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Plasma and Liver Selenium Levels in the Rat During Supplementation with 0.5, 2, 6, and 15 ppm Selenium in Drinking Water

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ABSTRACT

Plasma and liver selenium of Wistar rats were determined after 1, 3, and 6 mo supplementation with 0.5, 2, 6, or 15 ppm selenium as sodium selenite in drinking water. Plasma selenium was not different from control values at additional intake of 0.5 ppm but increased above usual levels at higher intakes. A highly significant correlation was observed between the total quantity of selenium ingested and plasma selenium after 1 mo treatment ($r = 0.99$, $p < 0.01$), but was less pronounced after 3 and 6 mo (0.94, $p < 0.05$, and 0.78, $p < 0.05$, respectively). The decrease in plasma selenium with time of treatment was more pronounced at higher intakes. There was also a highly significant correlation between total selenium intake and liver selenium concentration ($r = 0.99$, $p < 0.01$) after 1 mo of treatment, but this time liver selenium did not change with time, and the correlation remained highly significant throughout the investigation. Liver selenium therefore appears as a more sensitive and more representa-

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tive measure of selenium intake than plasma selenium. Most supplements did not affect body weight and survival of animals, except when the diet was supplemented with 15 ppm for 6 mo; however, alterations in biochemical parameters concerning lipid status and hepatic function were observed at levels above 2.0 ppm.

Index Entries: Selenium; plasma; liver; rat; intake; supplementation; toxicity.

INTRODUCTION

The selenium requirement for mammals was first demonstrated by Schwarz and Foltz in 1957 (*1*). However, the biochemical basis for its essentiality was only established in 1973 with the discovery of its presence in the active site of the mammalian peroxide-destroying enzyme, glutathione peroxidase (*2*). The element is not only active in the preservation of cell membranes and constituents against oxidative damage, but it also takes part in several other important metabolic processes, some of which were only recently discovered, such as the peripheric deiodination of thyroid hormones (*3*).

The dietary levels of selenium causing toxicity and those required to prevent deficiency differ greatly according to animal species (*4*). The range of dietary selenium tolerated by animals appears to be fairly narrow, yet distribution of the element in soil is broad (*5*). On the other hand, the dose/response effects of selenium on experimental animals still remain a controversial problem. Results from many laboratories are generally not comparable because of quite different experimental conditions. Therefore, the influence on biological fluid or tissue selenium levels of parameters such as intake level of selenium and duration of supplementation remains inadequately documented (*6*).

The present study is devoted to the effects on plasma and liver selenium concentrations in rats of selenite supplements at several doses and for several times of treatment.

MATERIALS AND METHODS

Animals, Diet, and Selenium Supplementation

A total of 133 male Wistar rats (Charles River, Gulbenkian Institute of Sciences, Oeiras, Portugal), 3 mo old at the beginning of the experiment and weighing 430 ± 40 g, were maintained in standard conditions (20 ± 2°C and 40 ± 5% humidity) and received *ad libitum* a basal diet (ref. 891-G, Fabricas Triunfo, Coimbra, Portugal) containing 0.26 mg/kg ppm selenium. They were divided in 7 groups and some of them received selenium supplements ranging from 0.5 to 15 ppm via drinking water. Selenium containing solutions were obtained by dissolution of Na_2SeO_3 ·

$5H_2O$ (p.a. Merck, Darmstadt, Germany) in distilled water. Since previous experiments demonstrated that animals receiving the high dose supplements drank significantly less water than nontreated ones, it was necessary to obtain controls for rats supplemented with 6 and 15 ppm that then received the same amount of water as animals treated with 6 and 15 ppm. Group I (n = 36) received no selenium supplements and served as controls for groups II and III; group II (n = 20) was supplemented with 0.5 ppm; group III (n = 17) received water with 2 ppm; group IV (n = 15) received no supplements and served as controls for group V; group V (n = 15) was supplemented with 6 ppm; group VI (n = 15) received no supplements and were controls for group VII; and finally group VII (n = 15) was supplemented with 15 ppm. Each of these groups were divided in three subgroups that received selenium supplements for 1, 3, and 6 mo. Food intake, water consumption, and body weights of animals were regularly recorded.

Sample Collection and Analysis

At the end of each period, animals were anesthetized with diethyl ether and their blood was collected by cardiac puncture with heparinized syringes. Blood was centrifuged at 1500*g* for 15 minutes at 4°C for collection of plasma and red blood cells. The liver was dissected and washed with isotonic saline. All samples were stored in liquid nitrogen until analysis.

Selenium concentration of plasma and aqueous solutions was determined by a direct electrothermal atomic absorption spectrometric procedure with Zeeman background correction and in the presence of copper–magnesium as matrix modifier (*7*). Diet and livers were lyophilized, ground, and homogenized and selenium was determined on an aliquot by an electrothermal atomic absorption spectroscopic procedure after digestion in acid medium and extraction with an aromatic *o*-diamine (*8*). Accuracy of both methods was previously established (*7,8*).

Statistics

Differences between means in the different groups were tested for significance by the Student's *t*-test. Correlations between some variables were analyzed by linear regression.

RESULTS

Table 1 reports mean values for the daily total quantity of selenium ingested per rat over the whole experimental period, calculated by summing the contributions of food intake (data not shown) and of water consumption. In comparison to control group I, a small decrease in the quantity of solid food intake could be observed in control groups IV and

Table 1
Total Quantity of Selenium Ingested/Rat/d in the Different Groups

Group	Selenium supplement, ppm	Number of rats	Water or solutions taken/rat/d, mL	Total selenium ingested/rat/d, μg
I	0	24	32 + 8	9 ± 1
II	0.5	20	32 ± 4	24 ± 3
III	2.0	17	28 ± 5	65 ± 9
IV	0	15	20 ± 2	7 ± 2
V	6.0	15	20 ± 4	125 ± 28
VI	0	15	15 ± 2	6 ± 2
VII	15.0	15	14 ± 3	211 ± 50

Values are the mean ± SD.

VI, where water consumption had to be restricted according to the quantity of water consumed by selenium supplemented animals.

After 1, 3, and 6 mo of treatment, the body weight of rats supplemented with 0.5 and 2 ppm selenium (groups II and III, respectively) did not differ from control rats (group I) (Fig. 1). On the contrary, a weak decrease in body weight of animals in group V (6 ppm) and a more pronounced decrease in animals from group VII (15 ppm) was observed in comparison with control rats from group I. However, a decrease was also observed in their respective controls with a restricted water intake (groups IV and VI). This trend seems therefore not to be owing to selenium supplementation but to the decrease in the quantity of water ingested by the animals (Table 1). There was in general no influence of selenium dietary treatment on rat survival, except in rats supplemented with 15 ppm after 6 mo of treatment. In this case, only 90% of the animals survived the treatment.

Mean plasma selenium of control animals (groups I, IV, and VI) were significantly lower at 6 mo than at 1 or 3 mo ($p < 0.05$, Table 2), suggesting a decrease in plasma selenium concentrations with time in spite of the constant selenium supply. Rats supplemented with 0.5 ppm selenium (group II) maintained their plasma selenium within the range of controls (Table 2) during the whole experiment. However, animals supplemented with 2, 6, or 15 ppm had mean plasma selenium levels clearly above those observed in control groups. These groups received water ad libitum, or they consumed restricted amounts of water. A tendency for decreasing plasma selenium concentrations with time was also observed in all supplemented animals and occurred earlier (generally significant at 3 mo) than in control animals (Table 2). The correlation in all animals between total quantity of ingested selenium (solid diet and beverages) and plasma selenium was highly significant after 1 mo of

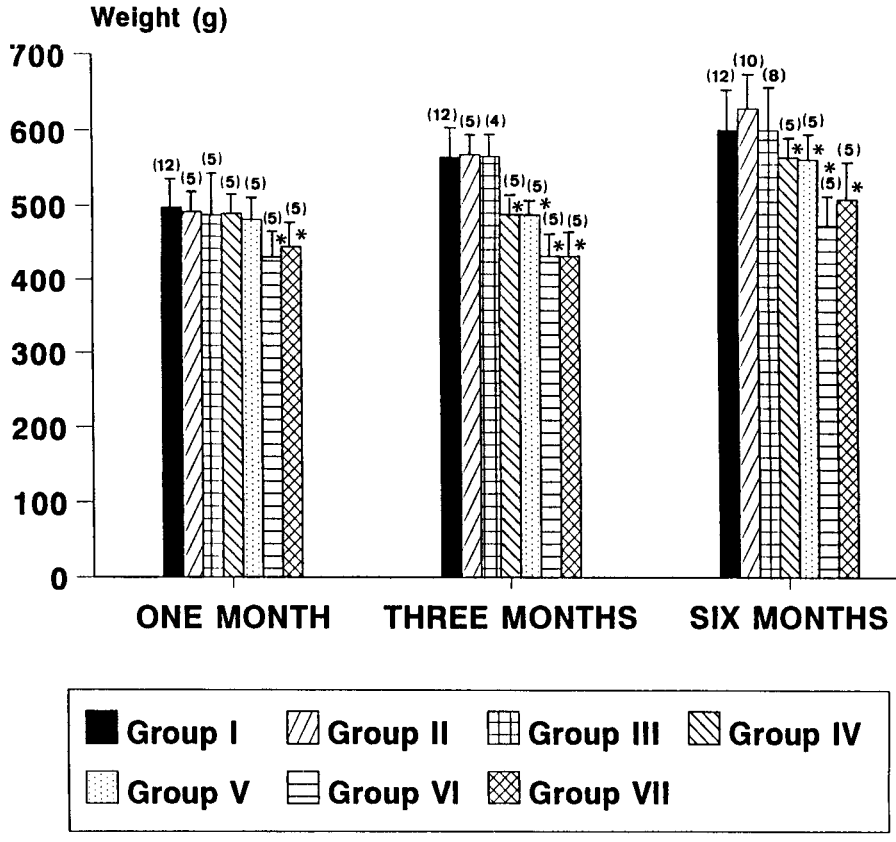

Fig. 1. Mean body weight (g) in the different groups after 1,3, and 6 mo of treatment. Values represent the mean ± SD and in parentheses the number of rats. The *t*-test in comparison to group I: * $p < 0.01$.

treatment; however, the correlation coefficient became lower when examined after 3 and 6 mo of treatment (Table 3).

In opposition to the trend observed for plasma selenium, liver selenium concentrations in all control animals (groups I, IV, and VI) were higher after 6 mo observation in comparison to the values at 1 or 3 mo observation in comparison to the values at 1 or 3 mo ($p < 0.05$, Table 4). As far as selenium-supplemented animals are concerned, liver selenium was significantly higher in comparison to controls in all treated groups, even in the animals receiving the lowest supplementary intake (0.5 ppm). Moreover, mean liver selenium in treated animals appeared more stable as time went on than the corresponding plasma selenium levels, although some fluctuations were apparent. The relationship between the total quantity of selenium ingested and liver selenium showed a highly significant positive correlation not only after 1 mo, but also after 3 and 6 mo (Table 3).

Table 2
Plasma Selenium Concentration in the Different Groups After 1, 3, and 6 Mo of Treatment

Group	Selenium, ppm	Plasma selenium conc. (μg/L) 1 mo	3 mo	6 mo
I	0	544 ± 69 (12)	544 ± 69 (12)	477 ± 67 (12)
II	0.5	507 ± 62 (5) *$p < 0.05$	556 ± 28 (5) *NS	526 ± 66 (10) *NS
III	2.0	744 ± 32 (5) * $p < 0.001$	665 ± 15 (4) *$0.02 < p < 0.05$	570 ± 30 (8) *$p < 0.05$
IV	0	520 ± 50 (5) *NS	530 ± 48 (5) *NS	421 ± 56 (5) *NS
V	6.0	918 ± 107 (5) *$p < 0.001$ **$p < 0.001$	861 ± 135 (5) *$p < 0.001$ **$p < 0.001$	898 ± 53 (5) *$p < 0.001$ **$p < 0.001$
VI	0	560 ± 55 (5) *NS	570 ± 51 (5) *NS	428 ± 83 (5) *NS
VII	15.0	1303 ± 198 (5) *$p < 0.001$ ***$p < 0.001$	888 ± 115 (5) *$p < 0.001$ ***$p < 0.001$	709 ± 58 (5) *$p < 0.001$ ***$p < 0.001$

Values are the mean ± SD and in parentheses the number of rats. Asterisks indicate the significance of *t*-test of the examined group as compared to group I*, group IV**, or group VI***.

DISCUSSION

The results showing opposite trends with time of mean plasma- and liver-selenium in rats with usual diets (control groups) confirmed results of previous experiments in larger groups of animals (unpublished data). Our data are also in agreement with the reports of a significant decrease in plasma selenium levels in elderly subjects (*9*) and the increase in liver selenium-dependent glutathione peroxidase activity in older rats (*10*).

The decrease in plasma selenium levels with time in treated animals that occurred in spite of a constant quantity of ingested selenium, as well as the moderate and generally insignificant increase in liver selenium levels during treatment, suggest that a homeostatic regulatory mechanism exists for this element, with no significant accumulation of the element in the liver even when animals were treated with high doses of selenium. According to the literature, there is little evidence that the intestinal absorption of selenite is the step where selenium homeostasis is regulated (*11*). Mechanisms of homoeostasis seem to be achieved through higher selenium excretion via the kidneys (*12–14*), but at higher selenium intake breath also becomes an important pathway for detoxification (*15*).

Table 3
Correlation between Total Ingested Selenium and Selenium Concentration in Plasma and Liver in all Groups After 1, 3, and 6 Mo of Treatment

Time of treatment, mo	Regression coeficient	
	Plasma	Liver
1	0.99 ($p < 0.01$)	0.99 ($p < 0.01$)
3	0.94 ($p < 0.05$)	0.99 ($p < 0.01$)
6	0.78 ($p < 0.05$)	0.99 ($p < 0.01$)

The degree of significance is in parentheses.

Table 4
Liver Selenium Concentration in the Different Groups After 1, 3, and 6 Mo of Treatment

Group	Selenium, ppm	Liver selenium conc. (μg/g dry wt)		
		1 mo	3 mo	6 mo
I	0	2.6 ± 0.3 (12)	2.6 ± 0.3 (12)	3.2 ± 0.4 (12)
II	0.5	3.7 ± 0.5 (5) *$p < 0.01$	3.9 ± 1.3 (5) *$p < 0.01$	4.6 ± 0.4 (10) *$p < 0.01$
III	2.0	8.3 ± 1.3 (5) *$p < 0.001$	6.7 ± 1.4 (4) *$p < 0.001$	7.1 ± 1.9 (8) *$p < 0.001$
IV	0	2.5 ± 0.2 (5) *NS	2.5 ± 0.3 (5) *NS	2.9 ± 0.3 (5) *NS
V	6.0	9.9 ± 1.5 (5) *$p < 0.001$ **$p < 0.001$	9.3 ± 1.9 (5) *$p < 0.001$ **$p < 0.001$	8.8 ± 0.6 (6) *$p < 0.001$ **$p < 0.001$
VI	0	2.5 ± 0.3 (5) *NS	2.5 ± 0.3 (5) *NS	3.0 ± 0.3 (5) *NS
VII	15.0	16.1 ± 1.4 (5) *$p < 0.001$ ***$p < 0.001$	17.3 ± 2.3 (5) *$p < 0.001$ ***$p < 0.001$	16.4 ± 3.7 (5) *$p < 0.001$ ***$p < 0.001$

Values are the mean ± SD and in parentheses the number of rats. Asterisks indicate the significance of *t*-test of the examined group as compared to group I*, group IV**, or group VI***.

The highly significant positive correlation observed between the total quantity of selenium ingested and liver selenium concentration that, contrary to plasma selenium, is independent of treatment time, suggests that selenium concentration in this organ is a more sensitive and more representative measure of selenium intake than is plasma selenium level. These results are in accordance with other studies showing that this

organ is an important pool for selenium that fills and empties when the dietary selenium intake goes up or down (*16*).

Concerning the safety of the supplements, most of them did not affect body weight and survival of animals, except when diet was supplemented with 15 ppm for 6 mo. Based on these indicators, therefore, it seems that Wistar rats tolerate doses from 0.5 to 6 ppm selenium, as sodium selenite, given in drinking water for 6 mo. Similar results were obtained in Syrian Hamsters (*4*), which tolerated dietary selenium from 0.05 to 5 ppm for 25 wk without detrimental effects, but levels above 10 ppm selenium in their diet became toxic (*4*, *17*). Looking at more sensitive indices of selenium toxicity (*18*), we elsewhere described that at supplemental intakes ≥2.0 ppm some toxic manifestations concerning biochemical parameters of lipid status and hepatocyte function occurred, i.e., an increase in plasma total cholesterol and a decrease in plasma esterification rate together with an ultrastructurally characterized loss of microvilli (*18*). These observations allowed us to conclude that supplemental selenium intake ≥2.0 ppm (total intake above 65 μg/d) could be toxic in the long run.

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