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Fecal selenium excretion is regulated by dietary selenium intake¹

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Abstract

Whole-body selenium is regulated by excretion of the element. Reports of studies carried out using isotopic tracers have led to the conclusion that urinary selenium excretion is regulated by selenium intake but that fecal excretion is not. Because of the limitations of tracer studies, we measured urinary and fecal selenium excretion by mice with selenium intakes ranging from deficient to almost toxic. Tissue and whole-body selenium concentrations increased sharply between deficient and adequate selenium intakes, reflecting tissue accumulation of selenium in the form of selenoproteins. Once the requirement for selenium had been satisfied, a 31-fold further increase in intake resulted in less than doubling of tissue and whole-body selenium, demonstrating the effectiveness of selenium excretion by the mouse. Urinary selenium excretion increased with increases in dietary selenium intake. Fecal selenium excretion, which was 20% to 30% of the selenium excreted in the physiological range, responded to moderately high selenium intake but did not increase further when selenium intake was increased to even higher levels. Thus, fecal selenium excretion contributes to regulation of whole-body selenium at physiological selenium intakes. The pattern of its response to the full spectrum of selenium intakes was different from the urinary excretion response, suggesting that the mechanisms of fecal and urinary routes of excretion are different.

Keywords

selenium homeostasis; fecal selenium excretion; tissue selenium regulation

Introduction

Selenium homeostasis mechanisms are important because the element is both an essential micronutrient and a potential toxin. Absorption of dietary forms of selenium is highly

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Statement of Author Contributions

All authors participated in the design of the study and reviewed the manuscript; LFCP, AKM, and TDS carried out the experiments; RFB and KEH analyzed the data and wrote the manuscript.

efficient and not influenced by the amount ingested.^{1, 2} Thus, regulation of whole-body selenium depends on excretion of the element.

Selenium is excreted in urine, in feces, and by other routes, which include exhaled breath and loss of hair and skin cells. Most studies of selenium excretion have been carried out with single doses of ⁷⁵Se tracers in animals. Those studies showed that urinary ⁷⁵Se excretion was directly related to selenium intake but that fecal ⁷⁵Se was not.^{3, 4} Breath ⁷⁵Se excretion became significant only at high selenium intakes.^{5, 6} Thus, based on the early studies, the urinary route appeared to be the primary means of regulating whole-body selenium under physiological conditions and the breath route became important under potentially toxic conditions. The fecal route did not appear to be regulated.

In those early studies, isotope-labeled selenium, usually ⁷⁵Se-labeled selenite, was used as a selenium tracer. Based on current knowledge of selenium metabolism, the ⁷⁵Se-selenite would have principally traced pools of selenium that were metabolically most active. It would not have adequately labeled selenoprotein pools that turn over slowly. Therefore it would not have represented all selenium pools in proportion to their selenium content.

To evaluate the influences of all regulated selenium pools on the routes of selenium excretion, we carried out an experiment that determined the effect of selenium intake on its excretion without using a tracer. The dietary form of selenium that was fed to the mice was selenite, which, after reduction, enters selenium pools directly. Selenomethionine, a major dietary form of selenium, was excluded from the study because its selenium is present in the methionine pool until the parent molecule is catabolized.⁷ Thus, the metabolism of selenium that is present as selenomethionine is not influenced by the size of regulated selenium pools and would not respond to efforts of the animal to regulate selenium. Use of it would complicate interpretation of the experiment.

Groups of mice were fed differing amounts of selenium for long periods of time to achieve a steady state before excretion of selenium was assessed. The results show that excretion by urine and by feces both respond to dietary selenium intake in the physiological to moderately high range of intake, suggesting that both routes have physiological significance.

Methods and Materials

Animals

Male C57BL/6 mice were used for this study. They were maintained in the Vanderbilt animal facility with a 12-h light/dark cycle and *ad libitum* access to food and tap water. Five *Torula* yeast-based diets were prepared to our specifications⁸ by Harlan Teklad (Madison, WI) to be selenium deficient (diet A) or to contain 0.25 mg selenium/kg (diet B), 2 mg selenium/kg (diet C), 4 mg selenium/kg (diet D), or 8 mg selenium/kg (diet E). Selenium concentrations of the diets were measured (Table 1) and those measured concentrations were used in all calculations.

Mice in groups A, B, and C were weaned onto the study diets. Mice in group D were switched from selenium-deficient diet (4 mice) or diet containing 0.25 mg selenium per kg (2 mice) to the study diet 210 days after weaning. Mice in group E were switched from diet containing 2 mg selenium per kg to the study diet 22–37 days after weaning. All mice were fed the study diets for at least 60 days before being studied—A for 275 days, B for 180 days, C for 157 days, D for 60 days, E for 175 days.

Protocol

The mice were studied using 6 metabolic cages, one for each mouse. Mice were acclimated to the cages for 3 days before the experiment was performed. During the acclimation period, 24-hour food intake was measured. On the fourth day the experiment was performed. Food intake was measured and urine and feces were collected. Urine was collected in a tube and the funnel surface under the mouse was washed into a separate tube by spraying water from a squirt bottle onto dried urine spots. On the fifth day and after the 24-hour excreta collections, mice were anesthetized with isoflurane and exsanguinated by removal of blood from the inferior vena cava using a syringe and needle. The blood was allowed to clot and serum was removed after centrifugation. The clot was discarded. The liver was removed. The Vanderbilt University Institutional Animal Care and Use Committee approved the study protocol.

Selenium assay

Selenium assays were performed using the method of Koh and Benson⁹ as modified by Sheehan and Gao.¹⁰ Urine, urine washdown, feces, serum, liver, and carcass were digested and assayed as described previously.¹¹ The urine value reported was the sum of the urine and urine wash down values.

Statistics

Statistical comparisons between groups were made using Prism 4 for Macintosh Version 4.0c software program (GraphPad Software, Inc.). Tukey's Multiple Comparison Test was applied after 1-way ANOVA.

Results

Dietary selenium intakes of diet groups

Food intakes of all mice on the day of experiment were similar to intake during acclimation except for one mouse in diet group D. That mouse had low food intake (1.0 g) on the day of study and appeared to be ill. Its data were removed from the experiment. One mouse in diet group C was excluded from the experiment because its perineum was injured, presumably by fighting.

Measured selenium concentrations of the diets were used to calculate the amounts of selenium ingested on the day of study (Table 1). The selenium ingestion of the diet B group (selenium-adequate) was 36-fold that of the diet A group (selenium-deficient). In comparison with diet B, 9 times as much selenium was ingested from diet C (moderately high-selenium), 15 times as much from diet D (high-selenium), and 31 times as much from diet E (almost toxic-selenium). Thus, a broad range of selenium intakes, ranging from deficient to almost toxic,¹² was studied.

Regulation of tissue selenium concentrations

As expected, selenium concentrations of serum, liver, and the whole body (comprising serum, liver, and carcass but excluding the blood clot) increased more than 10-fold in mice fed selenium-adequate diet (diet B) when compared with mice fed selenium-deficient diet (diet A) (Fig 1). This increase can be attributed to the increase in selenoproteins that is known to occur under these conditions.

Once animals are selenium replete, further increase in selenium intake does not increase selenoproteins.^{13, 14} The 31-fold increase in selenium intake between the groups fed diets B and E (Table 1) produced less than a doubling of tissue selenium concentrations (Fig 1)—

45% in serum, 69% in liver, and 84% in the whole body. This demonstrates that homeostatic mechanisms restrict the accumulation of excess ingested selenium.

Effect of dietary intake on selenium excretion by urinary, fecal, and other routes

Excretion of selenium was measured directly in urine (including the urine wash down) and in feces. The assumption was made that the mice were in selenium steady state with excretion equal to intake. This allowed estimation of excretion through 'all other routes' by subtracting urine and feces selenium from dietary selenium intake on the day of study.

Figure 2 shows that urinary and fecal selenium excretions by selenium-deficient mice (diet A) were at the limit of detection (see inset). The calculated excretion by 'all other routes' was negative, reflecting the limits of this calculation.

Excretion by urinary and fecal routes appeared to increase when mice were fed the selenium-adequate diet B (see inset of Fig 2) but those increases were not statistically significant in the context of comparisons among all diet groups. The next increment in intake, to moderately high (diet C), caused statistically significant increases in both urine and feces selenium. Urinary selenium excretion increased with each additional increase in dietary intake (diets D and E) but fecal selenium excretion did not increase in those groups. These results show that urinary selenium excretion was responsive to dietary selenium intake throughout the range studied. Fecal selenium excretion behaved in a different manner from urinary selenium excretion. It increased until selenium intake became moderately high (diet C) but reached a plateau at that level and had no further response to higher selenium intake. Thus, excretion by urine and feces appear to be regulated differently.

The calculated excretion by 'all other routes' increased significantly only in the group consuming the largest amount of selenium. Thus, selenium excretion by pathways other than urine and feces became statistically significant only when selenium intake was almost toxic.

Discussion

In this study, fecal selenium excretion was regulated by dietary selenium intake. Moreover, the amount of selenium excreted in feces was physiologically significant: 22% of the amount ingested from diet B (selenium-adequate) was excreted in feces and 30% of that ingested from diet C (moderately high-selenium) was excreted in feces. The percentages decreased at higher intakes because fecal selenium excretion plateaued at moderately high-selenium intake (diet C). This pattern of response to selenium intake was different from the response by urinary excretion, which continued to increase as long as dietary intake increased. Thus, both fecal and urinary pathways are physiologically relevant at selenium intakes expected in free-living animals but the importance of the fecal pathway diminishes at higher selenium intakes.

Previous studies have not detected regulation of fecal selenium excretion.^{3, 15} Turnover of intestinal mucosal cells, which contain selenium in the form of selenoproteins, is likely to contribute to fecal selenium excretion and the selenium in those cells would be expected to be different between diets A and B (Fig 1). However, the further increase in fecal excretion observed in mice fed diet C cannot be explained by such a passive mechanism because tissue selenium concentrations did not increase significantly between diets B and C. It seems likely that an active process is involved in fecal selenium excretion.

The results in this study on excretion of selenium in the urine and by 'all other routes' are in accord with studies carried out with tracers. Urinary excretion is the major route of selenium elimination and it is regulated from very low intakes to almost toxic intakes.^{3, 4, 16} 'All other

routes,' predominantly selenium in exhaled breath, becomes important as intake approaches toxic levels.

Methylated forms of selenium account for most selenium excretion in urine and breath. The volatile compound dimethyl selenide was identified 60 years ago in the breath of rats given large doses of selenium.¹⁷ Then trimethylselenonium ion was identified in rat urine.^{18–20} More recently, Suzuki's group showed that methylated selenosugars were synthesized in the liver and excreted in the urine.²¹ Thus, the physical nature of the methylated metabolites dictates their route of excretion: volatile metabolites appear in the breath and water-soluble ones appear in the urine.

Little is known about how selenium destined for excretion is distributed among the methylated excretory metabolites. It is clear, however, that volatile metabolites appear in significant quantities only when selenium intake is very high as was confirmed by this study.

Studies of this type are difficult to carry out and have limitations. Measurement of selenium excreted in urine can be underestimated because of incomplete collection. Some urine dries on the collection funnel of the metabolic cage and we attempted to collect it by washing down obvious dried spots, but it is likely that some urine remained on the funnel, decreasing urinary excretion values. Feces were collected in a separate compartment of the metabolic cage from urine but contamination by urine was possible. Our results in Fig 2 show, however, that fecal selenium values did not increase as did urine selenium values when diets D and E were fed. This suggests that urine contamination of feces was low. Finally, excretion by 'all other routes' was not measured directly. It was calculated by subtraction of urine and fecal selenium from estimated selenium intake. Thus, excretion by 'all other routes' is affected by errors in the other determinations and lacks precision. In spite of these potential difficulties, interpretable results were obtained.

In conclusion, the results presented here show that both urinary and fecal selenium excretory routes are regulated by selenium intake at deficient to moderately high selenium intakes. The fecal route did not respond to further increases in intake but the urinary route did. Excretion by 'all other routes' became statistically significant only when intake reached 'almost toxic' levels. These findings indicate that all routes of selenium excretion respond to intake of the element but that the pattern of response is distinct for each route. Characterization of fecal selenium excretion is needed.

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Figure 1.

Effect of dietary selenium intake on serum, liver, and whole-body selenium. Mice were fed diets that contained selenium levels from deficient (diet A) to almost toxic (diet E). Selenium was measured in serum, liver, and the whole body. Values are means ($n=5-6$) with 1 S.D. indicated by the bracket. An asterisk indicates that a value is significantly different ($p<0.05$) from the selenium-adequate (diet B) value.

Figure 2.

Effect of dietary selenium intake on its 24-h excretion by different routes. Mice were fed diets that contained selenium levels from deficient (diet A) to almost toxic (diet E). Excretion in urine and feces was determined and excretion by all other routes was calculated. Values are means ($n=5-6$) with 1 S.D. indicated by the bracket. An asterisk indicates that a value is significantly different ($p<0.05$) from the value preceding it.

Table 1Composition of the selenium deficient diet¹

component	g/kg diet
Torula yeast ¹	300
sucrose	568.3
corn oil	66.7
mineral mix ²	50
AIN-76A vitamin mix	10
DL-methionine	3
choline bitartrate	2

¹Originally published in reference 8.²Lake States Torula Dried Yeast, U.S.P. XIX, Type B, supplied by Lake States Division, Rhineland Paper Co., Rhineland, WI.³Composition (per kg mineral mix): 543g CaCO₃; 225.2g KH₂PO₄; 104.8g KCl; 59.69g NaCl; 25g MgCO₃; 16g MgSO₄; 20.5g ferric ammonium citrate; 3.44g MnSO₄·H₂O; 1g NaF; 0.9g CuSO₄; 0.1g CrCl₃·6H₂O; 0.08g KI.

Table 2

Diet groups of mice and their selenium intakes

diet group	group description	diet selenium concentration ^{*,†} mg/kg	n	weight [*] g	diet consumption [*] g/24 h	selenium intake from diet [*] μg/24 h
A	selenium deficient	0.006 ± 0.003	6	34 ± 3.9	4.0 ± 0.9	0.024 ± 0.006
B	selenium adequate	0.24 ± 0.10	6	35 ± 3.7	3.6 ± 0.4	0.87 ± 0.09
C	moderately high selenium	1.9 ± 0.2	5	35 ± 4.3	3.9 ± 0.4	7.4 ± 1.0
D	high selenium	4.3 ± 0.5	5	40 ± 1.9	3.0 ± 0.4	13 ± 1.8
E	almost toxic selenium	7.9 ± 1.6	6	37 ± 7.6	3.5 ± 0.5	27 ± 3.8

* Values are means ± S.D.

† n = 5–7.