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Influence of Selenium Deficiency on Vital Functions in Rats

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ABSTRACT

To clarify the relationship between selenium (Se) deficiency and functional disorders, the authors determined the Se concentration, anti-oxidant enzyme activity, and other parameters in rats fed a Se-deficient diet. Rats fed the Se-deficient diet showed a decrease in Se concentration and glutathione peroxidase (GSH-Px) activity in plasma, erythrocytes, heart, liver, and skeletal muscle from the first week after the initiation of the diet, an increase in heart lipid peroxide concentration from the second week, and an increase in liver glutathione S-transferase activity from the fourth week. From the twelfth week, a decrease in the growth rate in the rats fed the Se-deficient diet was observed. In spite of this growth impairment, no changes in electrocardiogram, muscle tone, degree of hemolysis, plasma biochemistry, or hematological values were detected. In summary, the authors found that a reduction of body Se is easily induced, but that the appearance of functional disorders following Se deficiency is difficult to detect in rats.

Index Entries: Selenium deficiency; antioxidant system; vital function.

INTRODUCTION

Selenium (Se), as an essential component of glutathione peroxidase (GSH-Px, EC 1.11.1.9), plays a critical role in protecting aerobic organisms

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from oxygen radical-initiated cell injury *(1)*. In recent years, some broader metabolic disturbances have also been identified as a result of Se deficiency *(2)*. Both epidemiological and etiological evidence indicate that a Se deficiency can cause cardiomyopathy *(3,4)*, but it appears that Se deficiency alone is not sufficient to explain the cause of some disturbances, even though it is an essential condition for the development. For example, biochemical evidence of Se deficiency, such as low blood Se level and GSH-Px activity, have often been reported in patients receiving long-term total parenteral nutrition *(5–17)*. However, not all of these patients develop cardiomyopathy *(11–17)*.

Little detailed information is available on the relationship between Se deficiency and biological functional disorders. The main purpose of the present investigation was to study the influences of Se deficiency on biological antioxidant systems and vital functions to clarify the relationship between Se deficiency and biological functional disorders, using rats fed a Se-deficient diet for 1, 2, 4, 8, and 20 wk.

MATERIALS AND METHODS

Animals and Diets

Sixty weanling male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan) weighing 70–80 g were randomly and equally divided into two groups, and fed diets containing adequate or deficient levels of Se (0.5 ppm [Se adequate] or <0.02 ppm [Se deficiency]) from 0–20 wk. The diet compositions are listed in Table 1. The rats were housed individually in stainless-steel cages with a raised wire bottom. The temperature was maintained at 22–24°C. Diet and deionized water were given *ad libitum*.

Sample Collection and Preparation

After 0, 1, 2, 4, 8, and 20 wk, the muscle tone of each rat was determined in their nonanesthetized state. Then, ECG was recorded under sodium pentobarbital-anesthesia (35 mg/kg, ip); blood was drawn from the abdominal aorta into heparinized syringes, and plasma and erythrocytes were obtained by a conventional method. The heart, liver, soleus muscle, and extensor digitorum longus (EDL) muscle were removed and weighed, and were stored in liquid nitrogen together with plasma and erythrocytes before analysis.

Assay for Muscle Tone

The wire maneuver method *(18)*, the inclined method *(18)*, and the rotating rod method *(19)* were used for assaying the muscle tone. For the wire maneuver method, the rat was obliged to grasp a suspended horizontal wire with its forelimbs, and then whether the rat dropped from the wire or not was examined. For the inclined method, the rat was

Table 1
Composition of the Diet Used in this Study

Ingredient	(values in g/100 g diet) Se-deficiency	Se-adequate
Torula yeast	33.0	33.0
β-cornstarch	5.0	5.0
Cellulose powder	5.0	5.0
Corn oil	5.0	5.0
Sucrose	47.2	47.2
DL-Methionine	0.3	0.3
Vitamin mixture[1]	1.0	1.0
Salt mixture[2]	3.5	3.5
Sodium selenite pentahydrate	0	0.00017

[1]Vitamin mixture contained (mg/100 g diet): vitamin A acetate, 0.8 (500,000 IU/g); vitamin D_3, 0.025 (100,000 IU/25 mg); vitamin B_1 hydrochloride, 0.6; vitamin B_2, 0.6; vitamin B_6 hydrochloride, 0.7; vitamin B_{12}, 0.001; D-biotin, 0.02; vitamin E acetate, 5; menadione sodium bisulfate complex (MSBC) containing 52% vitamin K_3, 0.096; folic acid, 0.2; calcium pantothenate, 1.6; nicotinic acid, 3; choline bitartrate, 200; sucrose, 787.4.

[2]Selenium-free mixture contained (mg/100 g diet): $CaHPO_4$, 1750; NaCl, 259; $K_3C_6H_5O_7 \cdot H_2O$, 770; K_2SO_4, 182; MgO, 84; KIO_3, 0.035; $MnCO_3$, 12.25; $ZnCO_3$, 5.6; $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$, 1.05; Fe-citrate (17% Fe), 21; $CrK(SO_4)_2 \cdot 12H_2O$, 1.925; sucrose, 413.14.

placed on a commercial instrument (Shinano, Tokyo, Japan), the floor was inclined slowly, and the angle at which the rat slipped down was measured. For the rotating rod method, the rat was placed on a commercial rod instrument (Rota rod treated mill for rats 7700; Ugo Basille, Milano, Italy), the rod was rotated at 5 revolutions per min, and whether the rat dropped down from the rod or not was examined for 10 s.

Analysis of ECG

The ECG (lead II) was recorded using a polygraph system (RM6000; Nihon Kohden, Tokyo, Japan). For each ECG, the following parameters were measured by means of the MacLab™-data recording system (Scope v3.2; AD Instruments Pty., Australia): heart rate, SaT, QaT, QRS, and PQ intervals. The heart rate was calculated from R-R intervals.

Assay of Se

Samples were wet-ashed with a nitric acid/perchloric acid mixture in a borosilicate test tube placed in hot block bath (TPB-62; Advantec Tokyo, Tokyo, Japan). Se concentration was determined by gaseous hydride generation (HFS-2; Hitachi, Tokyo, Japan) -atomic absorption spectrometer (Z-8100; Hitachi, Tokyo, Japan), using a standard curve technique *(20)*.

Biochemical Analysis

For assay of thiobarbituric acid-reactive substance (TBARS), tissues were homogenized under an argon gas flow to lessen the oxidative-reductive change. The TBARS level was determined by the fluorometric method of Ohkawa et al. *(21)* with a slight modification, i.e., the addition of 0.0125 vol ethanolic 2% butylated hydroxytoluene to the reaction solution to prevent further peroxidation of lipids during the assay. The activity of glutathione peroxidase (GSH-Px) with hydrogen peroxide as the substrate was measured spectrophotometrically by monitoring glutathione disulfide formation through concomitant oxidation of NADPH *(22)*. The activity of glutathione S-transferase (GST) was measured according to the method of Habig et al. *(23)*, using 1-chloro-2, 4-dinitrobenzene as the substrate. Proteins were determined by the method of Lowry et al. *(24)* using bovine serum albumin as the standard.

Assay of Plasma Biochemistry

For assay of plasma biochemistry: glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), glucose (GLU), creatine phosphokinase (CPK), creatinine (CRE), total protein (TP), total cholesterol (T-CHO), inorganic phosphorus (IP), phospholipid (PL), total bilirubin (T-BIL), triglyceride (TG), transferrin (Tf), albumin (ALB), iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and chlorine (Cl), an autoanalyzer (7150-E; Hitachi, Tokyo, Japan) was used.

Assay of Hematology

An automated blood cell counter (M-2000; Toua Iyou Denshi, Hyogo, Japan) was used for assay of erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes, and platelets.

Hemolysis Test

Ten-percent erythrocyte suspension was mixed with the same volume of distilled water. This mixture was incubated aerobically under constant shaking for 30 min in a water bath maintained at 37°C. The extent of hemolysis was measured spectrophotometrically as reported by Yamamoto et al. *(25)*. The hemolysis rate was expressed in the percentages of maximal hemolysis.

Statistics

Data were analyzed using the unpaired Student's *t*-test in determining the significance of difference between two means. A two-tailed probability of ±5% was taken to indicate significance. All data are expressed as mean ± standard error.

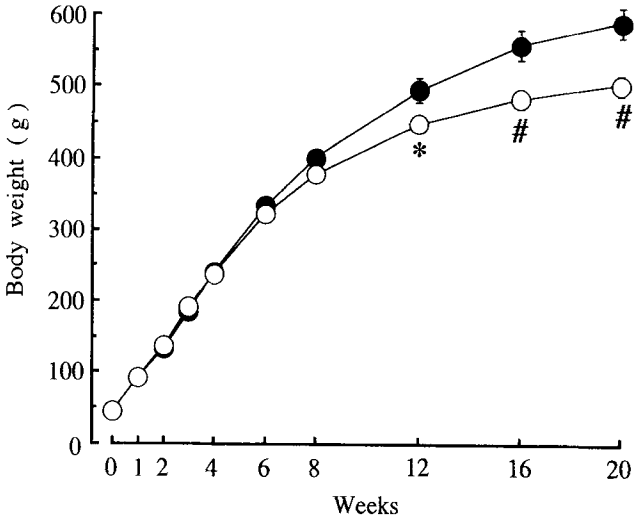

Fig. 1. Changes in growth curves of rats fed a Se-deficient or Se-adequate diet. The results are mean ± SE of 5–6 rats in each group. Significant difference from normal diet group: $*p < 0.05$, $\#p < 0.01$. ●: Se-adequate diet. ○: Se-deficient diet.

RESULTS

The growth curves of the two groups are presented in Fig. 1. Until eight weeks after the initiation of the experimental diets, no significant difference in body weights was detected between the two groups. However, at 12 wk or longer, the body weights were significantly lower in the rats fed the Se-deficient diet compared with those fed the Se-adequate diet.

Figures 2 and 3 show the time course of Se concentrations and GSH-Px activities in heart, liver, soleus muscle, EDL muscle, erythrocytes, and plasma, respectively, in two groups fed the experimental diets for 20 wk. Se concentrations in all aforementioned samples from rats fed the Se-deficient diet were already decreased significantly at one week of feeding, compared with those from rats fed the Se-adequate diet. These differences became progressively greater with time. At 20 wk of feeding, the Se-concentration, except for that in the EDL muscle, in the rats fed the Se-deficient diet decreased to less than 5% of those in the rats fed the Se-adequate diet. Similarly, the GSH-Px activities in the rats fed the Se-deficient diet significantly decreased in a time-dependent manner, and decreased to a barely detectable level at 20 wk of feeding. Figure 4 shows the time course of lipid peroxidation in the two groups. There were no significant differences in the TBARS concentration between the Se-deficient diet and -adequate diet groups, except for in the heart. The TBARS concentration in the heart of rats fed the Se-deficient diet was two times the value in the rats fed the Se-adequate diet at each mea-

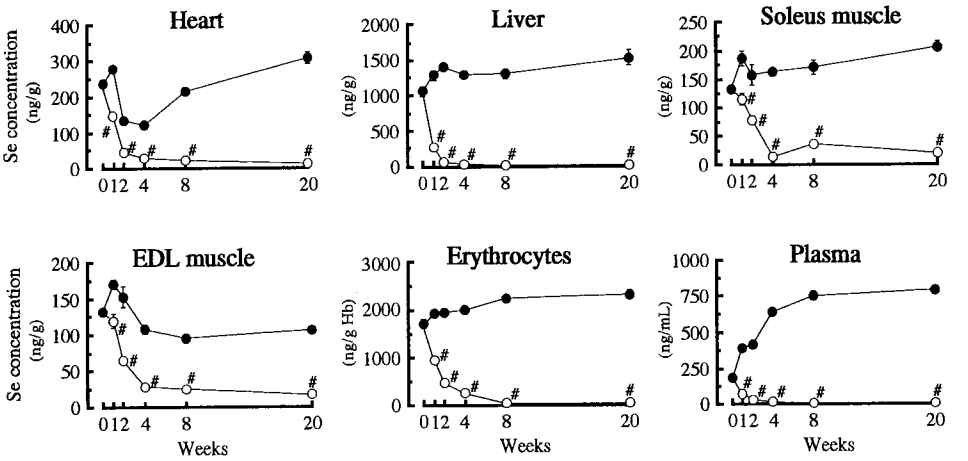

Fig. 2. Changes in Se concentration of heart, liver, soleus muscle, EDL muscle, erythrocytes, and plasma in rats fed a Se-deficient or Se-adequate diet. ●: Se-adequate diet, ○: Se-deficient diet. The results are mean ± SE of 5–6 rats in each group. Significant difference from normal diet group: #$p < 0.01$.

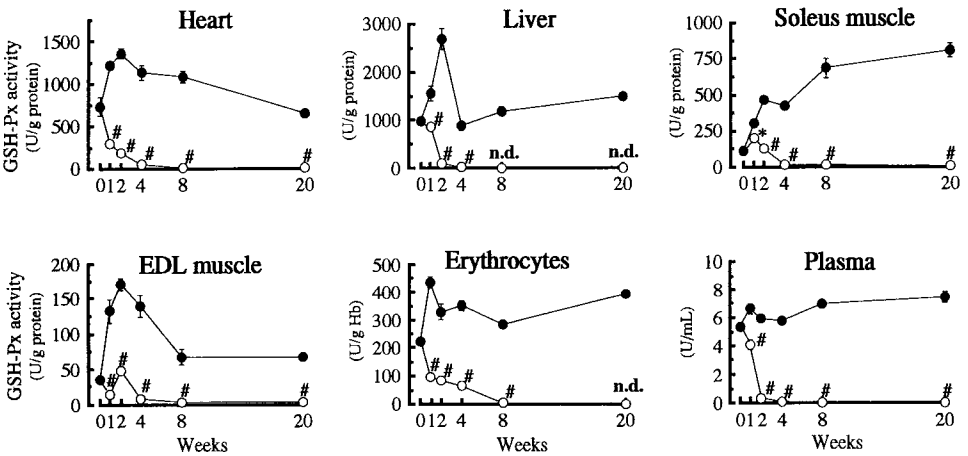

Fig. 3. Changes in GSH-Px activity of heart, liver, soleus muscle, EDL muscle, erythrocytes, and plasma in rats fed a Se-deficient or Se-adequate diet. ●: Se-adequate diet, ○: Se-deficient diet. The results are mean ± SE of 5–6 rats in each group. 1 U = 1 μmol NADPH oxidized/min. Significant difference from normal diet group: *$p < 0.05$, #$p < 0.01$. n.d.: not detection.

suring time point. The time course of GST activity is shown in Fig. 5. The GST activity of liver in the rats fed the Se-deficient diet was about two times the value in the rats fed the Se-adequate diet for 4, 8, and 20 wk. There were no remarkable differences in the GST activity in heart, EDL muscle, erythrocytes, and plasma between the Se-deficient rats and the Se-adequate rats.

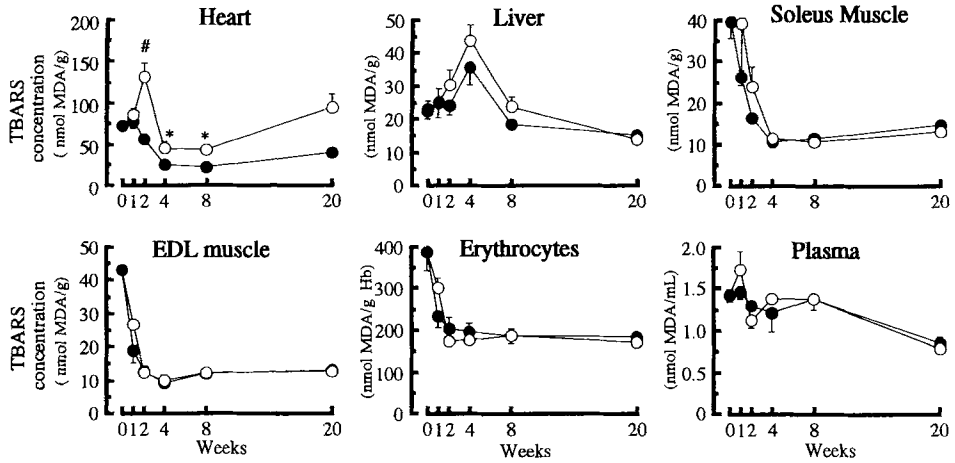

Fig. 4. Changes in TBARS concentration of heart, liver, soleus muscle, EDL muscle, erythrocytes, and plasma in rats fed a Se-deficient or Se-adequate diet. ●: Se-adequate diet, ○: Se-deficient diet. The results are mean ± SE of 5–6 rats in each group. Significant difference from normal diet group: $*p < 0.05$, $\#p < 0.01$.

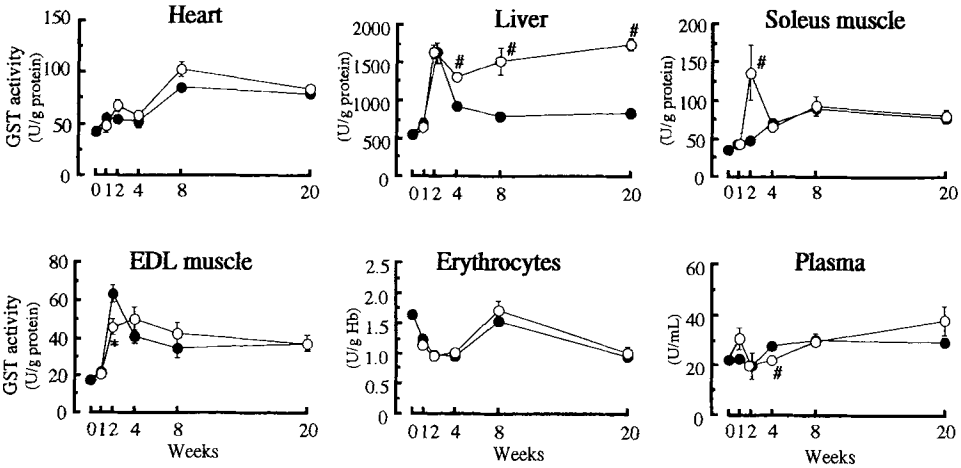

Fig. 5. Changes in GST activity of heart, liver, soleus muscle, EDL muscle, erythrocytes, and plasma in rats fed a Se-deficient or Se-adequate diet. ●: Se-adequate diet, ○: Se-deficient diet. The results are mean ± SE of 5–6 rats in each group. Significant difference from normal diet group: $*p < 0.05$, $\#p < 0.01$. 1 U = 1 μmol CDNB-complex produced/min.

The plasma biochemistry, hematology, ECG parameter, degree of hemolysis, and muscle tone parameters were not altered remarkably in the rats fed the Se-deficient diet for 1, 2, 4, 8, and 20 wk as compared

Table 2
Plasma Biochemistry in Rats Fed a Se-deficient or Se-Adequate Diet for 20 wk

	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	BUN (mg/dL)	GLU (mg/dL)	CPK (IU/L)	T-CHO (mg/dL)
Se-adequate	78.6 ± 7.5	29.4 ± 1.7	151.0 ± 17.0	14.23 ± 0.33	170.1 ± 7.0	891.3 ± 148.0	90.9 ± 4.2
Se-deficient	77.0 ± 7.4	39.3 ± 3.8	148.3 ± 7.1	15.19 ± 0.43	155.4 ± 4.9	813.5 ± 270.6	114.3 ± 4.1 #
	PL (mg/dL)	T-BIL (mg/dL)	ALB (g/dL)	IP (mg/DL)	CRE (mg/dL)	TP (g/dL)	TG (mg/dL)
Se-adequate	198.2 ± 5.4	0.501 ± 0.033	2.77 ± 0.07	7.14 ± 0.49	0.53 ± 0.02	5.52 ± 0.07	309.3 ± 28.7
Se-deficient	215.8 ± 10.4	0.399 ± 0.085	2.68 ± 0.06	7.50 ± 0.38	0.43 ± 0.02 #	5.57 ± 0.12	213.7 ± 43.5
	Tf (mg/dL)	Fe (mg/dL)	Ca (mg/dL)	Mg (mg/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
Se-adequate	347 ± 10	190.4 ± 14.5	10.20 ± 0.62	1.75 ± 0.08	139.4 ± 0.8	5.53 ± 0.47	103.1 ± 0.3
Se-deficient	349 ± 12	182.6 ± 17.5	9.62 ± 0.03	1.90 ± 0.03	139.7 ± 0.5	5.51 ± 0.31	103.7 ± 0.5

BUN: blood urea nitrogen, GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, ALP: alkaline phosphatase, CRE: creatinine, Tf: transferrin. The results are mean ± SE of 4–6 rats in each group.

Significant difference from normal diet group: #$p < 0.01$.

Table 3
Hematological Data in Rats Fed a Se-Deficient or Se-Adequate Diet for 20 wk

	Se-adequate	Se-deficient
Erythrocytes ($10^4/\mu L$)	814 ± 15	789 ± 17
Hemoglobin (g/dL)	14.7 ± 0.2	14.2 ± 0.3
Hematocrit (%)	40.5 ± 0.5	38.9 ± 0.9
MCV (fL)	49.9 ± 0.9	49.4 ± 0.6
MCH (pg)	18.0 ± 0.4	17.8 ± 0.2
MCHC (g/dL)	36.1 ± 0.2	36.0 ± 0.3
Leukocytes ($10^2/\mu L$)	67.5 ± 12.1	59.5 ± 7.3
Platelets ($10^4/\mu L$)	98.9 ± 2.2	91.8 ± 6.3

The results are mean ± SE of 5–6 rats in each group.

Table 4
ECG Parameters in Rats Fed a Se-Deficient or Se-Adequate Diet for 20 wk

	Heart rate (beat/min)	SaT (msec)	QaTc (msec)	QRS (msec)	PQ (msec)
Se-adequate	296 ± 15	14.5 ± 1.3	1.95 ± 0.12	19.5 ± 1.3	59.6 ± 5.4
Se-deficient	305 ± 20	16.3 ± 1.7	1.73 ± 0.15	19.3 ± 1.5	54.2 ± 3.6

The results are mean ± SE of 5–6 rats in each group.

Table 5
Muscle Tone in Rats Fed a Se-Deficient or Se-Adequate Diet for 20 wk

	Wire maneuver method (No. of rats which dropped/rats tested)	Inclined method (Angle with which rats slipped down)[1]	Rotating rod method (No. of rats which dropped/rats tested)
Se-adequate	0/6	47.5 ± 4.0	0/6
Se-deficient	0/6	49.1 ± 4.2	0/6

[1]The results are mean ± SE of 6 rats.

with the rats fed the normal diet. By way of example, each result at 20 wk of feeding is shown in Table 2 (plasma biochemistry), Table 3 (hematology), Table 4 (ECG parameter), Table 5 (muscle tone), and Table 6 (hemolysis).

Table 6
Hemolysis Rate in Rats Fed a Se-Deficient or Se-Adequate Diet for 20 wk

	Hemolysis rate (%)
Se-adequate	29.4 ± 0.3
Se-deficient	32.5 ± 0.3

The results are mean ± SE of 6 rats in each group.

DISCUSSION

The results of the present study demonstrated a distinctive relationship between the antioxidant system and functional disorder in rats fed a Se-deficient diet. Although many investigators have presented various reports concerning the effect of Se deficiency on antioxidant systems, there have been very few studies on the effect of Se deficiency on not only antioxidant systems, but also biological functions. As far as we know, this is the first report presenting data on features of both the antioxidant systems and the biological functions under Se deficiency.

The TBARS concentration in the heart was increased by Se deficiency from the second week. The increased TBARS concentration strongly suggests an acceleration of lipid peroxidation. This is because of the decrease in heart GSH-Px (antioxidant enzyme) activity induced by Se deficiency. However, in spite of the decrease in liver GSH-Px activity from the first week in rats fed the Se-deficient diet, the liver TBARS concentration did not increase as it did in the heart. It seems reasonable to speculate that the absence of change in the liver TBARS concentration is associated with the increase in liver GST, which was not observed in the heart. GST activity increased in the Se-deficient rat livers up to about 100% of level in the Se-adequate rat livers. GST is known to possess detoxification activity through the conjugation of glutathione as well as GSH-Px activity *(23)*. It is generally believed that, as a family of enzymes that catalyze the conversion of organic peroxides to corresponding alcohols, GST compensates for the depletion of cellular GSH-Px under Se deficiency, thereby reducing the oxidative stress *(26–28)*.

Se deficiency remarkably decreased the GSH-Px activity in the skeletal muscle of rats in the present experiment from the first week, but did not alter the GST activity. Thus, although Se deficiency impaired antioxidant capacity in skeletal muscle, lipid peroxidation was not observed throughout the experiment period. This difference in lipid peroxidation between skeletal muscle and heart may be explained by the extent of oxidative stress imposed upon the two organs. Chance et al. *(29)* reported that the cardiac antioxidant defense system is critical because the heart is

a highly oxidative organ, and resting myocardium produces a steady-state concentration of 0.3–0.6 nmol H_2O_2/min/mg protein.

It is difficult to monitor the Se-deficient signs in rats, such as cardiomyopathy, weakness of muscle strength and muscle pain, which are easily observed in humans. Therefore, the authors measured the ECG (using a polygraph system) and the muscle tone (using the wire maneuver method, the inclined method and the rotating rod method) in place of the Se-deficient signs in humans. However, although the dietary Se deficiency remarkably decreased the GSH-Px activity in tissues, it did not alter either the ECG or the muscle tone. In addition, the plasma biochemistry and the hematology were not affected, too. Vitamin E, catalase, and superoxide dismutase (SOD) status were not measured in the present study, but it has been reported that Se deficiency enhances the SOD activity in the heart *(30)*. The Se-deficient diet in this study contained an adequate amount of vitamin E. Therefore, the compensatory effects of these antioxidants was thought to be the reason that abnormalities of the ECG, muscle tone, plasma biochemistry, and hematology were not observed in the Se-deficient rats throughout the experiment period. In addition, the difference in oxygen consumption among the tissues would be contained as one of the reasons. However, no abnormality of ECG was found in spite of the increased heart TBARS concentration. Thus, it is thought that cardiac function was not influenced by a TBARS increase of this degree. In other words, the authors' results suggest that more lipid peroxidation in the heart is necessary to induce an abnormal ECG. Many investigators have shown that depletion of Se in tissues did not necessarily lead to morphological and physiological abnormalities in the heart *(31–34)*, and the authors' results support their findings. It was found in a previous study that Se deficiency enhanced the abnormal ECG induced by adriamycin, a free radical generator; this implies that Se is one of the factors that determines myocardial susceptibility to oxidant stress (data not shown).

Skeletal muscle is commonly categorized as red muscle and white muscle, which consume a large and a small quantity of oxygen, respectively. The soleus muscle, as typical red muscle, and the EDL muscle, as typical white muscle, were used in the present study, but Se deficiency did not alter the lipid peroxidation in either of these skeletal muscles throughout the experiment period. Skeletal muscles are also equipped with both enzymatic and nonenzymatic scavenging systems to eliminate oxygen radicals. In a state of weakened oxidant defense, such as lowered GSH-Px activity, the increases of oxidative stress might elicit widespread damage to constituents of the cell such as membrane lipids, mitochondria, and DNA. It is conceivable that severe physical exercise, which increases total body oxygen uptake by more than 10-fold, can be potentially harmful to skeletal muscle *(35)*. Actually, physical exercise at high intensity has been shown to cause free radical-mediated tissue and cell damage in skeletal muscle. Ji et al. *(36)* reported that skeletal muscle

mitochondrial lipid peroxide content in Se-deficient rats was elevated by an acute bout of exercise. Lipid peroxidation may have been inducible in the rat skeletal muscle in the authors' experiment, especially in the red muscle, which consumes a large quantity of oxygen, if the rats had been subjected to an acute, severe exercise task.

The pliability and elasticity of erythrocytes' membranes are essential to accommodate their continual passage through the circulation for a long time. To keep erythrocytes flexible and durable, membrane phospholipids and cytoskeletal proteins must be protected from oxidative damage by antioxidants such as vitamin E and by enzymatic detoxification system components such as GSH-Px, catalase, and SOD *(1)*. Therefore, it is thought that the reduction of GSH-Px activity under Se deficiency enhances the susceptibility of erythrocytes to hemolysis. In fact, Kim et al. *(37)* and Suzuki et al. *(38)* have reported that a decrease in vitamin E content and GSH-Px activity enhances the susceptibility of erythrocytes to the t-BuOOH-induced hemolysis in rats. However, this increase in susceptibility to hemolysis with a remarkably low GSH-Px activity was not observed in the present study, in which the low osmotic pressure method was used. This difference may be because the method the authors used is an experiment system in which erythrocytes hemolyze when the constitution of their membrane is altered, but not when the antioxidant defense system alone is altered. It is no exaggeration to say that the low osmotic pressure method rather than the t-BuOOH method is appropriate to evaluate the dysfunction of erythrocyte membrane. Neither an increase in the TBARS level in erythrocytes nor a change in hematological values were observed in the present study. In addition, it has been reported by Suzuki et al. *(38)* that morphology, phospholipid, and fatty acid composition, and cytoskeleton proteins of erythrocytes are not altered by Se deficiency. Thus, it is likely that the function of the erythrocyte membrane is maintained, in spite of the finding that the antioxidant defense system was attenuated by Se deficiency from the first week. It is possible, however, that the erythrocyte membrane is damaged when it is exposed to severe free radicals stress such as hard exercise, diseases, and drugs. Suzuki et al. *(38)* also reported that t-BuOOH induced hemolysis, and that the change in membrane composition was more sensitive in Se-deficient rats than in Se-adequate rats.

Electromyocardial and mechanical characteristics of the rat heart were not substantially affected by dietary Se deficiency when vitamin E intake was adequate *(39)*. Therefore, the present data showing that the myocardium was not significantly damaged with dietary Se deficiency most likely reflect a compensatory function of vitamin E, which exceeded 100 IU/kg in the rat diet. This heart hypothesis could also apply to the liver, skeletal muscle, and erythrocytes.

The authors recently found that Se deficiency enhanced an adriamycin-induced abnormal ECG *(40)*. It has been also reported that not all

patients receiving total parenteral nutrition develop the clinical signs (cardiomyopathy, weakness of muscle strength, and muscle pain) of Se deficiency, although these patients have a low blood Se level and GSH-Px activity *(11–17)*. The onset of disorders and side effects of drugs are associated with reactive free radicals *(41)*. From these findings, free radicals stress by disorder and/or drugs as well as Se deficiency, is supposed to be required for development of the clinical signs of Se deficiency. This speculation was strongly supported by the results of the present study.

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REFERENCES

1. R. F. Burk, Protection against free radical injury by selenoenzymes, *Pharmac. Ther.* **45,** 383–385 (1990).
2. R. F. Burk, Recent developments in trace element metabolism and function: Newer roles of selenium in nutrition, *J. Nutr.* **119,** 1051–1054 (1989).
3. National Research Council, "Selenium: Its Biological Function." Washington, DC, National Research Council, pp. 81–82 (1976).
4. P. D. Whanger, China, a country with both selenium deficiency and toxicity: some thoughts and impressions, *J. Nutr.* **119,** 1236–1239 (1989).
5. H. W. Lane, A. O. Barroso, D. Englert, S. J. Dudrick, and B. S. MacFadyen, Jr., Selenium status of seven chronic intravenous hyperalimentation patients, *JPEN* **6,** 426–431 (1982).
6. S. S. Baker, R. H. Lerman, S. H. Krey, K. S. Crocker, E. F. Hirsh, and H. Cohen, Selenium deficiency with total parenteral nutrition: reversal of biochemical and functional abnormalities by selenium supplementation: a case report, *Am. J. Clin. Nutr.* **38,** 769–774 (1983).
7. S. Jacobson and L-O. Plantin, Concentration of selenium in plasma and erythrocytes during total parenteral nutrition in Crohn's disease, *Gut* **26,** 50–54 (1985).
8. H. W. Lane, C. A. Lotspeich, C. E. Moore, J. Ballard, S. T. Dudrick, and D. C. Warren, The effect of selenium supplementation on selenium status of patients receiving chronic total parenteral nutrition, *JPEN* **11,** 177–182 (1987).
9. H. J. Cohen, M. R. Brown, D. Hamilton, J. Lyons-Patterson, N. Avissar, and P. Liegey, Glutathione peroxidase and selenium deficiency in patients receiving home parenteral nutrition: time course for development of deficiency and repletion of enzyme activity in plasma and blood cells, *Am. J. Clin. Nutr.* **49,** 132–139 (1989).
10. K. Sando, M. Hoki, R. Nezu, Y. Takagi, and A. Okada, Platelet glutathione peroxidase activity in long-term total parenteral nutrition with and without selenium supplementation, *JPEN* **16,** 54–58 (1992).
11. A. M. van Rij, C. D. Thomson, J. M. McKenzie, and M. F. Robinson, Selenium deficiency in total parenteral nutrition, *Am. J. Clin. Nutr.* **32,** 2076–2085 (1979).
12. C. L. Kien and H. E. Ganther, Manifestations of chronic selenium deficiency in a child receiving total parenteral nutrition, *Am. J. Clin. Nutr.* **37,** 319–328 (1983).
13. R. J. Baptista, B. R. Bistrian, G. L. Blackburn, D. G. Miller, C. D. Champagne, and L. Buchanan, Utilizing selenious acid to reverse selenium deficiency in total parenteral nutrition patients, *Am. J. Clin. Nutr.* **39,** 816–820 (1984).

14. R. D. Watson, R. A. Cannon, G. S. Kurland, K. L. Cox, and R. C. Frates, Selenium responsive myositis during prolonged home total parenteral nutrition in cystic fibrosis, *JPEN* **9,** 58–60 (1985).
15. R. A. Johnson, S. S. Baker, J. T. Fallon, E. P. Maynard, III, J. N. Ruskin, Z. Wen, K. Ge, and H. J. Cohen, An occidental case of cardiomyopathy and selenium deficiency, *N. Engl. J. Med.* **304,** 1210–1212 (1981).
16. C. R. Fleming, J. T. Lie, J. T. McCall, J. F. O'Brien, E. E. Baillie, and J. L. Thistle, Selenium deficiency and fatal cardiomyopathy in a patient on home parenteral nutrition, *Gastroenterology* **83,** 689–693 (1982).
17. R. A. Quercia, S. Korn, D. O'Neill, J. E. Dougherty, M. Ludwig, R. Schweizer, and R. Sigman, Selenium deficiency and fatal cardiomyopathy in a patient receiving long-term home parenteral nutrition, *Clin. Pharm.* **3,** 531–535 (1984).
18. S. Courvoisier, R. Ducrot, and L. Julou, Nouveaux aspects experimentaux de l'activite centrale des derives de la phenothiazine, Psychotropic Drugs, S. Garattini and V. Ghetti, ed., Elsevier, Amsterdam, pp. 373–391 (1957).
19. N. W. Dunhan and T. S. Miya, A note on a simple apparatus for detecting neurological deficit in rats and mice, *J. Am. Pharm. Assoc.* **46,** 208, 209 (1957).
20. K. Sekine, M. Kimura, and Y. Itokawa, Method for the determination of selenium in biological samples by hydrogen selenide evolution electrothermal atomic absorption, *Jpn. J. Hyg.* **39,** 807–811 (1984).
21. H. Ohkawa, N. Ohishi, and K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem.* **95,** 351–358 (1979).
22. D. E. Paglia and W. N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70,** 158–169 (1967).
23. W. H. Habig, M. J. Pabst, and W. B. Jakoby, Glutathione S-transferase, *J. Biol. Chem.* **249,** 7130–7139 (1974).
24. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, Protein measurement with the folin phenol reagent, *J. Bio. Chem.* **193,** 265–275 (1951).
25. Y. Yamamoto, E. Niki, J. Eguchi, Y. Kamiya, and H. Shimasaki, Free radical chain oxidation of erythrocyte ghost membranes and its inhibition, *Biochem. Biophys. Acta* **819,** 29–36 (1985).
26. K. E. Hill, R. F. Burk, and J. M. Lane, Effect of selenium depletion and repletion on plasma glutathione and glutathione dependent enzymes in the rat, *J. Nutr.* **117,** 99–104 (1987).
27. R. Lawrence, L. K. Parkhill, and R. F. Burk, Hepatic cytosolic non-selenium-dependent glutathione peroxidase activity: its nature and the effect of selenium deficiency, *J. Nutr.* **108,** 981–987 (1978).
28. L. L. Ji, F. W. Stratman, and H. A. Lardy, Antioxidant enzyme system in rat liver and skeletal muscle: Influences of selenium deficiency, acute exercise and chronic training, *Arch. Biochem. Biophys.* **263,** 150–160 (1988).
29. B. Chance, H. Sies, and A. Boveris, Hydroperoxide metabolism in mammalian organs, *Physiol. Rev.* **59,** 527–605 (1979).
30. L. L. Ji, F. W. Stratman, and H. A. Lardy, Antioxidant enzyme response to selenium deficiency in rat myocardium, *J. Am. Coll. Nutr.* **11,** 79–86 (1992).
31. S. Kennedy and D. A. Rice, Selective morphologic alterations of the cardiac conduction system in calves deficient in vitamin E and selenium, *Am. J. Pathol.* **130,** 315–325 (1988).
32. O. Oster, M. Dahm, H. Oelert, and W. Prellwitz, Concentrations of some trace elements (Se, Zn, Cu, Fe, Mg, K) is in blood and heart tissue of patients with coronary heart disease, *Clin. Chem.* **35,** 851–856 (1989).
33. H. Ikram, I. G. Crozier, M. Webser, and C. J. Low, The role of selenium deficiency in occidental dilated cardiomyopathy, *NZ. Med. J.* **102,** 100–102 (1989).
34. J. R. Arthur, D. B. McPhail, and B. A. Goodman, Spin trapping of free radicals in homogenates of heart from selenium and vitamin E deficient rats, *Free Radical Res. Commun.* **4,** 311–315 (1988).
35. K. J. A. Davies, A. T. Quintanilha, G. A. Brooks, and L. Packer, Free radicals and tissue damage produced by exercise, *Biochem. Biophys. Res. Commun.* **107,** 1198–1205 (1982).

36. L. L. Ji, F. W. Stratman, and H. A. Lardy, Antioxidant enzyme system in rat liver and skeletal muscle, *Arch. Biochem. Biophys.* **263,** 150–160 (1988).
37. C. H. Kim, K. Yasumoto, T. Suzuki, and M. Yoshida, *tert*-Butyl hydroperoxide-induced hemolysis of α-tocopherol-decreased erythrocytes from selenium-deficient and selenium-adequate rats, *J. Nutr. Sci. Vitaminol.* **34,** 481–490 (1988).
38. T. Suzuki, C. H. Kim, and K. Yasumoto, Deterioration of membrane morphology, phospholipids, and cytoskeletal protein in rat erythrocytes exposed to *tert*-butyl hydroperoxide: protection by exogenous glutathione fails in selenium deficiency, *J. Nutr. Sci. Vitaminol.* **34,** 491–506 (1988).
39. J. Ringstad, P. M. Tande, G. Norheim, and H. Refsum, Selenium deficiency and cardiac electrophysiological and mechanical function in the rat, *Pharmacol. Toxicol.* **63,** 189–192 (1988).
40. A. Matsuda, M. Kimura, and Y. Itokawa, Influence of selenium deficiency on the acute cardiotoxicity of adriamycin in rats, *Biol. Trace Element Res.* **57,** 157–167 (1997).
41. B. Halliwell and J. M. C. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview, in *Methods in Enzymology,* vol. 186, L. Packer and A. N. Glazer, ed., Academic, San Diego, pp. 1–85 (1990).