

## Effects of Excess Selenomethionine on Selenium Status Indicators in Pregnant Long-Tailed Macaques (*Macaca fascicularis*)

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### ABSTRACT

Forty pregnant long-tailed macaques were treated daily for 30 d with 0, 25, 150, or 300  $\mu\text{g}$  selenium as L-selenomethionine/kg body weight. Erythrocyte and plasma selenium and glutathione peroxidase specific activities, hair and fecal selenium, and urinary selenium excretion were increased by and were linearly related to L-selenomethionine dose. Hair selenium was most sensitive to L-se-

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lenomethionine dose, with an 84-fold increase in the 300  $\mu\text{g}$  selenium/(kg-d) group relative to controls ( $r = 0.917$ ). Daily urinary selenium excretion (80-fold,  $r = 0.958$ ), plasma selenium (22-fold,  $r = 0.885$ ), erythrocyte selenium (24-fold,  $r = 0.920$ ), and fecal selenium (18-fold,  $r = 0.911$ ) also responded strongly to L-selenomethionine. Erythrocyte and plasma glutathione peroxidase specific activities increased 154% and 69% over controls, respectively. Toxicity was associated with erythrocyte selenium  $> 2.3 \mu\text{g/mL}$ , plasma selenium  $> 2.8 \mu\text{g/mL}$ , and hair selenium  $> 27 \mu\text{g/g}$ . Plasma, erythrocyte, and hair selenium concentrations may be useful for monitoring and preventing the toxicity of L-selenomethionine administered to humans in cancer chemoprevention trials.

**Index Entries:** Selenomethionine; selenium; glutathione peroxidase; *Macaca fascicularis*; long-tailed macaque; nonhuman primate; selenium status assessment; selenium toxicity, cancer chemoprevention.

## INTRODUCTION

The trace element selenium (Se) is required in very small amounts (approx. 0.1  $\mu\text{g/g}$  in the diet) to maintain the health of animals and humans (1). Se is also one of the most toxic trace elements, causing adverse effects in some species at a level of as little as 2  $\mu\text{g/g}$  (1). Many experimental studies have shown that dietary Se at levels of 0.5–5  $\mu\text{g/g}$  can protect laboratory animals from spontaneous, chemically-induced, and transplanted malignant tumors (2). Epidemiological studies have found significant inverse correlations between the incidence of many forms of human cancer and the geographical distribution of Se or tissue Se concentrations (3). Prophylactic Se supplementation has been advocated as a potential chemopreventive agent in human clinical trials (4,5). L-Selenomethionine (L-SeMet) has been proposed as a relatively safe form of Se to administer in clinical trials, in part because of its lower acute toxicity compared to inorganic Se (5). Chen and Clark have proposed Phase I clinical trials using 10–70  $\mu\text{g}$  organic Se/(kg-d) (mostly L-SeMet) in subjects at high risk of cancer, while monitoring for "signs of selenosis" (4). Because of the extremely long biological half-life in humans of Se from SeMet (6), it could take an unacceptably long time for selenosis to resolve, once established. Therefore, there is a need for other clinical parameters that could be monitored to warn of impending Se toxicity from L-SeMet administration.

Although Se concentrations in various tissues and the activity of the Se-dependent enzyme glutathione peroxidase (EC 1.11.1.9, GPx) have been studied extensively as indicators of Se nutritional status, relatively little is known about their responses in humans and nonhuman primates to toxic levels of Se, particularly Se in the form of L-SeMet. Se concentrations in hair and nails are sensitive indicators of dietary Se exposure in

humans, responding to wide variations in Se intake, however, they can be influenced by a variety of different factors, including the chemical form of dietary Se (7). GPx is a specific functional indicator of Se status, but is reported to be responsive over only a limited range of Se intakes (1,8).

Environmental Se contamination at the Kesterson National Wildlife Reserve in Central California caused embryonic deaths and severe birth defects in several species of aquatic wildfowl (9). Subsequent controlled studies showed that DL-SeMet was a more potent teratogen in mallard ducks than was inorganic Se (10). Since L-SeMet is a major form of Se in the human diet (11-13), the situation at Kesterson raised public health concerns over the safety of pregnant women consuming elevated levels of Se and prompted the state of California to sponsor a study on the potential developmental toxicity of L-SeMet in nonhuman primates (14,15). The study presented here was part of this developmental toxicity study of L-SeMet. This study was designed to describe the responses of Se status indicators to toxic doses of L-SeMet and to address the question of whether Se status indicators can be useful as indicators of L-SeMet toxicity.

## METHODS

### *Animal Treatments and Diet*

Forty pregnant female long-tailed macaques (*Macaca fascicularis*) were housed individually in stainless steel cages and maintained in accordance with standards established by the Federal Animal Welfare Act and Institute for Laboratory Animal Resources. Animals were maintained on a 12 h light/dark cycle at a constant temperature of 22°C at 60% relative humidity.

Animals were fed Purina Monkey Chow (15% protein, Purina Mills, St. Louis, MO) twice daily and provided tap water, both ad libitum. The drinking water contained less than 1 ng Se/mL and the diet had a mean Se content of  $0.16 \pm 0.04$   $\mu\text{g/g}$ , giving an estimated daily dietary Se intake of 3  $\mu\text{g/kg}$  body weight. A powdered nutritional supplement produced for human consumption ("Meritene," Sandoz Nutritional Corp., Minneapolis, MN) was administered with water by nasogastric intubation and fresh fruit was given, as required, to minimize weight losses during periods of anorexia in those animals so affected.

The study was conducted in two phases. A preliminary study in nonpregnant female macaques estimated the maximum tolerated dose (the highest dose causing less than 10% body weight loss in 30 d) as 150  $\mu\text{g}$  Se (as L-SeMet)/(kg-d) (14). The highest dose in the present study, 300  $\mu\text{g}$  Se/(kg-d), was chosen to induce frank selenosis in the dams so that the effects of maternal toxicity could be distinguished from any specific teratogenic effects of L-SeMet.

The present study was conducted with 40 pregnant animals and L-SeMet was administered on gestation days 20–50. L-SeMet (seleno-L-methionine, #S-3132, Sigma Chemical Co., St. Louis, MO, 99% purity) solutions were prepared fresh each day in distilled water and were administered immediately by nasogastric intubation at a constant volume of 5 mL/(kg-d). Animals were randomly assigned to one of four treatment groups (10/group): controls (no supplemental L-SeMet); 25  $\mu\text{g Se}/(\text{kg-d})$ ; 150  $\mu\text{g Se}/(\text{kg-d})$ ; or, 300  $\mu\text{g Se}/(\text{kg-d})$ , where the L-SeMet doses are expressed as " $\mu\text{g Se as L-SeMet per kg body weight per day.}$ " One animal in the 150  $\mu\text{g Se}/(\text{kg-d})$  group was removed from the study when ultrasound examination on day 15 of L-SeMet treatment revealed a non-viable embryo. One animal in the 300  $\mu\text{g Se}/(\text{kg-d})$  group was removed from the study due to a spontaneous abortion on day 23 of L-SeMet treatment.

### **Sample Preparation and Analysis**

Approximately 2 mL of whole blood was collected by venipuncture (EDTA anticoagulant) once within three days prior to initiation of treatment, every 3 d during treatment, and every 10–14 d thereafter for 43 d post treatment. Plasma was separated by centrifugation and erythrocytes were washed in physiological saline and pelleted by centrifugation. Erythrocyte pellets and plasma were stored at  $-70^{\circ}\text{C}$  until analyzed.

Urine and feces were obtained from 2 or 3 animals in each group undergoing pharmacokinetic procedures on treatment days 1, 15, and 30 and were stored at  $-70^{\circ}\text{C}$  until analyzed. Hair samples were collected from all animals once within 3 d prior to initiation of treatments, then once every 3 wk for 9 wk. A 5 cm square patch of hair in the subscapular region of the back was clipped and the most proximal portion (approximately 0.5 cm) was stored in plastic bags at room temperature until analyzed. The hair sample (day 22–43) for one animal in the 300  $\mu\text{g Se}/(\text{kg-d})$  group was not obtained.

GPx activity in erythrocytes and plasma was assayed by the automated colorimetric method of Hawkes and Craig (16) using bovine erythrocyte GPx (Sigma, #G-6137) as the standard. Protein concentration was measured by an automated adaptation of the bicinchoninic acid method (17) using bovine serum albumin as the standard. Duplicate pooled human erythrocytes and duplicate pooled human plasma were included in each analytical run as long term quality control standards. GPx specific activity (S.A.) was expressed as micromoles of GSH oxidized per minute per g of protein.

Total Se was determined by a modification of Watkinson's fluorometric method (18). Briefly, 0.25 mL plasma or pelleted erythrocytes, up to 0.11 g hair, up to 2 mL urine, or up to 0.5 g feces or diet was mixed with 1 mL concentrated perchloric acid and 2.5 mL concentrated nitric acid and digested for 90 min at  $140^{\circ}\text{C}$ , then for 75 min at  $200^{\circ}\text{C}$  in  $16 \times$

125 mm tubes. Drinking water samples (500 mL) were made 1 mol/L in HNO<sub>3</sub> and concentrated by boiling to approximately 2 mL, then digested as described above. One mL 4 mol/L HCl was added to the digested samples, and the tubes were heated for 15 min at 150°C to reduce selenate to selenite. One mL 2 mol/L glycine base, 0.09 mol/L Na<sub>4</sub>EDTA, and 4 drops of 0.02% Cresol Red were added, and the pH was adjusted to pH 1.5–2.0 with 7 mol/L NH<sub>4</sub>OH (amber-orange). One and one-half mL 2 mol/L glycine (pH 1.75) was added, and the samples were diluted to 8 mL with distilled water. One mL 0.25% (w/v) recrystallized 2,3-diaminonaphthalene (Sigma) in 0.1 mol/L HCl was added, and the mixture was heated for 45 min at 50°C. The fluorescent piaszelenol was extracted in 3 mL cyclohexane and the relative fluorescent intensity was measured at 370 nm excitation and 518 nm emission (LS-3B Fluorescence Spectrometer, Perkin Elmer, Norwalk, CT). Se standards were prepared from National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards, NBS) Standard Reference Material (SRM) 3149. Quality control standards (NBS-SRM 1577 or NIST-SRM 1577a-Bovine Liver, NIST-RM 8431-Mixed Diet, NIST-SRM 2670-Toxic Metals in Urine, NIST-SRM 1643b-Trace Elements in Water, and duplicate pooled human erythrocytes, plasma, and urine) were included in each analytical run, as appropriate.

In all analyses, the analysts were blinded to the identity of the samples during analysis and data collection.

### **Statistical Analysis**

Quality control data were analyzed and charted using NWA Quality Analyst software (Northwest Analytical, Portland, OR). Linear regression, analysis of variance, t-tests, and Fisher's LSD tests were conducted using BMDP Solo statistical analysis software (BMDP Statistical Software, Inc., Los Angeles, CA). Data were transformed by logarithmic or power functions, as needed, to ensure homogeneity of variance before conducting the analyses of variance. A probability level of 0.05 or less was considered significant.

## **RESULTS**

### **Time Course of Se Status Indicators**

All of the Se status indicators showed clear dose- and time-dependent increases with L-SeMet treatment (Figs. 1–7). Erythrocyte Se and GPx S.A. (Figs. 1 and 2) exhibited a delay of several days before increases became noticeable. Erythrocyte GPx S.A. was lower in the 300 µg Se/(kg-d) group than in the 150 µg Se/(kg-d) group (Fig. 2 and Table 1). Plasma Se concentration (Fig. 3) showed a prompt increase at initiation of L-SeMet treatment. At the two highest L-SeMet doses, plasma Se

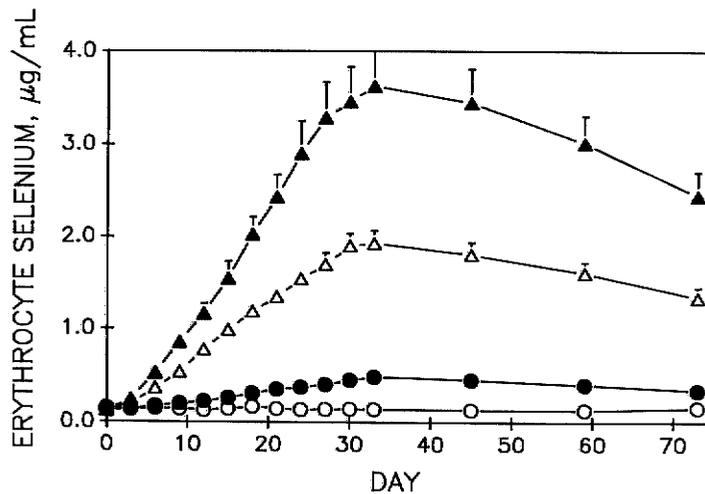


Fig. 1. Time course of erythrocyte Se concentrations. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

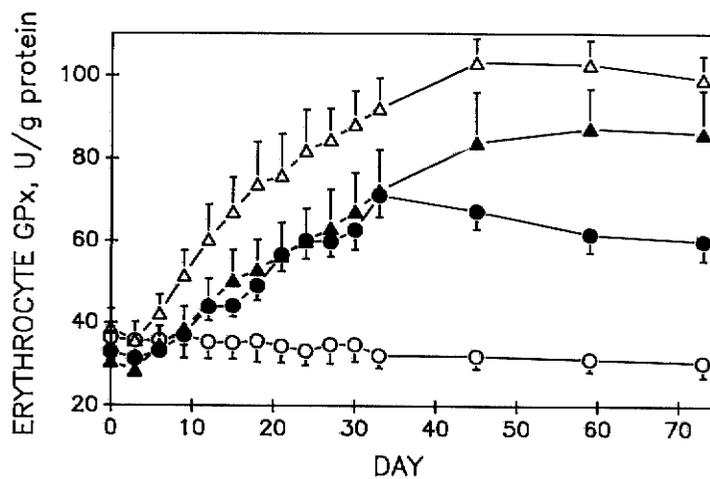


Fig. 2. Time course of erythrocyte GPx S.A.s. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

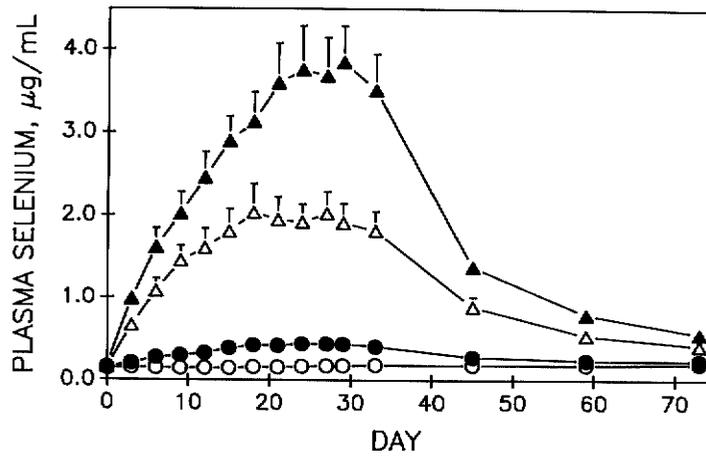


Fig. 3. Time course of plasma Se concentrations. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

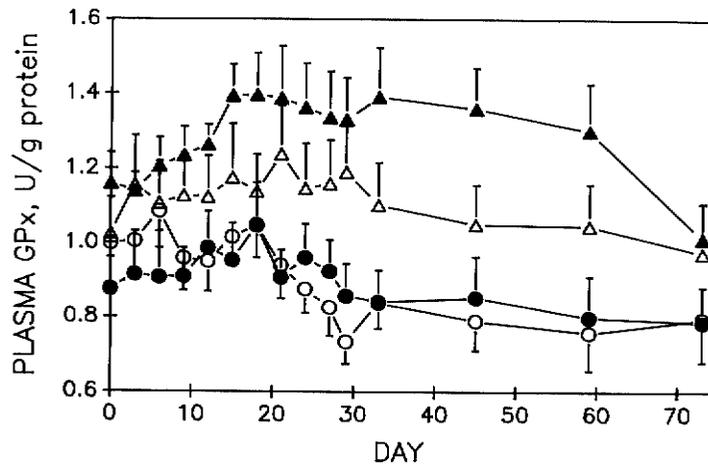


Fig. 4. Time course of plasma GPx S.A.s. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

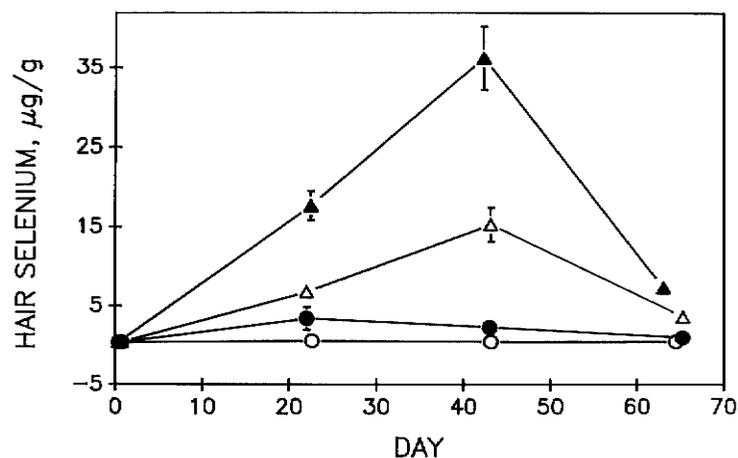


Fig. 5. Time course of hair Se concentrations. Data are plotted at the days that sampling occurred, but represent the Se accumulated in the hair since the last sampling. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

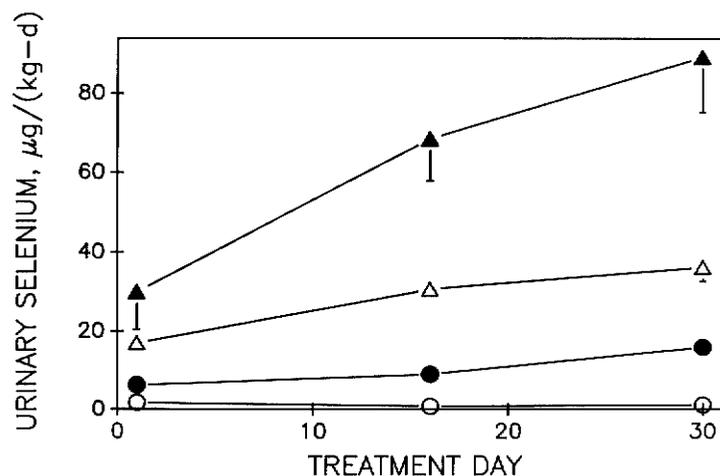


Fig. 6. Time course of daily urinary Se excretion. Data displayed are the group means  $\pm$  the standard errors of the means, expressed as  $\mu\text{g Se}$  excreted per day per kg body weight from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

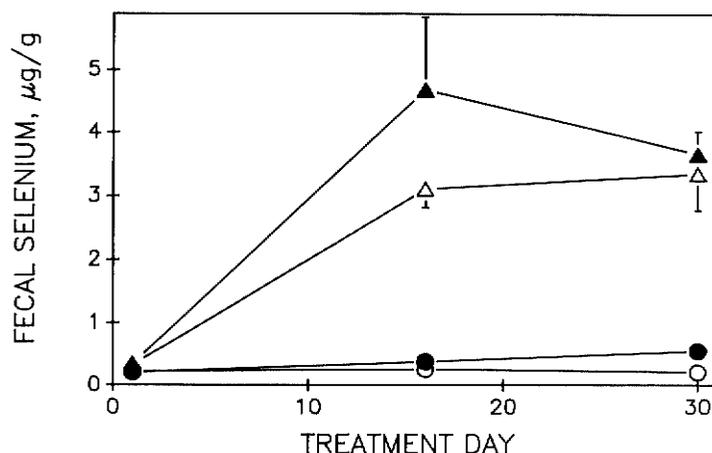


Fig. 7. Time course of fecal Se concentrations. Data displayed are the group means  $\pm$  the standard errors of the means from animals receiving daily L-SeMet treatments on days 1–30. Control group (○); 25  $\mu\text{g Se}/(\text{kg-d})$  group (●); 150  $\mu\text{g Se}/(\text{kg-d})$  group (Δ); and 300  $\mu\text{g Se}/(\text{kg-d})$  group (▲).

appeared to plateau by day 21. Compared to erythrocyte GPx S.A. (Fig. 2), plasma GPx S.A. (Fig. 4) increased only slightly with duration of treatment. Plasma Se (Fig. 3) decreased promptly when treatment was discontinued, such that the plasma Se in the 300  $\mu\text{g Se}/(\text{kg-d})$  group had decreased to 12% of its maximum level by day 73. In contrast, erythrocyte Se (Fig. 1) decreased much more slowly, such that the 300  $\mu\text{g Se}/(\text{kg-d})$  group had decreased to only 67% of its maximum level by day 73. In plasma and erythrocytes, GPx S.A. decreased more slowly than Se concentration after the end of L-SeMet treatment, such that by day 73 the GPx S.A.s of the 300  $\mu\text{g Se}/(\text{kg-d})$  group were 73 and 98%, respectively, of their maximum levels.

Hair Se (Fig. 5) and daily urinary Se excretion (Fig. 6) appeared to increase linearly with duration of L-SeMet treatment. Fecal Se (Fig. 7) appeared to plateau between days 15 and 30, especially in the 150 and 300  $\mu\text{g Se}/(\text{kg-d})$  groups.

### ***Se Status Indicators and Toxicity Signs at the End of Treatment***

The mean and standard deviation of each of the 7 indicators in the 4 treatment groups at the conclusion of the 30 d treatment period are shown in Table 1. Analysis of variance revealed highly significant ( $p < 0.001$ ) effects of L-SeMet dose upon each of the indicators. Regression of each of the Se status indicators on the L-SeMet dose revealed significant

Table 1  
Effects of L-SeMet on Indicators of Se Status in Pregnant Female Long-Tailed Macaques

Indicator	Control	Treatment groups ( $\mu\text{g Se as L-SeMet/kg body wt-day}$ )			Correlation coefficient <sup>†</sup> (r)
		25	150	300	
RBC <sup>†</sup> Se, $\mu\text{g/mL}$	0.14 $\pm$ 0.02 <sup>a</sup> (N = 10)	0.45 $\pm$ 0.11 <sup>b</sup> (N = 10)	1.90 $\pm$ 0.40 <sup>c</sup> (N = 9)	3.46 $\pm$ 1.13 <sup>d</sup> (N = 9)	0.920***
RBC <sup>†</sup> GPx, U/g <sup>‡</sup>	34.7 $\pm$ 13.1 <sup>a</sup> (N = 10)	62.6 $\pm$ 15.3 <sup>b</sup> (N = 10)	88.2 $\pm$ 24.2 <sup>c</sup> (N = 9)	67.2 $\pm$ 28.2 <sup>b</sup> (N = 9)	0.383*
Plasma Se, $\mu\text{g/mL}$	0.18 $\pm$ 0.03 <sup>a</sup> (N = 10)	0.43 $\pm$ 0.11 <sup>b</sup> (N = 10)	1.91 $\pm$ 0.71 <sup>c</sup> (N = 9)	3.86 $\pm$ 1.50 <sup>d</sup> (N = 9)	0.885***
Plasma GPx, U/g <sup>‡</sup>	0.73 $\pm$ 0.19 <sup>a</sup> (N = 10)	0.86 $\pm$ 0.28 <sup>a</sup> (N = 10)	1.19 $\pm$ 0.39 <sup>b</sup> (N = 9)	1.24 $\pm$ 0.25 <sup>b</sup> (N = 9)	0.575**
Hair Se, $\mu\text{g/g}$	0.43 $\pm$ 0.22 <sup>a</sup> (N = 10)	2.30 $\pm$ 0.44 <sup>b</sup> (N = 10)	15.3 $\pm$ 6.5 <sup>c</sup> (N = 9)	36.2 $\pm$ 11.6 <sup>d</sup> (N = 8)	0.917***
Urine Se, $\mu\text{g/(kg-d)}$	1.12 $\pm$ 0.22 <sup>a</sup> (N = 3)	15.9 $\pm$ 5.0 <sup>b</sup> (N = 3)	36.2 $\pm$ 6.2 <sup>c</sup> (N = 3)	89.3 $\pm$ 20.0 <sup>d</sup> (N = 2)	0.958***
Fecal Se, $\mu\text{g/g}$ (wet weight)	0.21 $\pm$ 0.05 <sup>a</sup> (N = 3)	0.55 $\pm$ 0.20 <sup>b</sup> (N = 2)	3.35 $\pm$ 0.82 <sup>c</sup> (N = 2)	3.66 $\pm$ 0.51 <sup>c</sup> (N = 2)	0.911**

Values represent the means of determinations made at the conclusion of 30 d continuous oral administration of L-SeMet  $\pm$  standard deviation. Hair samples grown between day 22 and day 43 after initiation of L-SeMet treatment. Values within a row not sharing a common superscript were significantly different ( $p < 0.05$ ) using Fisher's LSD test.

<sup>†</sup>Correlation coefficient for regressions of indicators on the L-SeMet dose. Statistical significance of regression: \*\*\* $p < 0.0001$ ; \*\* $p < 0.001$ ; \* $p < 0.05$ .

<sup>‡</sup>RBC: erythrocytes.

<sup>§</sup>Specific activity: enzyme U/g protein (micromoles GSH oxidized/min/g protein).

( $p < 0.05$ ) linear trends. The correlations with L-SeMet dose (Table 1) were strongest for the tissue Se concentrations and weaker for the GPx S.A.s. Hair Se, daily urinary Se excretion, erythrocyte Se, and plasma Se were significantly different between all four treatment groups (Table 1). There were fewer significant differences between the group means for fecal Se and erythrocyte GPx S.A., and fewer still for the weakly responding plasma enzyme.

All of the groups showed a decrease in their mean body weights (not shown) during L-SeMet treatment, however, the percentage body weight losses were significantly greater than the controls only in the 300  $\mu\text{g Se}/(\text{kg-d})$  group (15). Se toxicity was evidenced by anorexia and vomiting in the 300  $\mu\text{g Se}/(\text{kg-d})$  group and the mean percentage body weight loss was 3 times greater than in the 150  $\mu\text{g Se}/(\text{kg-d})$  (maximum tolerated dose) group, despite administration of dietary supplements (15). There was considerable inter-individual variation within each dose group in the values for erythrocyte Se, plasma Se, and hair Se (Table 1), with several animals in the 150  $\mu\text{g Se}/(\text{kg-d})$  group having higher values than some animals in the 300  $\mu\text{g Se}/(\text{kg-d})$  group. However, the percentage body weight losses in animals that had Se status indicator values at day 30 of treatment greater than 2.3  $\mu\text{g/mL}$  erythrocyte Se, 2.8  $\mu\text{g/mL}$  plasma Se, or 27  $\mu\text{g/g}$  hair Se were significantly greater ( $p \leq 0.01$ , *t*-test) than the percentage body weight losses in animals with lower values of these indicators, regardless of dose. In addition, these three Se status indicators were each significantly correlated with the percentage body weight loss: erythrocyte Se  $r = 0.598$ ,  $p = 0.0001$ ; plasma Se  $r = 0.650$ ,  $p < 0.0001$ ; and hair Se  $r = 0.463$ ,  $p < 0.005$ . Fecal Se, daily urinary Se excretion, and the two GPx S.A.s were not significantly associated with percentage body weight loss.

Figure 8 shows the relative responses of the Se status indicators to L-SeMet dose, with each parameter expressed as a percentage of its respective control value to illustrate the relative sensitivity of each indicator to L-SeMet. Relative to their respective control levels, hair Se and daily urinary Se excretion displayed the greatest increases with L-SeMet dose, and the erythrocyte and plasma GPx S.A.s the smallest.

## DISCUSSION

The delay of several days before erythrocyte Se and GPx S.A. began to increase (Figs. 1 and 2) may reflect a requirement for erythropoiesis in order for Se to be incorporated into the erythrocytes from L-SeMet. Similar results have been obtained in human studies where delays of 2–3 wk for erythrocyte Se and 2–4 wk for erythrocyte GPx were observed in supplementation experiments with various forms of Se (19–22). The shorter delays observed in the present study may be related to the very high doses of L-SeMet administered and are consistent with the shorter erythrocyte lifetime in monkeys of 90–100 d vs 120 d in humans (23).

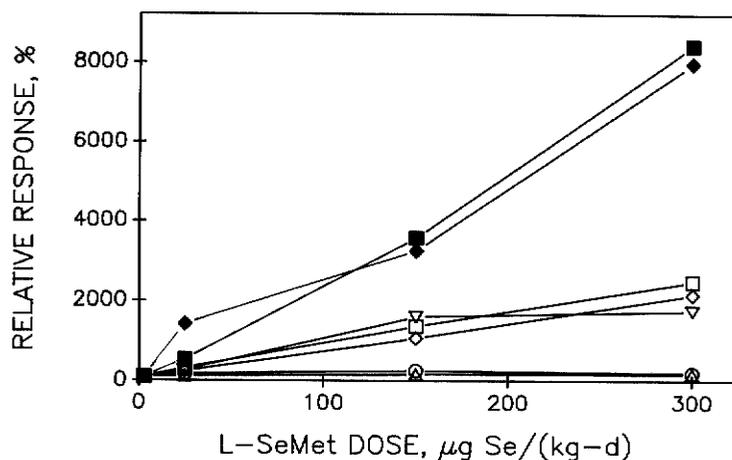


Fig. 8. Relative responses of Se status indicators to L-SeMet intake. Data from day 30 at the end of treatment (days 22–43 for hair Se). Only the group means are displayed for clarity. Data for each indicator are expressed as a percentage of the control value for that indicator. Plasma GPx S.A. ( $\Delta$ ), erythrocyte GPx S.A. ( $\circ$ ), fecal Se concentration ( $\nabla$ ), erythrocyte Se concentration ( $\square$ ), plasma Se concentration ( $\diamond$ ), daily urinary Se excretion ( $\blacklozenge$ ), and hair Se concentration ( $\blacksquare$ ).

The plateau of plasma Se at days 21–30 (Fig. 3) suggests that plasma may have achieved a steady state Se concentration, whereas erythrocyte Se (Fig. 1) was still increasing at the end of treatment (day 30). A similar plateau of plasma Se was observed in humans supplemented with 100–200  $\mu\text{g Se/d}$  as high-Se wheat bread (presumably L-SeMet) with maximum plasma Se concentrations occurring after 5–6 wk of supplementation (24).

The fact that the erythrocyte GPx S.A. continued to increase in the 150 and 300  $\mu\text{g Se/(kg-d)}$  groups after cessation of treatment (Fig. 2) suggests that Se deposited initially in some other pool, presumably nonspecifically substituted for methionine in proteins (11,25), continued to be made available for erythrocyte GPx synthesis for several weeks. This is consistent with reports that Se from SeMet has a longer biological half-life than Se from selenocysteine (the form in GPx) in mice (26) and a longer half-life than inorganic Se in rhesus monkeys (27), rats (28), and hamsters (29). In rats, the Se in GPx continued to increase after cessation of SeMet administration, while the Se in rat hemoglobin decreased (30), consistent with the fact that erythrocyte GPx S.A. continued to increase in the 150 and 300  $\mu\text{g Se/(kg-d)}$  groups after cessation of L-SeMet treatment (Fig. 2) even though the erythrocyte Se was decreasing (Fig. 1).

Of the indicators tested, erythrocyte and plasma GPx S.A. had the smallest relative responses to L-SeMet. This was not unexpected because

enzymes are typically subject to physiological control, and since L-SeMet is known to substitute freely for methionine in protein synthesis, rather than being directed exclusively to synthesis of selenoproteins (11,25,31). Nevertheless, the increase in erythrocyte GPx S.A. was surprisingly large. Several lines of evidence indicate that the increase in erythrocyte GPx S.A. was not caused by nonenzymatic effects of high tissue Se nor to methodological problems. First, the time courses of total Se and GPx S.A.s were not parallel in either erythrocytes or plasma, with total Se decreasing more rapidly than the GPx S.A.s after cessation of treatment (Figs. 1-4). Second, the increases in erythrocyte GPx S.A. in the 150 and 300  $\mu\text{g Se}/(\text{kg-d})$  groups were confirmed in blood samples from several animals by reassay of the GPx using the independent method of Paglia and Valentine (32), as modified by Tappel (33). Finally, the elevated erythrocyte GPx activity in the 150  $\mu\text{g Se}/(\text{kg-d})$  dose group was more than 95% inhibited by 5 mmol/L iodoacetic acid, a specific inhibitor of selenoenzymes at neutral pH (16,34,35), demonstrating that essentially all of the observed GPx activity was caused by Se-dependent GPx.

A controlled study in rats (36) and several epidemiological and dietary intervention studies in humans (1,8,37-39), concluded that GPx activity increased in response to dietary Se up to the Se requirement of the species, but tended to plateau with higher levels of Se. The response of GPx has never been studied at the extremely high doses employed in the present study, judging from the available literature. The increase in GPx activity tends to plateau at Se levels above the nutritional requirement, but the slope does not go to zero. A slope that appears insignificant when viewed at a scale of 0 to 3  $\mu\text{g Se}/(\text{kg-d})$  may appear very different when viewed at a scale of 3 to 300  $\mu\text{g Se}/(\text{kg-d})$ , as in the present study. The rate of increase of erythrocyte GPx S.A. observed with increasing L-SeMet dose in macaques can be compared to a similar study in rats fed supplemental SeMet (30). If it is assumed that the Se requirement of macaques is similar to humans, then the RDA of 70  $\mu\text{g Se}/\text{d}$  would correspond to a Se requirement for macaques of about 1  $\mu\text{g Se}/(\text{kg-d})$ . When the dietary Se of rats was increased from an adequate level to 4  $\mu\text{g Se}$  (as DL-SeMet)/g of diet (about 40 times the rat requirement, [1]), the erythrocyte GPx S.A. increased by 40% (30), consistent with the results of the present study where increasing the Se dose from an adequate level to 150  $\mu\text{g Se}$  (as L-SeMet)/(kg-d) (about 150 times the human requirement) caused a 154% increase in erythrocyte GPx S.A.

It is of interest to note that the mean erythrocyte GPx S.A. in the 300  $\mu\text{g Se}/(\text{kg-d})$  group was significantly lower than in the 150  $\mu\text{g Se}/(\text{kg-d})$  group (Table 1). The possibility that the decreased erythrocyte GPx S.A. in the 300  $\mu\text{g Se}/(\text{kg-d})$  group was associated with L-SeMet toxicity is suggested by several facts. First, the L-SeMet dose of 300  $\mu\text{g Se}/(\text{kg-d})$  was twice the estimated maximum tolerated dose of 150  $\mu\text{g Se}/(\text{kg-d})$  (14). Next, the animals in the 300  $\mu\text{g Se}/(\text{kg-d})$  group suffered percentage body weight losses 3 times greater than those in the 150  $\mu\text{g Se}/(\text{kg-d})$

group (15). Finally, the mean percentage body weight loss in animals in the 150 and 300  $\mu\text{g Se}/(\text{kg}\cdot\text{d})$  groups with erythrocyte GPx S.A. less than 68 U/g protein ( $12.6 \pm 8.3\%$ ), was 4 times greater ( $p < 0.02$ ) than in animals with GPx S.A. greater than 68 U/g protein ( $3.0 \pm 4.9\%$ ). While the actual mechanism underlying the decrease in GPx S.A. in the 300  $\mu\text{g Se}/(\text{kg}\cdot\text{d})$  group is not known, this observation fits well with the classical paradigm wherein nutritional status initially increases with increasing amounts of a nutrient, then plateaus as the nutritional requirement is exceeded, and ultimately decreases as the toxic level of the nutrient is reached.

The linear responses of tissue Se concentrations to L-SeMet dose in the present study are in agreement with studies in rats that found linear responses of hair Se, erythrocyte Se, plasma Se, and whole blood Se to 0–4  $\mu\text{g Se}$  (as SeMet)/g of diet (7,30). An 8 mo supplementation study of residents in a low Se area of China (40) found that erythrocyte Se, plasma Se, 12 h urinary Se excretion, and erythrocyte GPx increased linearly with DL-SeMet intake up to 90  $\mu\text{g Se}/\text{d}$ , whereas plasma GPx showed a weaker response, similar to the results of the present study. Consistent with the finding that hair Se had the greatest relative response to L-SeMet intake in the present study, hair Se in rats also showed a greater increase following administration of high levels of L-SeMet (up to 4  $\mu\text{g Se}/\text{g}$  diet), relative to controls, than did erythrocyte or plasma Se (7).

In terms of its relative sensitivity to L-SeMet dose and its significant association with percentage body weight loss, hair Se can be considered the most useful indicator of the toxic L-SeMet doses studied here. Two limitations of using human hair Se as an indicator are the necessarily long intervals between samplings and the possible confounding effects of the use of Se containing dandruff shampoos (41). Daily urinary Se excretion was nearly as sensitive as hair Se, but was not significantly associated with the percentage body weight loss. Erythrocyte and plasma Se showed 22–24-fold increases over the range of L-SeMet intakes studied. Although their relative sensitivities were less than for hair Se, erythrocyte and plasma Se were useful indicators of the toxic L-SeMet doses given and had stronger associations with percentage body weight loss than did hair Se.

Chronic selenosis in residents of seleniferous areas of China was associated with whole blood Se levels of 3  $\mu\text{g Se}/\text{mL}$  or greater and with hair Se levels of 20  $\mu\text{g Se}/\text{g}$  or greater (42). The results of the present study are consistent with these human data in that blood and hair Se levels in the 25 and 150  $\mu\text{g Se}/(\text{kg}\cdot\text{d})$  groups ( $\leq$  the maximum tolerated dose) were lower than the levels associated with signs of Se toxicity in humans in the Chinese study. At the highest L-SeMet dose in the present study, 300  $\mu\text{g Se}/(\text{kg}\cdot\text{d})$ , signs of Se intoxication were evident (15) and the mean blood and hair Se levels (Table 1) exceeded those associated with human toxicity in the Chinese study (42). Furthermore, the levels of erythrocyte Se, plasma Se, and hair Se associated with increased percent-

age body weight loss in the present study ( $>2.3 \mu\text{g/mL}$ ,  $> 2.8 \mu\text{g/mL}$  and  $>27 \mu\text{g/g}$ , respectively) are generally consistent with the cutoff values for humans identified in the Chinese study.

In conclusion, the present study has shown that hair, fecal, erythrocyte, and plasma Se concentrations, daily urinary Se excretion rate, and erythrocyte and plasma GPx S.A.s in pregnant long-tailed macaques responded to L-SeMet doses up to levels that elicited signs of toxicity. Hair Se was the most sensitive indicator of L-SeMet dose and hair Se, erythrocyte Se and plasma Se were significantly associated with body weight losses indicative of Se toxicity. These results indicate that these Se measurements in nonhuman primates are useful indicators of L-SeMet toxicity and they may therefore be useful for monitoring the accumulation of Se from L-SeMet and controlling the toxicity of L-SeMet administered to humans.

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