Effect of Acute Selenium Restriction on Whole Body Endogenous Selenium and the Selenite-Exchangeable Metabolic Pool in the Adult Rat

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The time course of changes in whole body endogenous selenium (Se_{end}) was investigated during a short-term (7-day) selenium restriction study in the adult rat. The method of continuous feeding with a stable isotope of selenium was used to permit normal intake of selenium while distinguishing between the dietary and endogenous components of body selenium. Additionally, the effect of short-term selenium restriction on the time course of the selenite-exchangeable metabolic pool (Se-EMP) was investigated. Two groups of adult male rats were intubated with the in vivo *stable isotope $^{74}SeO_3^{2-}$, then fed a Torula yeast diet (selenium <0.02 μg/g) and either deionized water (−Se group) or deionized water containing selenium as $^{76}SeO_3^{2-}$ (0.1 μg selenium/ml) (+Se group). Three animals from each group were killed at 24-hour intervals. Whole body Se_{end} and the estimated size of Se-EMP ($W_{Se\text{-}EMP}$) were determined using hydride generation-inductively coupled plasma mass spectrometry for isotopic measurements. Whole body Se_{end} decreased linearly in the +Se group ($Se°_{end}$: 54.4 μg; Se_{end} at 3 days: 49.3 ± 2.1; Se_{end} at 7 days: 45.2 ± 2.2). The decrease was exponential for the −Se group ($Se°_{end}$: 54.4 μg; Se_{end} at 3 days: 42.9 ± 0.3; Se_{end} at 7 days: 42.2 ± 0.7). The value of $W_{Se\text{-}EMP,pl}$ (μg) was 19.8 ± 0.6 at 1 day and 19.7 ± 1.0 at 7 days for the +Se group. The corresponding values for the −Se group were 15.7 ± 1.5 and 18.8 ± 0.4. All respective values of $W_{Se\text{-}EMP,pl}$ for the −Se group were significantly smaller than for the +Se group ($P < 0.05$), with the exception of values at days 6 and 7. The value of $W_{Se\text{-}EMP,urine}$ (μg) was 2.1 ± 0.2 at 1 day, increasing rapidly to 23.5 ± 1.5 at 7 days for the +Se group. The corresponding values for the −Se group were 3.0 and 23.1.*

Keywords: stable isotopes; selenium; pool size; adult rat.

Introduction

We recently proposed a new concept of potential value for the assessment of selenium status in humans.[1] The concept is based on the measurement of dilution of a single dose of selenite labeled with the stable isotope ^{74}Se, using either plasma or urine as the sampling compartment. We designated the so-calculated dilution space as the selenite-exchangeable metabolic pool (Se-EMP) and its estimated size as $W_{Se\text{-}EMP}$.[1] We have shown that this measurement is responsive to long-term (30-day) dietary selenium restriction in humans.[2]

Because of the complex nature of selenium compartments within the human body, we have begun to explore the quantitative relationship between this index and whole body selenium content, using the rat as our animal model. In earlier experiments (unpublished), we observed that $W_{Se\text{-}EMP}$ constitutes a significant fraction of whole body endogenous selenium, and that the estimated pool size varied depending on whether urine or plasma was used as the sampling compartment.

In the experiments reported here, we explored the relationship between acute (7-day) dietary selenium restriction, the estimated value of $W_{Se\text{-}EMP}$, and quan-

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titative changes in whole body endogenous selenium in the adult rat.

Materials and methods

Animal experiments

Animals used in this study consisted of adult male Fischer rats (F344) who, prior to arrival in our laboratories, had been fed only natural sources of selenium without added selenite. On arrival, the rats were quarantined for 1 to 2 weeks, being fed laboratory chow without selenite (0.25 μg selenium/g, Teklad, Madison, WI, USA). They were then randomized into groups of three, and each group was housed in polycarbonate boxes. Animals were fed Torula yeast diet (#170698, Teklad), selenium content by analysis 0.004 ± 0.001 μg/g (listed <0.02), throughout the experiments *ad libitum*. They were cared for under standard conditions of temperature, humidity, and lighting.

The animals were assigned at random to two experimental groups: selenium-restricted (−Se group, n = 24; initial body weight, 262 ± 23 [SD] g) and selenium-replete (+Se group, n = 24; initial body weight, 294 ± 10 g).

The +Se group was given only deionized water (*ad libitum*) for 12 hours prior to intubation with the *in vivo* ^{74}Se-selenite label. They were intubated twice, 4 hours apart, each time with a ^{74}Se-selenite solution (first dose, 1.05 ± 0.06 μg selenium; second dose, 1.38 ± 0.07 μg selenium; [mean ± SEM]; ^{74}Se/selenium = 0.77). Each dose was delivered with a 1-ml plastic syringe fitted with a 15- to 20-cm length of tubing (1.4 mm i.d., PE-200, Clay Adams, Parsippany, NJ, USA). The amount delivered was determined by weight loss of syringe. Thus, each animal received, on the average, approximately 2.5 μg selenium, which corresponded roughly to their normal daily intake. After 1 hour from the second intubation, the animals were fed the Torula yeast diet and given deionized water (no added selenium). After 24 hours from the first intubation, they were given deionized water containing 0.1 μg selenium/ml as ^{76}Se-selenite (96.79 wt%) until the end of the experiment. This was done to prevent initiation of any conservation mechanisms that would occur from dietary restriction of selenium.[3] Thus, these animals received their normal selenium requirements (0.1 μg selenium/ml water) throughout the 7 days of the protocol, without any selenium of natural isotopic composition (other than contaminant).

Three animals were killed for measurement of baseline values on the day of intubation (t = 0), but without receiving the label. Thereafter, three animals were killed at each 24-hour interval until the end of the experiment. Seventeen hours prior to death, the three animals were placed in Nalgene metabolic cages for collection of urine. Blood was collected (5 ml), separated into plasma and red blood cells (Sorvall GLC-2, Sorvall, Wilmington, DE, USA), and, together with the whole carcass, was saved (−20°C) for isotopic analysis. Whole carcass, plasma, and urine were analyzed for the ratios $R_{74/77}$ and $R_{82/77}$.[4] Whole carcass was also spiked with ^{82}Se and analyzed for $R_{82/77}$.

The −Se group was treated the same as the +Se group, with the following differences. The doses of ^{74}Se that were intubated corresponded to 1.75 ± 0.07 and 1.94 ± 0.26 μg selenium (^{74}Se/selenium = 0.77) for the two intubations. Following the intubations, animals received no selenium in their drinking water for the remaining days of the experiment, and urine samples were obtained from only two animals at each time of death. Therefore, the major difference between this and the preceding experiment was a lack of ^{76}Se supplement in the drinking water for the animals of this experiment during the 7 days following administration of the *in vivo* label, which was expected to result in the initiation of selenium-conservation mechanisms.[3]

Concept and terminology

The concept and terminology of the selenite-exchangeable metabolic pool (Se-EMP), its size ($W_{Se\text{-}EMP}$), procedures for its measurement, and calculations have been previously described in detail.[1] However, the following brief description is necessary for understanding the results presented in this report:

$$W_{Se\text{-}EMP,t} = k\,[{}^{74}Se^{*}_{r,t}]/[R_{74/77,t} - R^{\circ}_{74/77}] \qquad (1)$$

$W_{Se\text{-}EMP,t}$ is the size of Se-EMP at time t after dosing (μg); k is a constant; $^{74}Se^{*}_{r,t}$ is the amount of administered label retained by the animal at time t (μg); and $R^{\circ}_{74/77}$, $R_{74/77,t}$ is the isotope ratio for baseline and at time t for the sampling compartment of interest, respectively (wt/wt).

Isotopic measurements were performed by hydride generation/inductively coupled plasma mass spectrometry (HG/ICP-MS) as described previously.[4] This method permits quantitative measurement of the two stable isotopes ^{74}Se and ^{77}Se with an accuracy of approximately 1%.[4] From these analyses, quantitative information concerning ^{74}Se* (^{74}Se originating from the administered label) and endogenous selenium (Se_{end} = selenium content of the whole body or organ originating from the selenium present prior to intubation) is obtained according to previously described procedures.

Results

The primary purpose of this work was to establish the relationship between $W_{Se\text{-}EMP}$ and whole body Se_{end} under the conditions of normal (0.1 μg selenium/ml drinking water) and short-term selenium restriction (<0.02 μg selenium/g diet), and to determine the extent of changes in whole body endogenous selenium under these two conditions of selenium intake. A major feature of the analytic method used for these analyses[4] was the ability for accurate (approximately 1%) measurement of whole body endogenous selenium.

The quantitative relationship between whole body Se_{end} and $W_{Se\text{-}EMP}$, with either plasma or urine as the sampling compartment, is shown in *Figure 1*. Whole

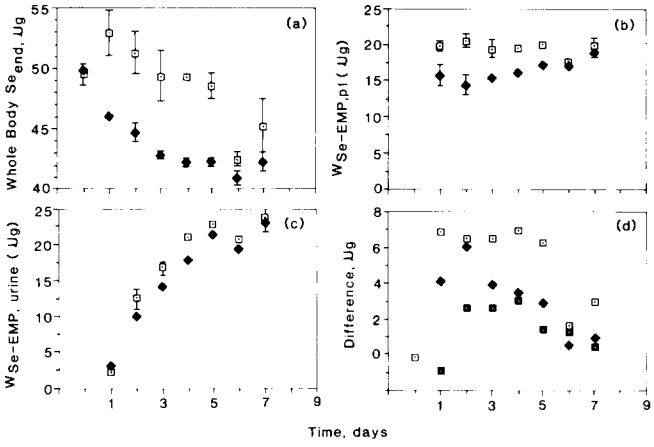

Figure 1 Time course of (a) whole body Se_{end}, (b) the measured size of selenite-exchangeable metabolic pool with plasma as the sampling compartment ($W_{Se-EMP,pl}$), (c) the same, with urine as the sampling compartment ($W_{Se-EMP,urine}$) for the +Se and −Se experiments, and (d) the time course of their difference. All data in panels a through c are given as mean ± 1 SEM, except for the urine data for the −Se experiment (panel c). Error bars for panel d have not been given (see text for explanation). Panels a, b, and c: ⊡, +Se group; ◆, −Se group. Panel d: ⊡, Se_{end}; ◆, $W_{Se-EMP,pl}$; ■, $W_{Se-EMP,urine}$.

body Se_{end} (*Figure 1a*) decreased during the course of both experiments. Data for baseline and day 6 of the +Se group experiment appeared too low compared with the other values for the experiment. The gut contents of baseline animals were cleaned thoroughly with saline solution to eliminate the chance of selenium from being included in the carcass analyses. Thus, all animals of this group were expected to have assimilated any residual selenium of natural isotopic composition which had been consumed up to the time of intubation, except for the baseline group. Because the baseline animals of the −Se group were also treated in an identical manner as those in the +Se group, the observed quantitative similarity between their Se_{end} supports the suggestion that the observed values are related to removal of gut contents. On day 6 of the +Se group experiment, we had difficulties with cannulation and lost a considerable amount of blood.

Eliminating the data for baseline and day 6 of the +Se group experiment, Se_{end} appeared to decrease at a linear rate ($y = 53.8 - 1.19t$; $r^2 = 0.95$). Thus, at the time of intubation, these animals had 53.8 µg selenium, of which approximately 2 to 4 µg would have been related to gut contents. Extrapolation of the linear equation to 12 hours prior to intubation yields 54.4 µg as the true whole body selenium content (Se_{end} + gut content) prior to fasting the animals.

The animals of the −Se group lost their Se_{end} at a strikingly different rate compared with those of the +Se group (*Figure 1a*). The initial rate of loss was much higher, approximating an exponential pattern. The difference in the carcass selenium content of the corresponding animals from the two experiments was highly significant for the period t = 1 to 5 days ($P < 0.001$). After the initial rapid loss, lasting until day 3, the rate of loss was much lower for the −Se group.

In *Figure 1b*, we have plotted the time course of W_{Se-EMP} with plasma as the sampling compartment ($W_{Se-EMP,pl}$). The value for $W_{Se-EMP,pl,+Se}$ (µg selenium) was 19.8 ± 0.6 at t = 24 hours. At t = 7 days, the corresponding value was 19.7 ± 1.0. The highest value was 20.5 ± 0.9. Except for the value at t = 6 days, there was no statistically significant difference among the estimates ($P > 0.1$). In contrast, $W_{Se-EMP,pl,-Se}$ was initially (t = 24 hours, µg) 15.7 ± 1.5, appeared to decrease to 14.4 ± 1.3 ($P > 0.1$), then continued to increase to the final value of 18.8 ± 0.4 ($P < 0.05$ cf. 14.4 ± 1.3). When the corresponding values for W_{Se-EMP} between the two experiments were compared, the values for the −Se group were significantly smaller than the corresponding values for the +Se group ($P < 0.05$), except for those at t = 6,7 days. Thus while the values of $W_{Se-EMP,pl,+Se}$ remained constant during the 7 days of the experiment, those for $W_{Se-EMP,pl,-Se}$ first decreased to a minimum (t = 2 days), then continued to increase. At t = 6,7 days, the corresponding values for both experiments were equal.

The values of W_{Se-EMP}, using urine as a sampling compartment, have been plotted in *Figure 1c* for the two experiments. The size of the pool increased rapidly, in both experiments, from the initial value of 2 to 3 µg to the final values of 23.5 ± 1.5 (+Se group) and 23.1 (−Se group). Because urine from only two animals was sampled in the latter experiment, no estimates have been given for variance of the mean. While no tests of statistical significance were run comparing the corresponding values of W_{Se-EMP} between the two experiments, all values for the −Se group were smaller than the corresponding values for +Se group, except for 24-hour and 7-day data (*Figure 1c*).

We plotted the difference in the whole body selenium content of the carcasses or that for W_{Se-EMP}, calculated for both sampling compartments, in *Figure 1d*. We have not included the associated error bars because they are expected to be large compared with the values themselves. For this reason, we also did not include statistical tests of significance for the data. Data from whole carcass analyses showed a difference between the two groups of approximately 7 µg selenium for the 1- to 5-day period, which decreased to 2 to 3 µg thereafter. The difference in W_{Se-EMP} was less than that based on true selenium content of the carcasses, but was systematically in the same direction. Furthermore, the difference values for W_{Se-EMP} were similar for both sampling compartments.

Discussion

No information is available concerning the early time course of whole body selenium in the adult rat fed a selenium-restricted diet, nor the normal rate of Se_{end} elimination in animals fed the selenium requirement. The data given here present the first experimental evidence concerning the normal rate of Se_{end} elimination from the whole carcass and the dramatic alteration that apparently occurs while the animal is being fed a

selenium-restricted diet for a short period of time (*Figure 1a*). Animals given supplemental selenium in the form ^{76}Se lost Se_{end} at the linear rate of 1.2 μg/d (2.2% of $Se°_{end}$ = 54.4 μg). At the end of 7 days, these animals had lost 9.2 μg (16.9% ± 0.8%) of their initial 54.4 μg Se_{end}. In clear contrast, when the animals were placed on a selenium-restricted Torula yeast diet, the initial loss of Se_{end} was considerably more rapid. Assuming that these animals also initially contained 54.4 μg Se_{end}, they lost 21% ± 0.1% of their Se_{end} during the initial 3 days following intubation. They showed no further losses for the remaining 4 days. By the end of the experimental period, the animals from this group had in fact lost a larger fraction of their whole body Se_{end} (22.4 ± 0.4%) than the animals fed supplemental ^{76}Se (16.9% ± 0.8%, $P < 0.01$).

The observations related to a high loss rate of Se_{end} in animals placed on a selenium-restricted diet (*Figure 1a*) were unexpected. It is commonly assumed that dietary selenium restriction triggers conservation mechanisms which limit selenium losses from the body.[5-8] This is supported by experiments based on turnover of exogenous selenite in animals[5,6] or humans,[7] or observations related to decreased urinary loss of selenium after restriction of dietary selenium.[7,8] The experimental results given here do not necessarily contradict the previous observations in that there are important experimental differences between the present experiments and the cited studies.[5-7] The observations made in the present experiments (*Figure 1a*) are related to the excretion of natural forms of selenium which had been consumed by the animal and had undergone some degree of metabolism in the animal prior to initiation of the experimental observations. We have termed this Se_{end} in an attempt to distinguish it from the selenium that entered the animals' bodies during the course of the experiment. In contrast, previous experiments[5,6] performed using rats and radioselenite have focussed on the retention of the radioselenite as influenced by changes in dietary selenite. If the metabolic fate of radioselenite were distinct from the rapid turning over component of Se_{end}, as is likely, the results of the cited investigations[5,6] would be expectedly different from those of the present study. Similarly, the observations based on changes in urine excretion of selenium due to alterations in its intake[7,8] are not comparable to the present results, as the former cannot distinguish between the component of urine selenium of dietary and endogenous origins.

With regard to any potential use of the proposed concept of selenite-exchangeable metabolic pool,[1] the data obtained in the present two experiments are encouraging. First, these data (*Figures 1b and c*) clearly show that $W_{Se\text{-}EMP}$ is a highly reproducible measurement, regardless of which sampling compartment is used. Second, even for a very mild selenium-restriction protocol, as was used in this experiment, the differences in $W_{Se\text{-}EMP}$ between the two experiments, albeit small, tracked the true changes in whole body Se_{end} (*Figure 1d*). Despite some differences, both sampling compartments detected these differences. Under such conditions of mild selenium restriction, other available indices of selenium status, such as plasma selenium concentrations or enzyme levels of glutathione peroxidase in plasma, may not indicate a measurable change. Further, it should also be noted that the differences observed in the animals of these two groups occurred during an ongoing mild state of selenium deficiency. Both groups were treated in the same manner prior to administration of the *in vivo* label ($^{74}SeO_3^{2-}$). This is distinct from the consequences of prior establishment of body selenium deficit, which would require the institution of selenium restriction for some time before administration of the *in vivo* label. Such experiments need to be carried out in the future.

Acknowledgments

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References

1 Janghorbani, M., Martin, R. F., Kasper, L. J., Sun, X. F., and Young, V. R. (1990). The selenite-exchangeable metabolic pool in humans; a new concept for the assessment of selenium status. *Am. J. Clin. Nutr.* (in press)

2 Martin, R. F., Young, V. R., and Janghorbani, M. (1989). Experimental selenium restriction in healthy adult humans: changes in selenium metabolism studied with stable isotope methodology. *Am. J. Clin. Nutr.* **49,** 854–861

3 Burk, R. F., Jr., Whitney, R. W., Frank H., and Pearson, W. N. (1968). Tissue selenium levels during the development of dietary liver necrosis in rats fed torula-yeast diets. *J. Nutr.* **95,** 420–428

4 Ting, B. T. G., Mooers, C. S., and Janghorbani, M. (1989). Isotopic determination of selenium in biological materials with inductively coupled plasma mass spectrometry. *Analyst* **114,** 667–674

5 Burk, R. F., Jr., Brown, D. G., Seely, R. J., and Scaief, C. C. (1972). Influence of dietary and injected selenium on whole-body retention, route of excretion, and tissue retention of $^{75}SeO_3^{2-}$ in the rat. *J. Nutr.* **102,** 1049–1056

6 Burk, R. F., Jr., Seely, R. J., and Kiker, K. W. (1973). Selenium: dietary threshold for urinary excretion in the rat. *Proc. Soc. Exp. Biol. Med.* **142,** 214–216

7 Kasper, L. J., Young, V. R., and Janghorbani, M. (1984). Short-term dietary selenium restriction in young adults: quantitative studies with the stable isotope $^{74}SeO_3^{2-}$. *Br. J. Nutr.* **52,** 443–455

8 Robinson, J. R., Robinson, M. F., Levander, O. A., and Thomson, C. D. (1985). Urinary excretion of selenium by New Zealand and North American human subjects on differing intakes. *Am. J. Clin. Nutr.* **41,** 1023–1031