36

Selenium intake and metabolic balance of 10 men from a low selenium area of China[1-3]

Xianmao Luo, MD, Huijuan Wei, MD, Chunlin Yang, Jiu Xing, Changhong Qiao, BS, Yimin Feng, Jun Liu, Zing Liu, BS, Qin Wu, Yongxing Liu, Barbara J Stoecker, PhD, Julian E Spallholz PhD, and Shiang P Yang, PhD

ABSTRACT Selenium intake and urinary and fecal Se excretion of 10 healthy men from a low Se area in China were determined for three consecutive days, in summer, fall, and winter of 1983, and the spring of 1984 while self-selected diets were being consumed. Mean daily Se intake was 8.8 μg/day with a range of 2.3-35.5 μg/day, and was far below the recommended range of safe and adequate Se intake of 50-200 μg Se/day (National Academy of Sciences/National Research Council). Mean urinary and fecal Se outputs were 3.7 and 3.4 μg Se/day, respectively. Mean Se balance during this time was +1.8 μg Se/day. Apparent absorption of Se approximated 57%. The low Se intake in this area is a cause for concern since the residents of Molimo may be at risk for Se deficiency diseases. *Am J Clin Nutr* 1985;42:31-37.

KEY WORDS Selenium balance, selenium intake, selenium status in China, human selenium nutrition

Introduction

Selenium (Se) has been known to be essential element for animals since the 1950's (1). During the past few years there has been increasing evidence of the nutritional essentiality of Se in humans. Perhaps the most important evidence was the isolation of the Se-containing enzyme glutathione peroxidase (GSH-Px) from human erythrocytes (2), furthermore, beneficial responses to Se supplementation have been observed in the setting of total parenteral nutrition (3), and in certain people living in low Se areas of the People's Republic of China (4, 5).

Present epidemiological studies suggest that low Se status might be related to an increased incidence of cancer (6) and cardiovascular diseases (7). In a review by Griffin (8), the association between Se nutrition and carcinogenesis in animals was emphasized, and epidemiological studies showing a correlation between low environmental selenium and human cancer were discussed. The practical value of Se for prevention of human cancer remains in doubt (8) and is currently a subject of research in China.

In 1980, the US National Research Council (9) established a safe and adequate range of daily Se intake of 50-200 μg Se for adults and children over 7 years of age. However, there have been relatively few investigations of the minimal Se requirement for humans, and information on the amount of Se actually consumed by varied population groups is still limited (10-14). Se intakes estimated on the basis of food puchased rather than food eaten probably indicate a higher intake of Se than would be expected in the general population (14).

[1] From the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, PRC (XL, HW, JX, CQ, YF, JL, ZL), and the Edemic Disease Institute, Weichang, PRC (CY, QW, YL), and Department of Food and Nutrition, Texas Tech University, Lubbock, TX (BJS, JES, SPY).

[2] Supported by the US Department of Agriculture Competitive Research Grant #59-2486-1-1-671-0, Cancer Insitute, Chinese Academy of Medical Sciences, Beijing, PRC, Endemic Disease Institute, Weichang, PRC, and Shaklee Corp, San Francisco, CA.

[3] Address reprint requests to: Dr Barbara Stoecker, Department of Food and Nutrition, Texas Tech University, Lubbock, TX 79409.

Received August 6, 1984.
Accepted January 8, 1985.

While Keshan disease is the best known Se deficiency disease of China, Kaschin-Beck disease, a lesser-known disease affecting mainly the joints of both animals and humans, has been recognized in China and is also preventable with dietary Se supplements (15). The site of the experiments reported here, Molimo, is a low Se area where these two diseases are prevalent. The present study was designed to estimate the daily Se intake and the metabolic balance of residents eating self-selected diets in Molimo. It was thought that these balance studies would provide a realistic measure of the actual Se consumption of people living in a Keshan and Kaschin-Beck disease area.

Methods

After an explanation of the experimental protocol to the people of Molimo, ten apparently healthy male adults were selected from volunteers for the study. Subjects gave informed consent and were compensated (3 renminbi per day). Study procedures were approved by the Texas Tech University Committee for Protection of Human Subjects and by the Department of Science and Education, Ministry of Health, People's Republic of China. **Table 1** describes the age, weight, and smoking status of the subjects. Duplicate meals, feces and 24-h urine samples were collected on each of three consecutive days in June, September, and December of 1983 and in March of 1984 (**Table 2**).

All subjects ate self-selected diets. No fish was eaten. Liver and kidney, which are good sources of Se, are not usually consumed by these subjects, and were not consumed during the study. Subjects were instructed to collect an exact duplicate of everything consumed each day and project staff assisted them at each meal. A three-day record with weights or volumes of food items eaten was additionally maintained for each person. Weighed aliquots of $^1/_{20}$ of each food item were composited, homogenized, and refrigerated on the same day of collection. This sampling technique was used because of the limited storage, handling, and transportation facilities in the village, and because the diets were very simple.

Urine and feces were measured or weighed in the morning after each 24-h period. As a preservative, a 2% solution of sodium benzoate was added to the samples after collection (1 ml for 10 g diet or feces or for 100 ml of urine). Samples were collected in acid-washed polyethylene bottles. All samples were subsequently refrigerated and transported to Beijing where they were frozen until analysis. Feces were dried at 60°C for 72 h, ground in a coffee mill, and then kept in a desiccator. Fasting blood samples were drawn via vena mediana cubiti early in the morning. About 6 ml blood was treated with 0.1 ml of 10.0% potassium oxalate and centrifuged, and plasma was stored at −20°C. The RBC were maintained in 2 ml ACD solution (Acid-Citrate-Dextrose, Citric acid 0.540 g, trisodium citrate 1.485 g, dextrose 1.687 g and water to 67.5 ml) at 4°C until analysis for GSH-Px activity (usually finished within 72 h).

TABLE 1
Age, weight, and smoking status of subjects*

n	Age	Weight	Smoking
	yr	*kg*	
10	24 ± 5 (19–33)	60.2 ± 6.4 (48–68)	8/10

* Mean ± SD.

TABLE 2
Diet collection schedule

Season	Sampling date
Summer	June 27–29, 1983
Fall	Sept 25–27, 1983
Winter	Dec 10–12, 1983
Spring	March 2–4, 1984

Selenium levels were measured from digestates of the diet, urine, and feces by a modification (16) of the 2,3-diaminonaphthalene fluorometric method of Watkinson (17). Reagent purification included removal of residual Se from H_2SO_4 with 48% HBr, redistillation of hexane, and removal of fluorescent substances from 2,3-diaminonapthalene with hexane. A standard reference material (kale) from the International Atomic Energy Agency (a generous gift of Dr Bowen, Reading University, UK) was used. Recoveries of added Se were: grain and diet, 96–102%; blood, 93%; urine, 103%; feces, 96%; and kale, 109%.

GSH-Px activities were assayed by a modification of the coupled method of Paglia and Valentine (18) using hydrogen peroxide as substrate. One unit of enzyme activity was defined as 1 micromole NADPH oxidized/min and the results were expressed as units/ml plasma or units/g Hb for RBC. GSH-Px activity using erythrocyte lysates as a measure of the Se status of humans may be hampered by human hemoglobin (19). Much of the Se in human erythrocytes (85–90%) is reported to chromatograph with hemoglobin exhibiting GSH-Px activity (19). In our assay procedure this GSH-Px activity of human hemoglobin contributed negligible amounts of GSH-Px-like activity when measured in the presence of sodium azide, using H_2O_2 as the substrate and monitoring oxidation of NADPH at 340 nm as described by Paglia and Valentine (18). In a pilot study in China, GSH-Px activity in human plasma or erythrocyte lysate was not found to be significantly different using either H_2O_2 or cumene hydroperoxide as substrate. The data were subjected to one-way analysis of variance and compared by Duncan's multiple range test (19).

Results

The distribution of the Se content of the 113 composite diets is shown in **Figure 1**. Se levels in all diets were far below the recom-

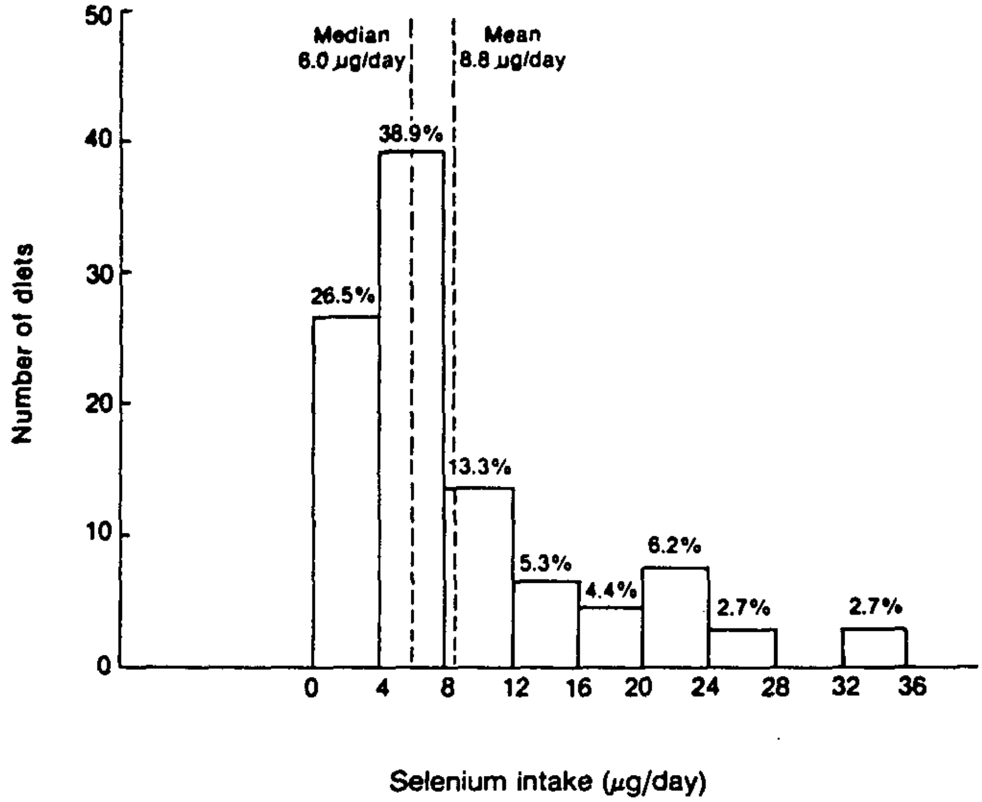


FIG 1. The percentage distribution of Se content of 113 diet composites from Molimo, China.

mended range of safe and adequate intake of 50–200 µg Se/day (9). The mean daily intake of Se was 8.8 µg with a range of 2.3–35.5 µg. Since the distribution was upwardly skewed because of a few relatively high Se levels, the median of 6.0 µg Se/day might be more representative than the mean daily Se intake. To our knowledge, the daily Se intake of subjects of Molimo is one of the lowest reported to date in the world (Table 3). The low daily Se intake resulted in the low Se status of the subjects (Table 4). Plasma and RBC GSH-Px activities were less than 60% of the levels seen in Beijing residents. Plasma and RBC Se concentrations were also extremely low compared to residents of Beijing and the USA.

As shown in **Table 5**, a seasonal variation of Se intake was observed. The Se intake in summer was significantly higher than in the other three seasons ($p < 0.05$). In the spring, the Se intake was less than in either the fall

TABLE 3
Selenium intake in different population groups

Country and area	Se intake	Reference
	µg/day	
Canada	168†	21
US, California	90–168*	22
Japan	100*	23
US, North Carolina	93†	24
US, Maryland	81‡	11
US, Washington, DC	71‡	25
United Kingdom, Britain	60*	26
New Zealand	56*	27
Finland	30*	28
New Zealand	24‡	10
China, Keshan disease area	11†	29
China, Molimo (Keshan disease area)	8.8‡	present study

* Calculation of Se content in food based on consumption information.
† Chemical analysis of diet composites prepared on the basis of food consumption information or idealized diets.
‡ Chemical analysis of diet composites collected from subjects.

TABLE 4
Selenium status of residents in different locations

Location	n	Se: Plasma (ng/ml)	Se: RBC (ng/g Hb)	GSH-Px: Plasma (units/ml)	GSH-Px: RBC (units/g Hb)	References
Molimo†	30	23.9 ± 1.1	100.9 ± 2.9	0.074 ± 0.005	14.0 ± 0.9	Present study
Beijing	20	93.4 ± 5.5	262.8 ± 24.9	0.136 ± 0.006	22.0 ± 1.2	Present study
USA	50	80.0 ± 10.0	740.0 ± 40.0	—	32.0 ± 2.5	30
USA	174	97.0 ± 2.3	810.0 ± 31.8	—	35.0 ± 1.2	31

* Mean ± SEM.
† Mean of analyses of blood samples collected from 10 subjects in Oct, Nov, and Dec of 1983.

or winter ($p < 0.05$). Se intake in the fall did not differ from that of the winter. The Se concentration of urine in fall was less than that in summer ($p < 0.05$) but was not significantly different from urine samples taken in the winter or spring.

Se in the feces of these subjects declined from 4.5 and 4.3 μg Se/day in summer and fall to 2.7 and 2.1 μg Se/day in winter and spring ($p < 0.05$). The mean balance of Se varied from +1.5 to +4.4 μg Se/day in summer, fall and winter, but decreased to −1.1 μg Se/day in spring. The means of apparent Se absorption in the four seasons did not vary significantly ($p > 0.05$). On the average, our subjects excreted 52% of their total body Se losses in urine and 48% in the feces with a Se balance of +1.8 μg/day. The mean apparent absorption of Se during the four seasons was 57.2%.

Table 6 shows the daily intake of some macronutrients. These macronutrient intakes did not vary significantly in summer, fall, and winter. Significant decreases ($p < 0.05$) during spring were observed in energy and protein intake (vs fall) and carbohydrate intake (vs fall and winter). Correlation between Se intake and the intakes of energy, protein, carbohydrate, and fat were positive but were not statistically significant ($0.1 > p > 0.05$).

Discussion

Diet composites actually prepared and consumed by the 10 subjects in Molimo, China, were analyzed in this study. The data, therefore, are believed to represent true Se intakes of the people of this area.

Data from New Zealand indicated that the minimum dietary Se requirement for maintenance of human health is probably not more than 20 μg/day (10). In China, Keshan disease is absent in areas where the dietary Se intake is at least 30 μg Se/day (5). On the average, self-selected diets in this study provided only 8.8 μg Se/day (median 6.0 μg) for men. Moreover, of these diets, 88 or 97% had Se levels less than 20 or 30 μg Se/day, respectively. It is likely that the residents of Molimo are at risk of Keshan and

TABLE 5
Selenium balance in 10 men during the four seasons*†

Season	n	Selenium: Intake (μg/day)	Selenium: Urine (μg/day)	Selenium: Feces (μg/day)	Selenium: Balance (μg/day)	Apparent‡ absorption %
Summer	10	13.3 ± 1.0^{a}	4.3 ± 0.3^{a}	4.5 ± 0.6^{a}	+4.4 ± 1.5^{a}	63.1 ± 6.5^{a}
Fall	10	9.2 ± 0.3^{b}	2.7 ± 0.3^{b}	4.3 ± 0.6^{a}	+2.2 ± 0.7^{ab}	52.4 ± 6.8^{a}
Winter	10	8.1 ± 1.4^{b}	3.9 ± 0.6^{ab}	2.7 ± 0.3^{b}	+1.5 ± 1.7^{ab}	57.7 ± 6.8^{a}
Spring	10	4.7 ± 0.4^{c}	3.8 ± 0.4^{ab}	2.1 ± 0.3^{b}	−1.1 ± 0.6^{b}	55.4 ± 6.8^{a}
Annual mean		8.8 ± 0.7	3.7 ± 0.2	3.4 ± 0.3	+1.8 ± 0.8	57.2 ± 3.3

* Mean ± SEM.
† Means in a column not sharing a common superscript are significantly different ($p < 0.05$).
‡ Apparent absorption, % = Se intake-fecal Se/Se intake × 100.

TABLE 6
Energy and macronutrient content of daily diets in four seasons in Molimo, China*†

Season	Energy	Protein	Carbohydrate	Fat
	kcal	*gm*	*gm*	*gm*
Summer	2530 ± 265[a]	75.0 ± 7.2[ab]	521 ± 56[a]	19.2 ± 1.9[a]
Fall	2637 ± 141[a]	81.2 ± 3.3[a]	540 ± 26[a]	18.6 ± 1.7[a]
Winter	2506 ± 88[a]	73.0 ± 3.1[ab]	536 ± 28[a]	16.7 ± 1.6[a]
Spring	1935 ± 220[b]	63.2 ± 6.9[b]	381 ± 4[b]	15.4 ± 1.5[a]
Annual mean	2402 ± 102	73.1 ± 2.8	495 ± 22	17.5 ± 0.8

* Mean ± SEM.
† Means in a column not sharing a common superscript are significantly different ($p < 0.05$).

Kaschin-Beck disease as a consequence of Se deficiency, and the low Se intake in this area is a cause for concern.

Located in a mountainous northern district of Hebei province, Molimo is an isolated area where locally grown food constitutes almost the entire diet. The amount of Se in the soil is so low that the major grains and beans contain only about 0.005 ppm of Se (except soybean which contains about 0.012 ppm of Se). The average Se level in vegetables is less than 0.003 ppm on a wet weight basis. Low Se in most foods is the reason that the Se levels are so low in the composite diets of our subjects, and in the people of Molimo in general.

Several factors may be involved in the seasonal variation of Se intake: (a) During summer, and especially in the fall, heavy field work compelled our subjects to eat 3 meals/day. In the winter and spring, only 2 meals a day were eaten. (b) In summer and fall, diets were usually supplemented with purchased wheat flour products from Henan Province whose Se level is about 0.1 ppm or 20 times that found in local staple foods. (c) During the spring, pickled Chinese cabbage was the main vegetable eaten, and it contained less Se than did fresh vegetables.

Selenium and macronutrient intake appeared to decline progressively during fall and winter and dropped to the lowest values in the spring. In this area, Keshan disease occurs mainly during the winter and early spring. The possible role of seasonal variation in Se and macronutrient intake may contribute to the onset of Keshan disease and should be further investigated.

The high individual variance in daily Se intake (from 2.3 to 35.5 μg) probably could be attributed to day-to-day variability in the consumption of relatively few products that are good sources of selenium. In the case of our subjects, as mentioned above, imported wheat flour products from the market might contribute to infrequent high Se intakes.

In our subjects, the mean daily fecal and urinary Se losses were only 7.1 μg. These values are lower than those reported for six young American men consuming formula diets who needed 54 μg Se/day to replace fecal and urinary losses (32), or for men consuming self-selected diets whose Se requirement for balance was calculated at 80 μg/day (14). Mean body weight of our subjects was 60 kg compared to the mean 75–76 kg reported by Levander and coworkers for male subjects (14, 32). Selenium intake needed to maintain balance has been suggested to be related to lean body mass. Our results also differ from those of four young New Zealand women who needed about 24 μg Se/day to maintain a positive Se balance (10). It is possible that people from extremely low-Se areas, like Molimo, maintain their Se balance but at a much lower Se level with some compensative mechanism conserving their body Se reserves. These data do not mean that the Se intake is enough to support optimal physiological function. In another study, daily supplements of 150 μg Se as Na_2SeO_3 for 30 days did not raise GSH-Px levels of RBC or plasma to those levels seen in Beijing residents (33).

Despite considerable seasonal variation in Se intake, urinary Se remained more or less constant, except in the fall when a significant drop in urinary Se was observed compared to that of summer. The decrease of fecal Se excretion in winter and spring reflected the decline of Se intake more markedly than did urinary Se loss. Levander and Morris (14) reported no seasonal variation in Se intake, but it is probable that the Chinese subjects had more seasonal variation in energy expenditure and food availability than did the North American subjects.

Our subjects excreted 52% of their total Se losses in the urine. This agrees closely with the 55% of total Se output via the urine calculated for four New Zealand women consuming diets which provided 24 μg Se/day (10). Percent of Se lost in the urine of our subjects was slightly lower than data obtained from two groups of American subjects (14, 32).

In spite of the low Se intakes, the apparent absorption of Se in our subjects (57 ± 3) remained similar to the 55 ± 5% apparent absorption calculated for four New Zealand women (10), the 62 ± 6% for six American young men (31) during Se repletion (32), and the 61 ± 2% and 68 ± 2% calculated for American men and women respectively, on self-selected diets (14). However, a linear regression of Se balance vs intake indicated that zero balance would be attained with 7.4 μg Se/day. This figure was much less than required for balance by either North American or New Zealand adults, even considering the slightly smaller body mass of Chinese subjects.

New Zealand workers had shown little loss of Se by routes other than urine and feces in their subjects consuming a diet with 24 μg of Se per day (10). Others have indicated that the sauna-induced sweat of North Americans contained low levels of Se (average of 1.3 μg/l) (34). Presumably, the net balance of our subjects should be less than +1.8 μg/day if dermal and respiratory losses of Se, particularly in summer and fall, were considered. ■

References

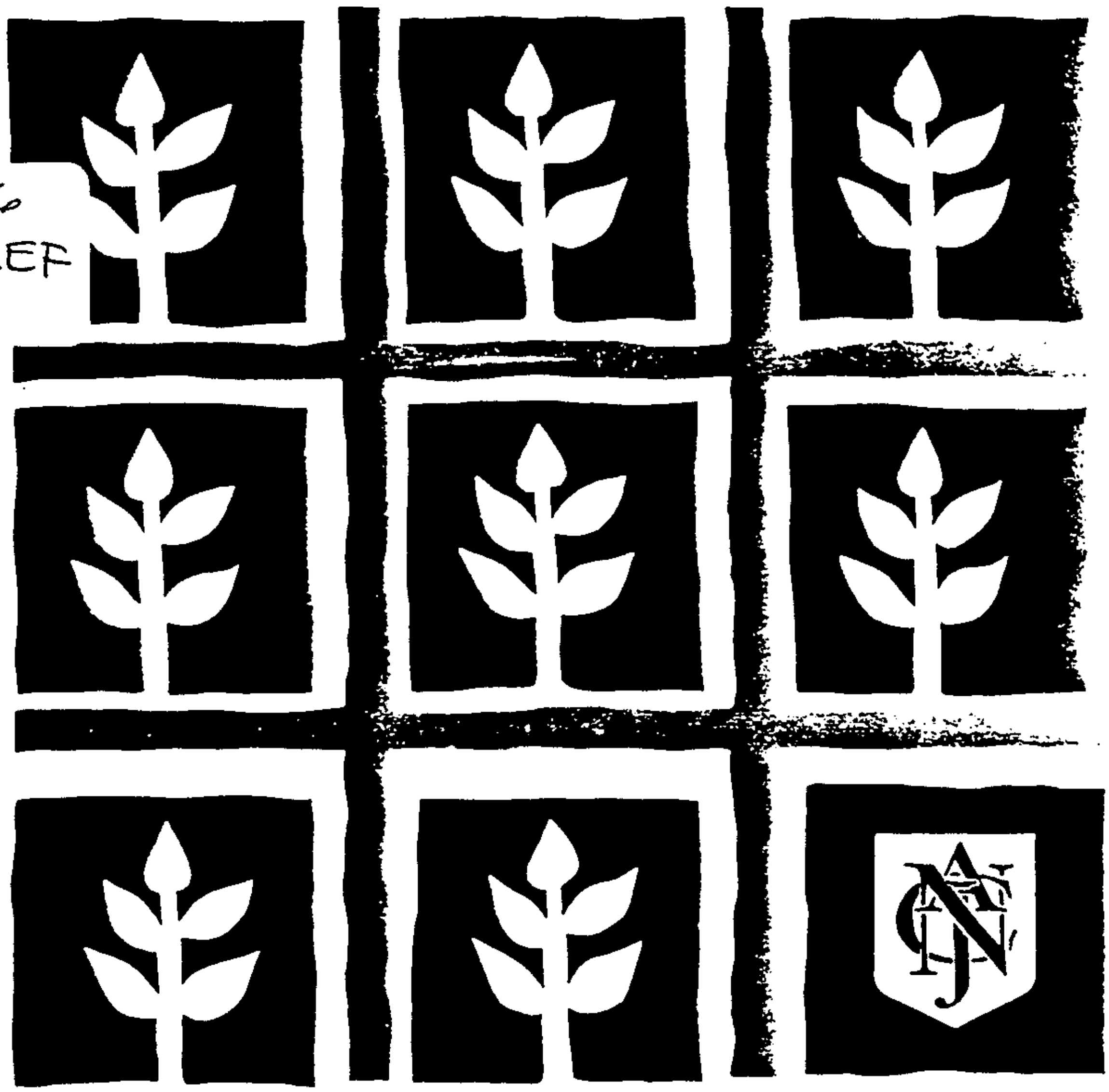
1. Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J Am Chem Soc 1975;79:3292-3.
2. Awasthi YC, Beutler E, Srivastava SK. Purification and properties of human erythrocyte glutathione peroxidase. J Biol Chem 1975;250:5144-9.
3. Van Rij AM, Thomson CD, McKenzie JM, Robinson MF. Selenium deficiency in total parenteral nutrition. Am J Clin Nutr 1979;32:2076-85.
4. Keshan Disease Research Group. Observations on effect of sodium selenite in prevention of Keshan Disease. Chin Med J (Engl Ed) 1979;92:471-6.
5. Keshan Disease Research Group. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. Chin Med J (Engl Ed) 1979;92:477-82.
6. Committee on Diet, Nutrition, and Cancer. Diet, nutrition and cancer. Washington, DC: National Academy of Sciences, 1982.
7. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982;2:1975-9.
8. Griffin AC. Role of selenium in the chemoprevention of cancer. Adv Cancer Res 1979;29:149-442.
9. Food and Nutrition Board. Recommended Dietary Allowances, 9th rev ed. Washington, DC: National Academy of Sciences, 1980.
10. Stewart RDH, Griffiths NM, Thomson CD, Robinson MF. Quantitative selenium metabolism in normal New Zealand women. Br J Nutr 1978;40:45-54.
11. Welsh SO, Holden JM, Wolf WR, Levander OA. Selenium in self-selected diets of Maryland residents. J Am Diet Assoc 1981;79:277-85.
12. Griffiths NM. Dietary intake and urinary excretion of selenium in some New Zealand women. Proc Univ Otago Med School 1973;51:8-9.
13. Levander OA, Morris VC, Moser PB. Dietary selenium (Se) intake and Se content of breast milk and plasma of lactating and nonlactating women. Fed Proc 1981;40:890.
14. Levander OA, Morris VC. Dietary selenium levels needed to maintain balance in North American adults consuming self-selected diets. Am J Clin Nutr 1984;39:809-15.
15. Hou SF, Zhu ZY, Ton JA. The relationship between the selenium dynamics in the course of human body growth and the Kaschin-Beck disease epidemiology. Acta Geographica Sinica 1984;39:75-85.
16. Keshan Disease Research Group, Chinese Academy of Medical Sciences. Study on selenium and etiology of Keshan disease. 1. Fluorometric method of micro Se determination in biological samples. Health Research (Chin ed). 1976;(2):175.
17. Watkinson JH. Fluorometric determination of selenium in biological material with 2,3-diaminonaphthalene. Anal Chem 1966;38:92-7.
18. Paglia OE, Valentine WN. Studies on the quantitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
19. Beilstein MA, Whanger PD. Distribution of selenium and glutathione peroxidase in blood fractions from humans, rhesus and squirrel monkeys, rats and sheep. J Nutr 1983;113:2138-46.
20. SAS Institute Inc. SAS User's Guide: Statistics, 1982 Edition. Cary, NC: SAS Institute Inc, 1982. 122 pp.
21. Thompson JN, Erdody P, Smith DC. Selenium content of food consumed by Canadians. J Nutr 1975;105:274-7.

22. Schrauzer GN, White DA. Selenium in human nutrition. Dietary intake and effects of supplementation. Bioinorg Chem 1978;8:303–18.
23. Sakurai H, Tsuchiya K. A tentative recommendation for making daily intake of selenium. Environ Physiol Biochem 1975;5:107–18.
24. Ganapathy S, Dhande R. Selenium content of omnivorous and vegetarian diets. Fed Proc 1976; 35:360.
25. Morris VC, Chansler MW, Levander OA. Selenium (Se) intake and metabolic balance in 9 adult subjects consuming self-selected diets. Fed Proc 1983;42:927.
26. Thorn J, Robertson J, Buss DH, Bunton NG. Trace nutrients. Selenium in British food. Br J Nutr 1978;39:391–6.
27. Watkinson JH. The selenium status of the New Zealanders. NZ Med J 1974;80:202–5.
28. Varo P, Koivistoinen P. Mineral element composition of Finnish foods, XII. General discussion and nutritional evaluation. Acta Agric Scand Suppl 1980;22: 165–171.
29. Yang GQ, Zhou RH, Sun SZ, Wang SS, Li SS. Endemic selenium intoxication of man in China and the selenium levels of human body and environment. Acta Nutrimenta Sinica 1982;4:81–9.
30. Goodwin WJ, Lane HW, Bradford K, et al. Selenium and glutathione peroxidase in patients with epidermoid carcinoma of the oral cavity and oropharynx. Cancer 1983;51:110–5.
31. Lane HW, Warren DC, Martin E, McCown J. Selenium status of industrial worker. Nutr Res 1983;3:805–17.
32. Levander OA, Sutherland B, Morris VC, King JC. Selenium balance in young men during selenium depletion and repletion. Am J Clin Nutr 1981;34: 2662–9.
33. Luo XM, Wei HJ, Yang CL, et al. Bioavailability of selenium to residents in a low-selenium area of China. Am J Clin Nutr (in press).
34. Levander OA, Sutherland B, Morris VC, King JC. Selenium metabolism in human nutrition. In: Spallholz JE, Martin JL, Ganther HE, eds. Selenium in biology and medicine. Westport, CT: AVI Publ Co, 1981:256–68.

The American Journal of CLINICAL NUTRITION

Official Journal of The American Society for Clinical Nutrition, Inc.

36
REF



ISSN 0002-9165

VOLUME 42 NUMBER 1 JULY 1985