PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 383–390 (1981)

Blood Selenium Levels and Glutathione-Peroxidase Activities in University and Chronic Intravenous Hyperalimentation Subjects[1] (41184)

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Abstract. The purpose of this study was to determine selenium concentration in erythrocytes and plasma and to compare these values to the measured erythrocyte glutathione-peroxidase activity for a university and a long-term intravenous hyperalimentation (IVH) population. In addition, we wished to determine if these chronic IVH patients were at risk for selenium deficiency. There was a positive correlation ($r = 0.67$) between erythrocyte selenium levels and erythrocyte glutathione-peroxidase activities in the university population. The patients receiving IVH had significantly lower mean selenium levels in erythrocytes and plasma and mean glutathione-peroxidase activities than the values from the university population. No correlation existed between erythrocyte selenium levels and erythrocyte glutathione-peroxidase activities in this IVH patient population. These chronic IVH patients when compared with the university population appear to be at risk for selenium deficiency.

Many symptoms which occur with selenium deficiency in various species of animals and the elusive role of selenium's relationship to vitamin E deficiency (1) can now be explained in part by the discovery (2) that selenium is essential for glutathione-peroxidase (EC 1. 11. 1. 9) activity in rodents (2–5), livestock (6–9), poultry (10, 11), and humans (12–20). The most accepted function of glutathione-peroxidase (GSH-Px) is to detoxify peroxides (1, 11).

The activity of glutathione-peroxidase decreases in tissues of animals raised on selenium-deficient refined diets and increases when animal diets are supplemented with selenium (2–6, 8, 10). The question to be addressed now is whether dietary selenium concentration has the same effect on glutathione-peroxidase activity in humans as it has on animals.

Studies of New Zealand human populations known to have low dietary selenium have demonstrated a positive correlation between whole-blood selenium levels and whole-blood glutathione-peroxidase activities (12–15). Reduced blood glutathione-peroxidase activities and selenium levels have been reported also in patients receiving short-term intravenous hyperalimentation (IVH) (15) and in patients consuming severely restricted diets (16, 17). Therefore, previously published evidence appears to demonstrate that blood glutathione-peroxidase activity is an indicator of selenium nutritional status. Methods for measuring and evaluating selenium status in humans are becoming increasingly more important. Chinese children consuming diets low in selenium have a higher incidence of a serious cardiac disease (Keshan disease) than children consuming normal diets (18–20). Selenium supplementation decreased the incidence of Keshan disease in this population. Furthermore, selenium has been hypothesized to have a role in the prevention of carcinogenesis (21).

The purpose of this study was to determine selenium concentration in erythrocytes and plasma and to compare these values to the measured erythrocyte glutathione-peroxidase activity for a university and a long-term IVH population. In addition, the selenium status of these two populations was evaluated by the methods used to identify selenium deficiency in animals:

[1] This research was supported by the Science and Education Administration of the U.S. Department of Agriculture under Grant 5901-0401-8-0086-0 from the Competitive Research Office.

0037-9727/81/070383-08$01.00/0

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blood selenium levels and glutathione-peroxidase activities.

The university population lived in a region of the United States where adequate dietary selenium from sea food, plants, and soil was available and the nine chronic IVH patients were ambulatory, lived at home, and had short-bowel syndrome.

Materials and Methods. *Subjects: University.* Students and staff from a small (2000 students) suburban religiously affiliated university were selected for this study. After completion of a medical history, subjects were selected who met all of the following criteria: (i) had no current disease or disorder; (ii) had blood pressure below 150/100 mm Hg; (iii) were not presently consuming any prescribed medication including oral contraceptives and had no recent history of chronic use of laxatives, antacids or aspirin; (iv) had not been subject to surgery or a blood transfusion within the last year; (v) had no current minor ailment such as a cold; and (vi) had not been out of the country recently. The one hundred subjects (52% male, 48% female) were questioned about their smoking and drinking habits. All subjects except three had normal weights for their heights (22). Eight percent of the subjects smoked more than one pack of cigarettes per day (23). Seventy-seven percent of the subjects consumed less than one alcoholic drink per week, while 98% consumed less than two alcoholic drinks per day. The mean age was 23.3 years (Table I) and mean hematocrit, hemoglobin, and blood pressure values were within normal ranges (24).

Subjects: IVH. All patients selected for the study had short-bowel syndrome, were outpatients, and received their intravenous solutions continuously over a 24-hr period (25, 26). The IVH solutions contained an amino acid solution (one of the following: FreAmine II, Travasol, or Aminosyn), an electrolyte solution containing zinc and copper in addition to all macroelements, dextrose, sterile water, and the water-soluble vitamins. The fat-soluble vitamins were given with the Intralipid and iron was given as imferon. Analysis of these solutions in this laboratory revealed no measurable selenium levels and thus are considered selenium free. This IVH population received no parenteral or enteral selenium and were thus candidates for selenium deficiency. Several of the patients did continue to eat, as tolerated, but in general this food was considered not to be absorbed because of documented large stools and their short bowel. The mean age of the subjects was 43 years (Table I) and seven of the nine subjects studied were male. The IVH patients had mean values for hematocrit, hemoglobin, and blood pressures that were normal with an occasional hematocrit or hemoglobin, and blood pressures that were normal with an occasional hematocrit or hemoglobin that was slightly low (Table I). All subjects were ambulatory, living at home and had normal weight for their height (22). These subjects have received at home total parenteral nutrition from 1 to 27 months prior to participating in this study. Blood was collected monthly. The IVH was discontinued for one subject (No. 4) because of medical improvement and two subjects expired (Nos. 1 and 9). Three subjects resided

TABLE I. DESCRIPTION OF THE UNIVERSITY SUBJECTS AND PATIENTS RECEIVING INTRAVENOUS HYPERALIMENTATION (IVH)

Descriptors	University			IVH		
	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range
Age (years)	23.2	6.2	17–57	43.4	15.2	21–65
Hematocrit (%)	43.4	3.7	35–51	38.1	5.2	30.8–42.9
Hemoglobin (g/dl)	13.8	1.8	9.5–17.6	11.6	1.6	9.9–14.7
Blood pressure						
Diastolic (mm Hg)	72.1	6.9	59–91	69.3	16.7	50–80
Systolic (mm Hg)	118.2	10.7	91–151	114.0	21.6	90–132

outside of the metropolitan area and returned to the outpatient clinic infrequently (Subjects 3, 7, and 8).

All subjects signed an informed consent form approved by The University of Texas Committee for the Protection of Human Subjects.

Blood preparation and analysis. A vena puncture was performed with a syringe and needle. Immediately, hematocrits and hemoglobins were determined on a portion of the whole blood. The remaining blood was maintained at 4°, treated with heparin, and centrifuged, and the plasma removed and frozen. The cellular fraction was rinsed twice with saline to remove the buffy coat (27). The cells were hemolyzed with cold deionized water. Hemoglobin values and selenium concentrations were measured on the hemolyzed blood.

Selenium levels were determined in the following manner. The hemolyzed cells were digested with concentrated nitric and 70% perchloric acids (28). Then 2 ml of 0.04 M EDTA was added to the digestate. The pH of the solution was adjusted to 1.0 with 7 M NH_4OH and made up to a constant volume (50 ml) with 0.1 M HCl. Each solution was incubated at 60° for 20 min with 0.1% 2,3 diaminonaphthalene (prepared daily), cooled, and extracted with 10 ml of reagent grade cyclohexane. The fluorescence of each extraction was measured at 366 nm excitation and 606 nm emission with a spectrofluorometer[2] (experimentally determined wavelengths for maximum excitation and emission intensity). The instrument was calibrated before each run by preparing a standard curve using extracts from solutions containing known amounts of selenium. This method of calibration was validated periodically by the method of standard addition. All chemicals, containers, and water were routinely checked for selenium contamination. All Se measurements which resulted in a fluorescence reading equal to the blank fluorescence reading were assigned as being at the limit of detection (LOD) which was 0.01 μg Se/g. All reported selenium values in this study were duplicated with a standard deviation of 0.0026.

A fraction of the hemolyzed red blood cells was removed and diluted with 0.02 M pH 7.0 potassium phosphate buffer for glutathione-peroxidase activity determination. The glutathione-peroxidase activity was measured by coupling of hydrogen peroxide with NADPH via glutathione reductase (29). The reaction mixture had the following composition: dialyzed glutathione reductase in 0.005 M phosphate buffer, 0.1 ml; 0.003 M EDTA, 0.02 M phosphate buffer, pH 7, 0.5 ml; 0.01 mM $NADPH_2$, 0.1 ml; 40 mM glutathione, pH 5.0, 0.05 ml; 0.10 mM sodium azide, 0.01 ml; water plus diluted buffered hemolyzed blood, 0.1 ml. After a 5-min preincubation at room temperature 12 mM hydrogen peroxide, 0.01 ml, was added. Every 30 sec for 5 min the concentration of $NADPH_2$ was measured spectrophotometrically at 340 nm. Blanks (solution containing all reagents but the sample) were measured before each set of unknowns. A matched set of optical glass cuvettes was used. The protein in each sample was determined by the method developed by Lowry *et al.* (30). Enzyme activity was expressed both as micromoles NADPH oxidized per minute per gram of hemoglobin and micromoles NADPH oxidized per minute per gram of protein.

Statistics. The University of Texas Computer and Statistical Package for Social Sciences was used (31) to calculate the analysis of variance and Pearson's coefficient, and for linear regression analysis.

Results. *University population.* Selenium levels in plasma and erythrocytes and glutathione-peroxidase activities in erythrocytes from university-associated males and females are shown in Table II. There was no significant difference in selenium levels or glutathione-peroxidase activities between males and females. The mean plasma level of selenium was 0.10 μg Se/g plasma while the mean erythrocyte selenium level was 0.73 μg Se/g of hemoglobin (Hb) or 0.65 μg Se/g of protein. Glutathione-peroxidase activities expressed as units per gram of hemoglobin or protein were 31 and 41, respectively. Units were defined as micromoles of NADPH oxidized per minute. The

[2] Varian SF-330.

TABLE II. SELENIUM LEVELS AND GLUTATHIONE-PEROXIDASE ACTIVITIES IN PLASMA AND ERYTHROCYTES OF THE UNIVERSITY POPULATION[a]

Measurements	Male	Female	Total
Erythrocyte selenium concentration (μg Se/g Hb)	0.76 ± 0.37	0.68 ± 0.39	0.73 ± 0.38
Erythrocyte selenium concentration (μg Se/g of protein)	0.64 ± 0.25	0.66 ± 0.28	0.65 ± 0.20
Plasma selenium concentration (μg Se/g plasma)	0.103 ± 0.030	0.088 ± 0.019	0.096 ± 0.026
Erythrocyte glutathione-peroxidase activity (units/g of protein)[b]	30.2 ± 6.4	31.8 ± 7.0	31.0 ± 6.7
Erythrocyte glutathione-peroxidase activity (units/g of Hb)[b]	40.2 ± 11.9	41.0 ± 12.5	40.5 ± 11.9

[a] Means ± SD.
[b] Units are equivalent to μmoles NADPH oxidized per minute.

erythrocyte selenium levels ranged from 0.2 to 1.8 μg Se/g of Hb and erythrocyte glutathione-peroxidase activities varied from 10.7 to 75.5 units/g of Hb. The correlation coefficient of the erythrocyte selenium concentrations compared to the erythrocyte glutathione-peroxidase activities was 0.67 with $P < 0.01$ for all subjects (Fig. 1). There was a low correlation ($r = 0.39$) between plasma and erythrocyte selenium levels while the correlation between plasma selenium concentration and erythrocyte glutathione-peroxidase activity was 0.30 (Fig. 2).

Patients receiving intravenous hyperalimentation. Mean selenium concentrations and glutathione-peroxidase activities for each patient receiving intravenous hyperali-

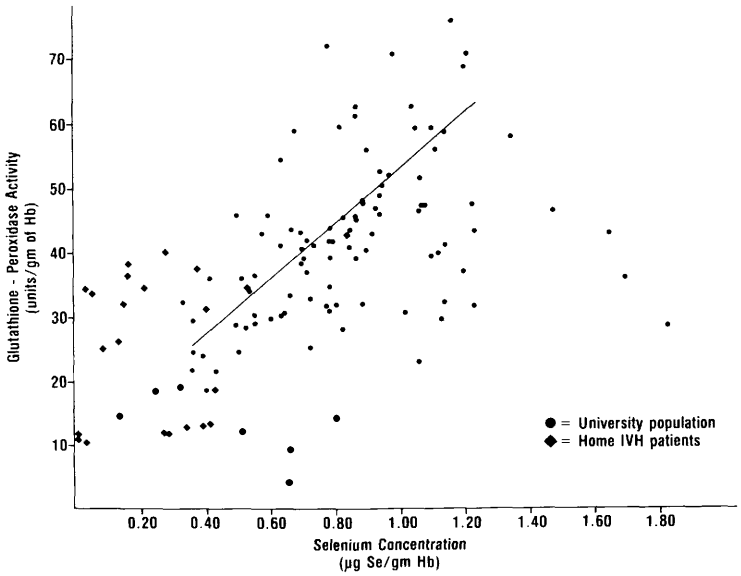

FIG. 1. Correlation between erythrocyte selenium levels and glutathione-peroxidase activity in the university population and patients receiving home intravenous hyperalimentation (IVH).

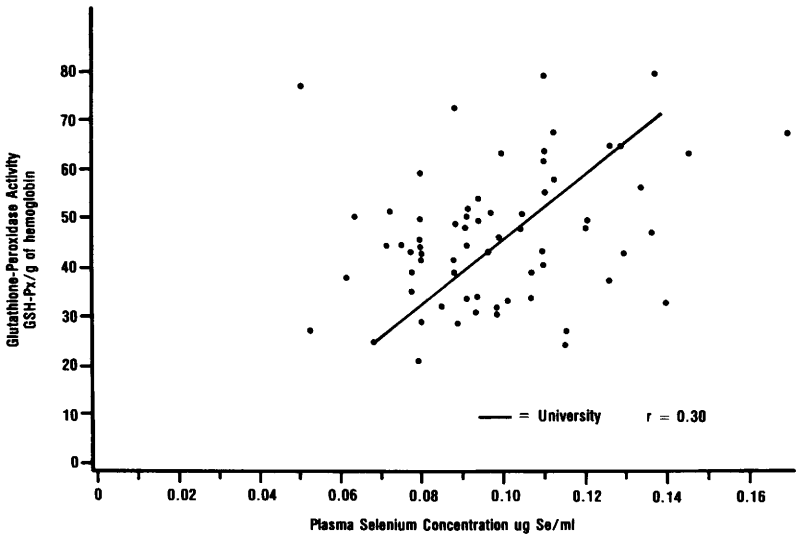

FIG. 2. Correlation between plasma selenium levels and erythrocyte glutathione-peroxidase activity in the university population.

mentation are shown in Table III. Subjects 2, 3, 6, and 9 had plasma selenium levels which were lower than the university population. All subjects except No. 3 had glutathione-peroxidase activities and erythrocyte selenium concentrations below the university population values. The mean of the erythrocyte selenium concentrations was 0.33 μg Se/g Hb with values ranging from the limit of detection to 0.84 μg Se/g Hb (Table IV). The plasma selenium levels ranged from the limit of detection (LOD = 0.01 ppm Se) to 0.15 μg Se/g plasma with a mean of 0.04 μg Se/g plasma. The mean erythrocyte glutathione-peroxidase activity was 24 units/g of Hb and the values varied from the blank value to 42 units/g Hb. There appears to be no relationship between the level of any of the selenium parameters and age, sex, or length of time the patient has been receiving hyperalimentation. A low negative correlation (−0.49) between selenium levels and glutathione-peroxidase activities in erythrocytes from patients receiving total parenteral nutrition (Fig. 1) existed.

Comparison of university population to

TABLE III. SELENIUM LEVELS AND GLUTATHIONE-PEROXIDASE ACTIVITIES FROM PATIENTS RECEIVING HOME IVH[a,b]

Subject No.	Sex	Age (years)	Number of collections[c] (months)	Time receiving IVH[d] (months)	Erythrocytes (μg Se/g Hb)	Erythrocytes (units GSH-Px/g Hb)[e]	Plasma (μg Se/g of plasma)
1	F	65	4	27	0.22 ± 0.22	25.75 ± 14.12	0.016 ± 0.024
2	M	31	4	9	0.25 ± 0.36	21.55 ± 17.74	0.077 ± 0.090
3	M	21	1	2	0.20	36.36	0.088
4	M	40	8	3	0.50 ± 0.32	24.80 ± 11.29	0.040 ± 0.030
5	M	30	8	3	0.27 ± 0.12	22.62 ± 9.87	0.030 ± 0.015
6	F	37	6	24	0.46 ± 0.24	28.48 ± 10.05	0.058 ± 0.018
7	M	50	1	1.5	0.24	10.72	0.022
8	M	58	2	1.0	0.23 ± 0.01	26.56 ± 11.41	0.027 ± 0.026
9	M	59	1	1	0.41	13.25	0.054

[a] Means ± SD, except in the case where only one measurement was made.
[b] IVH = intravenous hyperalimentation.
[c] One blood collection per month.
[d] Time patients receiving IVH prior to initiation of study.
[e] Units expressed as μmoles NADPH oxidized per minute.

TABLE IV. SELENIUM CONCENTRATIONS AND GLUTATHIONE-PEROXIDASE ACTIVITIES FROM UNIVERSITY SUBJECTS AND PATIENTS RECEIVING INTRAVENOUS HYPERALIMENTATION (IVH)

Measurement	University			Patients receiving IVH		
	Mean	SD	Range	Mean	SD	Range
Plasma Selenium concentration (μg Se/g of plasma)	0.095	0.027	0.050–0.139	0.043[a]	0.039	0[b]–.152
Erythrocyte Selenium concentration (μg Se/g of Hb)	0.73	0.33	0.20–1.80	0.33[a]	0.22	0[b]–0.84
Erythrocyte Glutathione-peroxidase activity (units/g of Hb)[c]	40.5	11.9	10.7–75.5	23.7[a]	11.7	0[b]–42

[a] University population significantly different from IVH population ($P < 0.01$).
[b] Below the limits of detection.
[c] Units are equivalent to μmoles NADPH oxidized per minute.

patients receiving intravenous nutrition. Mean selenium concentrations and glutathione-peroxidase activities for all of the university and IVH subjects are shown in Table IV. The IVH population had significantly lower mean selenium concentrations and glutathione-peroxidase activities compared to those found in the university population. The ranges for each selenium parameter of the IVH population overlapped with the corresponding ranges of values for the university population.

Discussion. The first objective of the research was to determine selenium concentrations in erythrocyte and plasma and to compare these values to the measured erythrocyte glutathione-peroxidase activity for both a university and a long-term IVH population. The plasma and erythrocyte selenium levels obtained in the university population were similar to values previously obtained from U.S. and European populations (16, 32, 33). The plasma selenium levels in the university population were twice those found in the New Zealand population (13, 34, 35). Previous research has indicated that this university population consumed between 50–250 μg Se/day[3] whereas it is reported that the New Zealand population consumed only about 50 μg Se/day (34, 35). The Chinese children (18, 19) from the region with a high incidence of Keshan disease had mean whole-blood selenium levels of 0.025 μg/g of blood with a dietary selenium level below 20 μg/day. Extrapolation of the university population plasma and erythrocyte selenium concentrations to whole-blood selenium indicates that the university population had selenium levels six times greater than those of the Chinese children from Keshan-disease-affected regions. The difference between the New Zealander, Chinese children, and university population blood selenium levels may be explained by dietary selenium levels.

The university-associated female population had glutathione-peroxidase activities and selenium levels nearly identical to the values of the university male population. The hematocrit and hemoglobin levels for females were slightly below those of the males but the lowest values were within the acceptable limits. A female population, which has a high incidence of anemia, may have different selenium levels and glutathione-peroxidase activities compared to males.

In general the glutathione-peroxidase activities in the erythrocytes were from one to four times greater than levels found in the New Zealand population (34). A positive significant correlation existed between the concentration of selenium and the level of glutathione-peroxidase activity in erythro-

[3] D. W. Lima and F. Sargent, Selenium content of foods. Master's Thesis. School of Public Health. University of Texas Health Science Center, Houston (1976), unpublished observation.

cytes for the university population. In addition, when the erythrocyte selenium levels were greater than 1.2 μg Se/g of Hb no correlation between glutathione-peroxidase activity and erythrocyte selenium levels was found. These data suggest that glutathione-peroxidase activity does not increase as the erythrocyte selenium level continues to rise. No correlation between the selenium levels in erythrocytes and the selenium levels in plasma was found. This lack of correlation may be due to more rapid fluctuations of selenium levels in plasma compared to erythrocytes. In addition a low significant positive correlation ($r = 0.30$) between plasma selenium levels and erythrocyte glutathione-peroxidase was observed. In our continuing evaluation of selenium status in an IVH population, we are measuring both glutathione-peroxidase activity and blood selenium levels. Therefore, the positive correlations between plasma or erythrocyte selenium concentration and glutathione-peroxidase activities indicate that the glutathione-peroxidase activities were generally related to the level of blood selenium in this healthy population.

The variance (standard deviation) of the selenium values in the university population was greater than the variance derived from rat (5) or the New Zealand (13) studies. Several of the individuals from the university and IVH group had extremely low selenium levels and three individuals in the university population had very high selenium levels. The ranges for the selenium levels and GSH-Px activity are reported in Table IV. The wider range of blood selenium levels and GSH-Px activities found in these populations may reflect the consumption of a range of dietary selenium wider than that found among the New Zealanders.

The patients receiving total parenteral nutrition had glutathione-peroxidase activities and selenium levels below those of the university population (Table IV). But unlike the university population, there was no correlation between the activity of glutathione-peroxidase and erythrocyte selenium levels. There was considerable variation of these levels within the IVH population (Fig. 1, Table III). Some of the erythrocyte samples had normal selenium levels, but low glutathione-peroxidase activity, while other samples had low concentrations of selenium but normal glutathione-peroxidase activity. Several times the selenium levels (Table III, Fig. 1) were below the limits of detection (LOD = 0.01 ppm Se) and the glutathione-peroxidase activities (Table III, Fig. 1) were at blank levels. Yet, in general, these chronic IVH patients have plasma selenium levels comparable to those of New Zealand surgical cancer patients and healthy blood donors (14). The New Zealand surgical patients and blood donors had lower mean erythrocyte selenium levels than the mean values for the chronic IVH patients. Three individual IVH patients had mean plasma selenium levels below the mean New Zealand levels suggesting that these patients may be at risk for selenium deficiency. These data suggest that selenium supplementation of these patients would result in an increased concentration of blood selenium and glutathione-peroxidase activity. Such a study is now under way in this laboratory. In addition, we are completing protein purification studies to determine if there are differences in the glutathione-peroxidase molecule of IVH patients as comparable to healthy individuals or if some other protein is affecting the assay for glutathione-peroxidase activity.

The authors wish to thank Diane Servance, Fred Salley, Lawrence Fan, Thomas Strawmyer, Napoleon Lee, and Sherrie Squyres for technical assistance and the pre-med club at Houston Baptist University for providing the subjects. Also, we appreciate the cooperation and assistance of the staff for the Home IVH program at Hermann Hospital, Houston, Texas.

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Received September 2, 1980. P.S.E.B.M. 1981, Vol. 167.