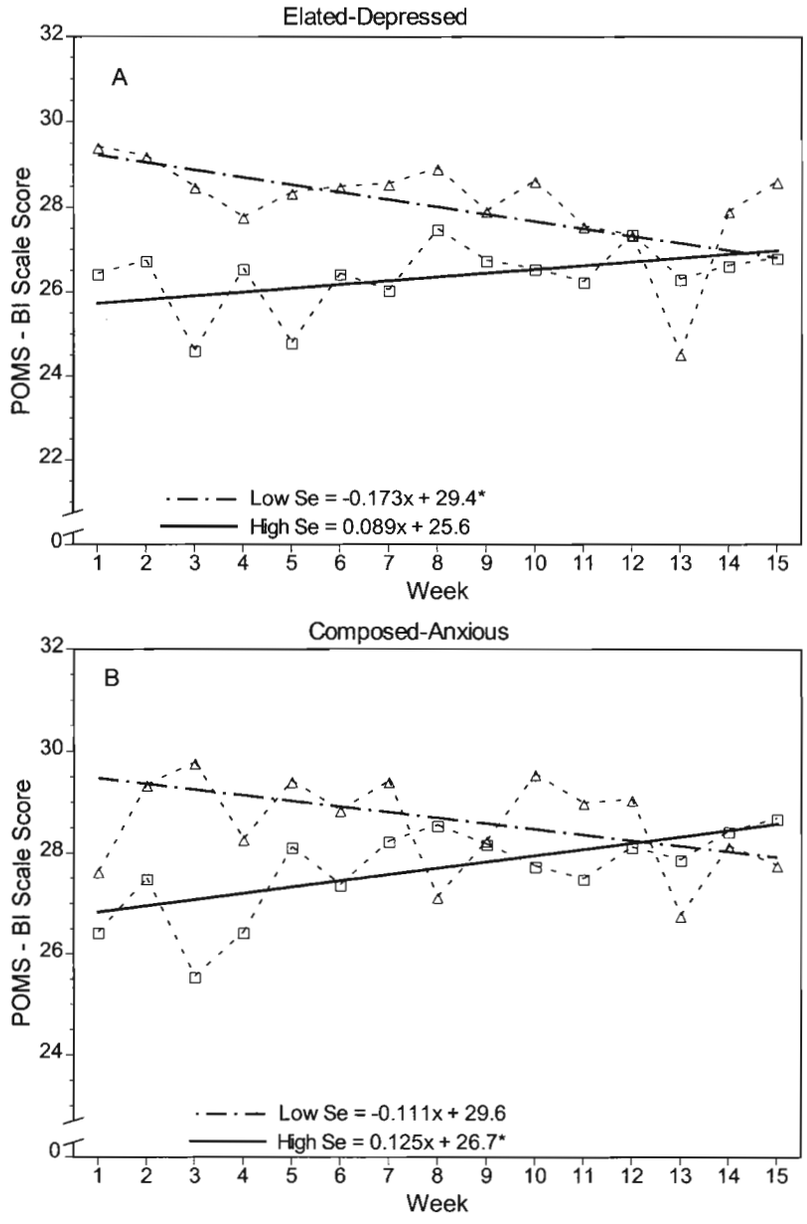

Fig. 2. Influence of dietary selenium status on mood scores of healthy men as measured with the POMS-BI. **A.** Elated/depressed subscore; higher score = more elated. **B.** Composed/anxious subscore; higher score = more composed. **C.** Confident/unsure subscore; higher score = more confident. **D.** Clearheaded/confused subscore; higher score = more clearheaded. **E.** Total mood disturbance; lower score = less mood disturbance. Equations of regression lines are listed in each graph; * = slope is different from 0 ($P < 0.05$). (Figure 2C, D and E overleaf.)

the seven measures, means were calculated for weeks 2–5 and 11–14 to obtain a more stable measure. Student's *t*-test was used to contrast the mood states of the two dietary groups. Pearson correlation coefficients were computed between clinical measures and measures of mood state for week 12 data, and tested for significance. GVAS and SBI responses were also converted to change scores prior to analysis for effects of Se intakes to achieve consistency with treatment of POMS-BI data. For each GVAS and SBI measure, means were calculated across all days in weeks 1 and 4, and separately, weeks 10 and 13. Statistical tests to analyze Se intake and status effects on GVAS and

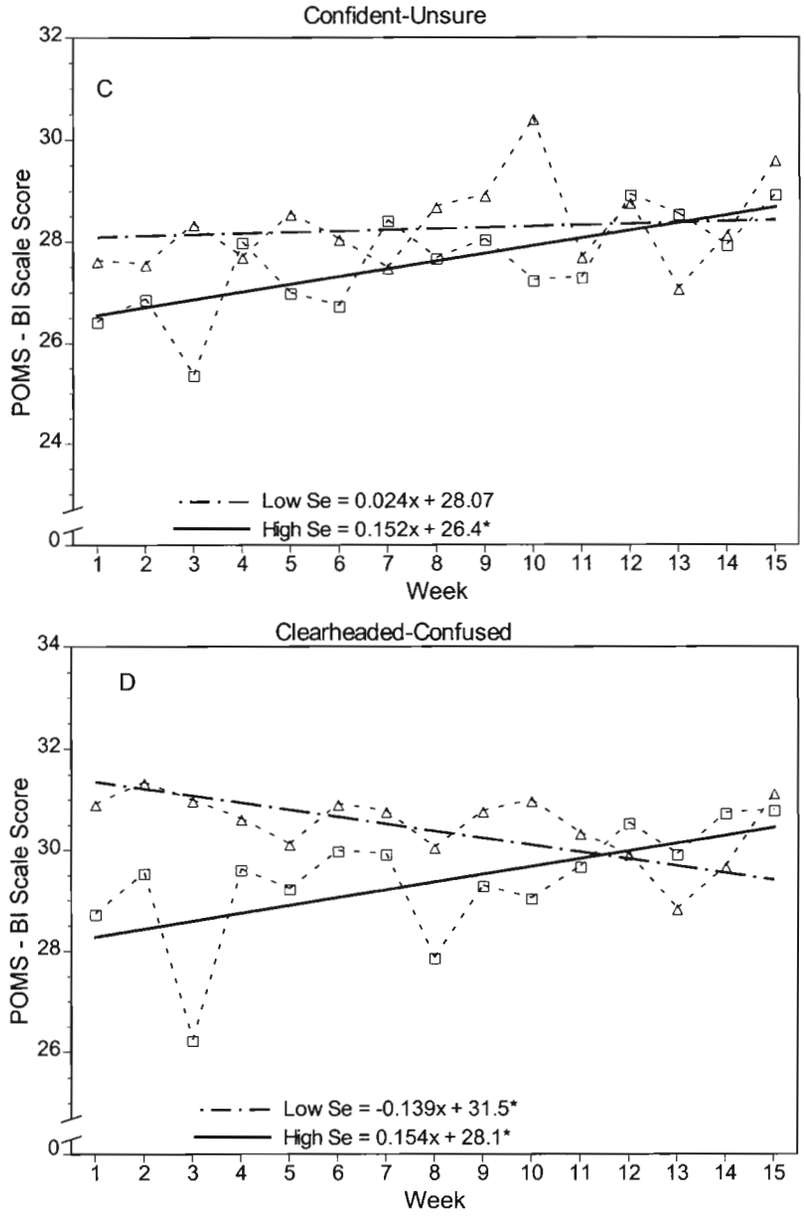

Fig. 2. Continued.

SBI responses were the same as those used to analyze the POMS-BI. Following discovery that initial mood states of the two treatment groups differed, slopes of the lines representing the relationship between mood states and time in treatment were tested for significance by the MIXED procedure in the statistical analysis package of SAS for Windows PC (Version 6.10).

RESULTS

Influence of Diet on Measures of Selenium Status

All variables were originally analyzed to determine if start date contributed significant variation (seasonal variation). When it was determined that it did not, it was removed from the statistical model. Plasma Se concentration (Fig. 1) was significantly

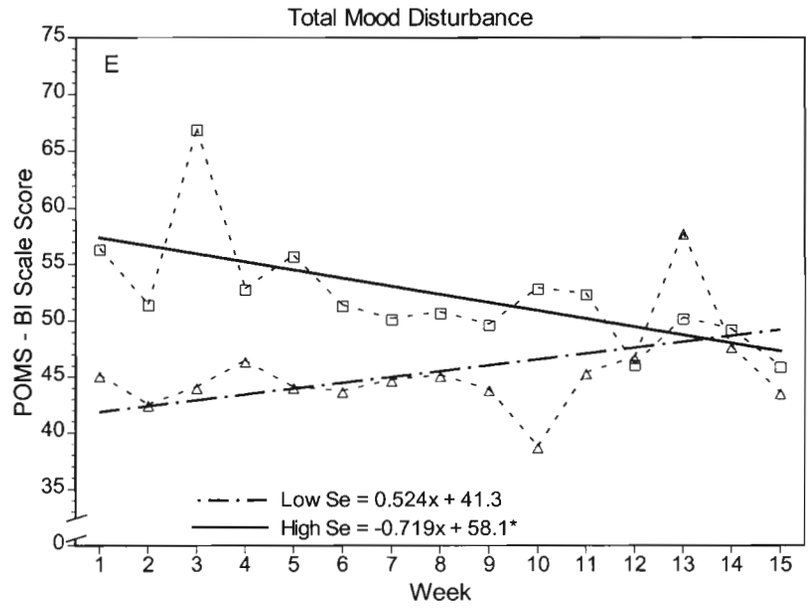

Fig. 2. Continued.

affected by diet; concentrations were not different at the beginning of the study, but were significantly different after 3 weeks. Plasma Se concentrations in subjects consuming the low Se diet were not significantly different at the end of the study (117.8 ± 2.5 μg/L) from the original baseline values; however, subjects consuming the high Se diet had significantly increased plasma Se concentrations (127.0 ± 2.3 μg/L).

The effect of diet on other measures of Se status are shown in Table III. Glutathione peroxidase in platelets was significantly lower in persons consuming the low Se diet, but the variability in this measure between timepoints makes this of questionable physiological significance. Glutathione peroxidase in plasma was not affected by dietary Se intake, but the ratio of plasma GSH-Px activity to plasma Se concentration tended to be higher ($P = .07$) in subjects consuming the low Se diet (1.42 ± 0.07 and 1.52 ± 0.07 for the high and low group, respectively). No other blood measures of Se status were significantly affected by diet, although free T3 concentrations tended ($P = 0.10$) to be lower in persons consuming the high Se diet.

Influence of Diet on Clinical Measures

Hematologic status and most measures of antioxidant status were not affected by diet (Table III). However, serum vitamin C and β-carotene were significantly higher in subjects consuming the low Se diet. These differences were probably the result of the different β-carotene and vitamin C concentrations in the two diets.

Influence of Diet on Selenium Balance

During the 21-day balance period, Se intake averaged 32.6 (low Se diet) and 226.5 μg/d (high Se diet) (Table IV). Compared to the low Se diet, urinary Se excretion was approximately threefold greater and fecal Se excretion was twofold higher for subjects on the high Se diet. Subjects fed the high Se diet were in positive balance of ~104 μg/d, whereas the low Se group was slightly negative (–6.6 μg/d).

TABLE V. Influence of Diet on Mood States Measured by the Profile of Mood States–Bipolar Form†

	POMS-BI scales						
Diet	Agreeable -Hostile	Clearheaded -Confused	Composed -Anxious	Confident -Unsure	Elated-Depressed	Energetic -Tired	Total mood Disturbance
High Se	102 ± 13[a]	106 ± 11*	106 ± 17	105 ± 10	107 ± 19*	109 ± 20	87 ± 41
Low Se	98 ± 12	96 ± 15	97 ± 17	99 ± 16	94 ± 14	97 ± 20	122 ± 102

†Change scores: (weeks 11–14)/(weeks 2–5) × 100.
[a]Mean ± standard deviation.
*Significant ($P < 0.05$) Se effect.

Influence of Diet on Psychological Measures

The POMS-BI measures showed that the diet high in Se significantly increased the clearheaded/confused, composed/anxious, and confident/unsure subscores, as well as significantly decreasing total mood disturbance. The low Se diet significantly decreased the clearheaded/confused and elated/depressed subscores (Fig. 2).

Table V shows the percent change in POMS-BI mood states from the first to the fourth month of the study; excepting total mood disturbance, numbers >100 indicate improvement. Although platelet GSH-Px activity was highly variable, it was significantly associated with all seven POMS-BI measures of mood states in the group fed low Se; higher activity was associated with more positive mood states. There were no other consistent relationships between the biochemical indices of Se status and mood states measured by the POMS-BI. GVAS and SBI responses (data not shown) were not significantly related to Se intake or biochemical indices of Se status, although several trends were apparent in the data.

DISCUSSION

One objective of this study was to feed people typical Western diets that differed in Se concentrations. Others have fed synthetic diets differing in Se content [22,23], but synthetic diets do not reflect the complexity of a self-selected diet. Because dietary Se comes predominantly from a few sources (wheat and meat), changing the Se content of the diet meant that diets would either have to be composed of the same ingredients with different Se contents, or be composed of different ingredients. We attempted the first approach by feeding diets that used meat high or low in Se; however, this did not result in a large enough difference between the diets. Consequently, we also altered ingredients in the diets that resulted in putting foods containing high amounts of Se on the high Se diet and putting foods with little Se on the low Se diet. This resulted in slightly different percentages of caloric intake coming from protein (10 vs. 13% for the low and high Se diet, respectively), carbohydrates (54 vs. 52%) and fat (36 vs. 35%). This situation does not allow for the comparison of dietary effects to be as clean as if identical diets had been fed. However, all ingredients used in the two diets were available at local grocery stores, and we believe this to be more reflective of normal North American diets that may contain different

amounts of Se than the synthetic diets that have been used in previous depletion studies [22,23].

The low Se diet did not change plasma Se concentrations, but the high Se diet significantly increased plasma Se after 1 week. Selenium concentrations in test subjects at the beginning of the study (120 μg/L) were within the normal range [24] and similar to those reported for other North American men [22]. By the end of the study, plasma Se concentrations for the high Se diet were near the upper end of the normal range (143 μg/L). Erythrocyte GSH-Px activity and Se concentrations were not significantly changed by dietary treatment. These results emphasize the difficulty in depleting Se in a subject with significant Se stores and also emphasize that plasma Se is a good indicator of short-term Se status [25].

Dietary Se intakes also resulted in substantial differences in Se balance. Subjects on the low Se diet were in essentially zero balance, whereas subjects consuming the high Se diet apparently retained >100 μg Se/d. An earlier North American study found that men required 70 μg/d for positive balance and were in negative balance when consuming 33–36 μg Se/d for 25 days [22]. Another North American study that fed men 11.4 μg/d for 34 days found a loss of ~18 μg/d [23]. Conversely, men from a low-Se area of China were in positive balance while consuming only 8.8 μg/d [30], and Dutch men consuming Se-enriched bread or meat for 5 weeks found positive balance when 43, but not 27 μg of Se was consumed per day [31].

The present study is more similar to the Dutch study [31] than other North American studies in that subjects were in only slightly negative balance while consuming 33 μg Se/d. This may be partially due to the length of time allowed for dietary adaptation. The present study allowed 85 days of adaptation before determining balance, which is 50 days longer than the longest previous North American depletion study [23]. Also, both the Dutch and the present study used a diet of natural foods, whereas the North American studies used synthetic diets. Despite these differences from previous North American studies, these data show that people living in an area of moderate to high Se intake can remain at almost zero Se balance while consuming a diet that provides much less Se on a daily basis than the current RDA.

The positive balance of 104 μg/d for people consuming the high Se was caused, in part, by greater apparent absorption. Apparent absorption for the low Se diet was 63%, but was higher (89%) for the high Se diet, a phenomenon previously reported by North American [22], Dutch [31], and New Zealander [32] studies. Although intakes of 200 μg of Se/d are not considered dangerous [33], the high positive balance in the present study indicates that people consuming relatively large amounts of Se on a daily basis may build up a considerable body burden of Se. The possible deleterious health implications of this, especially if Se supplements are abused, has been discussed [25].

Selenium is known to be important in the brain. Early work showed that Se was bound to protein in the brain [34], and Behne and co-workers [35] have shown that the brain has priority for Se when Se is limiting. More recently, Se infusion into the brain resulted in sleep inhibition in rats [5], although the dose infused was supraphysiologic. More importantly, Se supplementation has been shown to improve mood in humans supplemented with 100 μg of Se (as high Se yeast) per day for 5 weeks [7].

In this study, the high Se diet significantly improved mood, as measured by the POMS-BI. It was unfortunate that the random allotment of subjects to test diets

resulted, by chance, in subjects with more negative mood scores being assigned the diet high in Se. However, the primary objective of this study was to determine the efficacy of using stable isotopes to study selenium metabolism in humans, whereas assessment of the effects of selenium intakes and status on psychological function was initially viewed as secondary. Therefore, subjects were randomly assigned to treatment groups based only on Se status. As subjects were fed their respective diets, the group fed the high Se diet reported improved mood scores, and vice versa for the low Se group. It could be argued that the two treatment groups were regressing toward the mean. However, several factors argue against this; first, an analysis of slopes showed significant differences between treatments, but for any given measure of mood state (except clearheaded vs confused), only one group had a slope significantly different from 0. Second, regression toward the mean is far more likely to occur when measurements are made on only two occasions rather than when multiple measurements are made [36] as in this study. This is because measurement error is inversely related to the number of measurements and because models of regression toward the mean are largely based on two measurement studies. Third, several other measured mood states showed consistent, although insignificant, changes with different Se intakes. Finally, effects similar to those reported here have been observed in previous studies [7,37]. Thus we believe the data show real relationships between Se intakes and mood states. Nevertheless, future studies should avoid this potentially confounding factor by ensuring that the mood states of subjects in different groups are comparable prior to treatment. The finding that groups with similar Se status differed in mood prior to treatment suggests that mood may not be related to Se status but rather to Se intake.

The present findings are remarkably consistent with those from a controlled metabolic study recently reported by Hawkes and Hornbostel [37]. In that study, 11 healthy men living on a metabolic research unit were fed either 13 or 356 Fg Se/d for 99 days. Although Se intakes were not significantly related to mood states, a significant, positive relationship was found between erythrocyte Se concentrations and elated (vs. depressed), and aggreeable (vs. hostile) mood states in the group fed low Se. Thus the present study adds to the growing body of evidence that Se is involved in brain function and specifically affects mood. Different mood states may be affected in different individuals (and in different studies) but mood per se appears to be reliably affected. Further study is required to identify the mechanism through which Se intake influences mood states in otherwise healthy adults.

Because of the difference in dietary ingredients, Vitamin C and β-carotene concentrations were greater in the blood of subjects consuming the low Se diet, and this may be considered a confounding variable. However, two reasons mitigate the confounding effect. First, vitamin C and vitamin A were adequate in both diets; selenium was the only nutrient deficient in a diet. Second, although the mean vitamin C concentration was significantly higher in blood of subjects consuming the high Se diet, significantly elevated concentrations were found in only three of six sample periods. β-carotene concentrations in subjects on the high Se diet were significantly elevated at all time points, but the diets were balanced (from food tables) for vitamin A, which include β-carotene as a component of vitamin A, and by the end of the study blood retinol concentrations were not significantly different.

In summary, this study has found that feeding healthy young men diets high or low in Se for an extended period of time changes plasma Se concentrations, but has little

or no effect on other Se measures and measures of health and metabolism. Greater apparent absorption of Se was noted in individuals consuming high Se diets, and this, in part, resulted in considerable retention of Se on a daily basis. Subjects consuming only one-half the daily amount of Se recommended by the National Research Council [33] were in essentially zero daily balance. High Se intakes also significantly improved mood scores in subjects, a finding that has been reported elsewhere. The mechanism of the effect of Se intake on mood warrants further investigation.

REFERENCES

1. Levander O. Selenium. In Mertz W (ed): "Trace Elements in Human and Animal Nutrition," 5th ed, New York: Academic Press, 1985, pp 209–280.
2. Burk R, Hill K: Regulation of selenoproteins. In Olson R, Bier D, McCormick D (eds): "Annual Review of Nutrition," 13th ed, Palo Alto, CA: Annual Reviews, 1993, pp 65–81.
3. Rotruck J, Pope A, Ganther A, Swanson A, Hafeman D, Hoekstra W: Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588–590, 1983.
4. Burk RF, Hill KE: Some properties of selenoprotein P. Biol Trace Elem Res 33:151–153, 1992.
5. Takahata R, Matsumara H, Kantha S, et al: Intravenous administration of inorganic selenium compounds, inhibitors of prostaglandin D synthase, inhibits sleep in freely moving rats. Brain Res 623:65–71, 1993.
6. Benton D, Cook R: The impact of Se supplementation on mood. Biochem Pharma 29:1092–1098, 1991.
7. Benton D, Cook R: Selenium supplementation improves mood in a double-blind crossover trial. Psychopharmacology 102:549–550, 1990.
8. Lorr M and McNair DM: (1984) Manual. Profile of Mood States. Bi-Polar Form (POMS-BI), Educational and Industrial Testing Service, San Diego, 1984, pp 1–8.
9. Monk TH: A visual analogue scale technique to measure global vigor and affect. Psychiatry Res 27:89–99, 1989.
10. McCormack HM, Horne DJL, Sheather S: Clinical applications of visual analogue scales: A critical review. Psychol Med 18:1007–1019, 1988.
11. Lewis S, Hardison N, and Veillon C: Comparison of isotope dilution mass spectrometry with Zeeman background correction for determination of plasma selenium. Anal Chem 58:1272–1273, 1986.
12. Finley J, Vanderpool R, Korynta E: Use of stable isotopic selenium as a tracer to follow incorporation of selenium into selenoproteins. Proc Soc Exp Biol Med 210:270–277, 1995.
13. Finley J, Matthys L. Shuler T, and Korynta E: Selenium content of foods purchased in North Dakota. Nutr Res 1996 (in press).
14. Paglia D, Valentine W: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–169, 1967.
15. Aebi H: Catalase. In Berymeyer H (ed): "Methods of Enzymatic Analysis," Vol. 3, 3rd edition. New York: Academic Press, 1983 pp 273–286.
16. Winterbourn C, Hawkins R, Brain M, Carrell R: The estimation of red cell superoxide dismutase activity. J Lab Clin Med 85:337–341, 1975.
17. Sun V, Oberley L, Ying L: A simple assay for clinical assay of superoxide dismutase. Clin Chem 34/3:477–500, 1988.
18. Henry R: Determination of ascorbic acid. In Hoeber Medical Division (ed): "Clinical Chemistry Principles and Techniques." New York: Harper & Row, 1964, pp 715–719.
19. Milne D, Botnen J: Retinol, α-tocopherol, lycopene and α- and β carotene simultaneously determined by isocratic liquid chromatography. Clin Chem 32:874–876, 1986.
20. Born G: Aggregation of blood platelets by adenosine di-phosphate and its reversal. Nature 194:927–929, 1962.
21. Beutler E, Duron O, Kelly B: Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888, 1963.
22. Levander OA, Sutherland B, Morris B, King J: Selenium balance in young men during selenium depletion and repletion. Am J Clin Nutr 34:2662–2669, 1981.

23. Kasper L, Young V, Janghorbani M: Short-term dietary selenium restriction in young adults: Quantitative studies with the stable isotope 74SeO3. Br J Nutr 52:443–455, 1984.
24. "Clinical Guide to Laboratory Tests," 3rd ed. Philadelphia: W.B. Saunders, 1995.
25. Levander OA: Considerations on the assessment of selenium status. Fed Proc 44:2579–2583, 1985.
26. Berry M, Larsen P: The role of selenium in thyroid hormone action. Endocr Rev 13:207–219, 1992.
27. Pence B, Delver E, Dunn D: Effects of dietary selenium on UVB-induced skin carcinogenesis and epidermal antioxidant status. J Invest Dermatol 102:759–761, 1994.
28. L'Abbe M, Fischer P, Trick K, Campbell J, Chavez E: Dietary selenium and tumor glutathione peroxidase and superoxide dismutase activities. J Nutr Biochem 2:430–436, 1991.
29. Lin F, Thomas J, Girotti A: Hyperexpression of catalase in selenium-deprived murine L1210 cells. Arch Biochem Biophys 305:176–185, 1993.
30. Luo X, Wei H, Yang C, et al: Selenium intake and metabolic balance of 10 men from a low selenium area of China. Am J Clin Nutr 42:31–37, 1985.
31. Van Der Torre H, Van Dokkum W, Schaafsma G, Wedel M, Ockhuizen T: Effect of various levels of selenium in wheat and meat on blood Se status indices and on Se balance in Dutch men. Br J Nutr 65:69–80, 1991.
32. Robinson M, Rea H, Friend G, Stewart R, Snow P, Thomson C: On supplementing the selenium intake of New Zealanders: 2. Prolonged metabolic experiments with daily supplements of selenomethionine, selenite, and fish. Br J Nutr 39:589–600, 1978.
33. National Research Council. "Recommended Dietary Allowances," 10th ed. Washington, DC: National Academy Press, 1989.
34. Prohaska J, Ganther H: Selenium and glutathione peroxidase in developing rat brain. J Neurochem 27:1379–1387, 1976.
35. Behne D, Hilment H, Scheid S, Gessner H, Elger W: Evidence for specific Se target tissues and new biologically important selenoproteins. Biochim Biophys Acta 966:12–21, 1988.
36. Nesselroade J, Stigler S, Baltes P: Regression toward the mean and study of change. Psy Bull 88:622–637, 1980.
37. Hawkes W, Hornbostel L: Effects of dietary selenium on mood in healthy men in a metabolic research unit. Biol Psychiatry 37:1–8, 1995.