

Comparison of Selenite and Selenate Apparent Absorption and Retention in Infants Using Stable Isotope Methodology

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ABSTRACT

The inorganic selenium compounds selenite and selenate are used for selenium fortification of infant formulas. However, information on absorption and retention of selenium from these compounds is lacking. The purpose of this study was therefore to determine apparent absorption and retention of selenium from selenate and selenite added to a milk-based infant formula in healthy infants. Labeled test meals were prepared by addition of 10 μg Se as ^{76}Se -selenate or ^{74}Se -selenite to 500 mL formula. The two batches of labeled formulas were fed as alternate feeds during the first day of the balance period, followed by unlabeled formula. Selenium isotopes were determined in feces collected for 72h after intake and in 3 consecutive 24h collections of urine. Mean apparent absorption was 97.1% for ^{76}Se -selenate and 73.4% for ^{74}Se -selenite; mean difference 23.7% (range: 13.8%–35.7%; SD 6.8%, $p < 0.001$). Mean urinary excretion (% of ingested dose) was 36.4% (^{76}Se -selenate) and 9.7% (^{74}Se -selenite); mean difference 26.7% (range: 13.9%–36.5%; SD 5.9%, $p < 0.001$). Mean apparent retention of selenium from

^{76}Se -selenate and ^{74}Se -selenite was not significantly different, 60.7% (^{76}Se -selenate) versus 63.7% (for ^{74}Se -selenite). The average difference was -3.01% (range: -14.0% – 12.0% ; SD 9.4%, $p = 0.36$). Although apparent selenium absorption and urinary excretion differed for selenite and selenate, selenium was equally well retained by infants from both selenium compounds. We therefore concluded that Se fortification of infant formulas with selenate or selenite can be expected to have similar impact on the selenium nutritional status of term infants. (*Pediatr Res* 51: 71–75, 2002)

Abbreviations

GC-MS, gas chromatography mass spectrometry
GPx, glutathione peroxidase
HGAAS, hydride generation atomic absorption spectrometry
ID, iodothyronine deiodinase
Se, selenium

Selenium is an essential trace element for humans (1, 2). Biochemical evidence for its importance is demonstrated by its function in the selenoenzymes GPx, which protects cells against oxidative damage, ID, which catalyzes the conversion of T_4 into T_3 (3), and thioredoxin reductase, which plays a role in maintaining the redox environment of the cell (3, 4). Several other selenoproteins, such as selenoprotein P, have been identified, but clear evidence for their biologic function is lacking (5). Recent observations on the role of selenium in the emergence of viral mutations (6) and the impact of selenium supplementation on the incidence of certain cancers (7) add more evidence to its importance for human health.

Although selenium deficiency has not been observed in healthy term infants, infants may be at risk of deficiency when fed formulas with naturally low selenium content (8). Selenium re-

quirements during infancy are not well established. The World Health Organization recommends that infants consume 6 μg Se/d during the first 6 mo of life (2), whereas the U.S. Food and Nutrition Board set the Recommended Dietary Allowance at 10 $\mu\text{g}/\text{d}$ for this age group (1). The selenium content of infant formulas without added selenium has been reported to be lower than that of human milk (6–12). Differences in selenium status between breast-fed and formula-fed infants may reflect the difference in selenium content, but may also indicate better selenium absorption from human milk (13–15).

Fortification of infant formulas with selenite or selenate, the only currently approved compounds for selenium fortification of infant formulas, has been shown to improve the selenium status of formula-fed infants (14–18). Selenium bioavailability, using selenite as an extrinsic stable isotope tracer, has previously been studied in preschool children (18) and very low birthweight infants (19), but to date there are no data comparing selenite and selenate apparent absorption and retention in healthy term infants.

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The aim of the present study was to compare apparent absorption and retention of selenium in term infants fed infant formula with added selenite and selenate.

METHODS

Subjects. Nine healthy term infants (3 females, 6 males) living in or around Iowa City, IA, U.S.A. participated in the study. Their age at the time of the study ranged from 64 to 198 d and their body weight varied from 4.69 kg to 7.93 kg. All infants were formula-fed before enrollment into the study. Infants were admitted to the Lora N. Thomas Metabolic Unit, Iowa City, for the study (4 days). The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects. The study procedures were explained to both parents and written consent was obtained.

Experimental design. The study formula, a commercially available milk-based infant formula (Carnation Follow-Up[®], Carnation, Glendale, CA, U.S.A.) provided 32.5 g protein, 26.6 g fat, and 85.6 g carbohydrate per liter. The formula contained no added selenium, and its selenium concentration was 0.16 $\mu\text{mol Se/L}$. Labeled formula was prepared by the addition of 10 $\mu\text{g Se}$ as ⁷⁴Se-selenite or ⁷⁶Se-selenate to 500 mL formula about 15h before use. The labeled formulas were stored at 4°C until used.

The study formula without added stable isotope labels was fed to each infant for at least one week before the start of the study. The two 500 mL formula batches, labeled with ⁷⁴Se-selenite and ⁷⁶Se-selenate, respectively, were fed in alternation during the initial day of the balance study until all labeled formula was consumed. The order of the labeled formulas was alternated between the infants. During the remainder of the balance study, unlabeled formula was used. Intake of labeled and unlabeled formulas was determined by weighing feeding bottles before and after feeding. Regurgitated formula was collected with preweighed wipes and subtracted. Urine and feces were collected quantitatively for 72h using metabolic beds.

Stable isotope labels. Highly enriched elemental ⁷⁴Se (98.2% ⁷⁴Se, 1.8% ⁷⁶Se, <0.1% other Se isotopes), ⁷⁶Se (1.2% ⁷⁴Se, 98.5% ⁷⁶Se, 0.2% ⁷⁷Se, <0.03% other Se isotopes) and ⁸²Se (0.06% ⁷⁴Se, 0.62% ⁷⁶Se, 0.56% ⁷⁷Se, 1.76% ⁷⁸Se, 4.8% ⁸⁰Se, 92.2% ⁸²Se) were purchased from Isotec (St-Quentin, France) and converted into ⁷⁴Se-selenite, ⁷⁶Se-selenate, and ⁸²Se-selenite. Selenite was prepared by dissolving a precise amount of elemental Se (approximately 2.5–10 mg; weighed on a high precision analytical balance) into 1 mL of concentrated nitric acid. The solution was heated for 1 h at 60°C under nitrogen flow. The clear solution (approximately 0.2 mL) was made up to 20 mL with deionized water, filtered through a 0.22 μm Teflon filter, and stored at 4°C. Selenate labels were prepared from the selenite solution after evaporation to dryness at 100°C under nitrogen flow. Five mL of hydrogen peroxide were added to the white precipitate to oxidize selenite. The solution was slowly heated to 70°C and 0.2 mL of 2 M potassium hydroxide was added. The volume was reduced under nitrogen flow to about 1 mL at 70°C and the oxidation

step repeated three times. The solution was evaporated to dryness, the white precipitate re-dissolved in deionized water, and 0.1 mL concentrated nitric acid was added. Finally, the solution was made up to 20 mL, filtered through a 0.22 μm Teflon filter, and stored at 4°C.

Speciation of selenite and selenate stable isotope solutions was performed by continuous flow HGAAS based on the principle that only selenite, but not selenate, can be directly measured by HGAAS upon reaction with a sodium borohydride reductant solution (20).

Determination of selenate by HGAAS requires a hydrochloric acid reduction step before HGAAS analysis. For each isotope, stock solutions containing 1.27 mmol Se/L in 0.01 M HNO₃ were prepared.

Sample collection and handling. Fecal material and urine were collected separately for 72h as described by Fomon (21). Carmine red was used as a fecal marker to determine the start- and end-point of the fecal collection. Carmine red powder (50 mg) was added to a small portion of the first labeled test meal and the second dose of carmine red was fed 72h later. All fecal material was collected and included in the pooled fecal material for analysis of total Se and Se stable isotopes, from the first appearance of carmine red until but not including feces colored by the second dose of carmine red. Postbalance fecal samples were collected immediately after completion of the 72h balance period. Urine was collected in 24h pools. Pre and postbalance spot fecal samples were also collected. Capillary blood samples (0.5 mL) were collected in heparinized tubes before the start of the study for assessment of selenium status. Erythrocytes and plasma were separated by centrifugation (2,500 \times g, 15 min, 4°C) and stored at -80°C. All samples were shipped on dry ice to the Nestlé Research Center and stored at -40°C until analyzed.

Analytical methods. Total selenium levels and stable isotope ratios in samples of feces and urine were determined by GC-MS (22). Urine and fecal samples were acid digested using a mixture of nitric/perchloric acid (3:1) followed by the addition of hydrochloric acid to convert all selenium into selenite, which was then derivatized with 4-nitrophenylene-diamine to form the volatile piazselenole before analysis by GC-MS. Total selenium was determined by isotope dilution GC-MS using a ⁸²Se-selenite spike. The selenium analysis was validated against a standard reference material (SRM) NIST 2670 toxic metals in freeze-dried urine [National Institute of Standards and Technology (NIST), Gaithersburg, MD, U.S.A.].

Plasma and erythrocyte selenium concentrations were determined by continuous flow HGAAS (20) and expressed as $\mu\text{mol Se/L}$ and nmol Se/ $\mu\text{mol hemoglobin (Hb)}$, respectively. Se analyses were validated against commercial whole blood and serum quality control material (Seronorm: trace elements whole blood batch no. 030016; serum batch no. 010017; Nycomed, Oslo, Norway). Hb was analyzed by the standard cyanomethemoglobin technique.

Plasma and erythrocyte GPx activity were determined according to Belsten and Smith (23) and expressed per mg protein and per $\mu\text{mol Hb}$, respectively. Plasma protein levels were determined using the Pierce BCA colorimetric method (Rockford, IL, U.S.A.).

Calculation of apparent selenium absorption and retention. Absorption and retention of the selenium stable isotope labels were calculated according to Turnlund *et al.* (24). Apparent absorption of ^{74}Se and ^{76}Se was calculated as the difference between the administered dose and the amount of isotope excreted in feces. Apparent retention of ^{74}Se and ^{76}Se was determined based on apparently absorbed isotope minus urinary excretion of selenium stable isotopes during 72h. Apparent absorption of total selenium (added stable isotope labels of selenium plus native selenium) from the formula was calculated using the classical chemical balance approach as the difference between total selenium intake and total fecal excretion during the 72h balance period. Similarly, apparent retention of total selenium from the formula was calculated as total selenium apparent absorption minus total urinary selenium excretion during the 72h balance.

Statistical analyses. The statistical significance was assessed by two-way ANOVA, with isotope and order of administration as factors (25). All computations are performed using S-PLUS 2000, release 2 (Mathsoft Inc., Seattle, WA, U.S.A.).

RESULTS

Subject's selenium status. Baseline values (mean \pm SD) of Se status parameters were plasma Se, $0.84 \pm 0.17 \mu\text{mol Se/L}$ (range: 0.64–1.16); erythrocyte Se, $0.333 \pm 0.109 \text{ nmol Se}/\mu\text{mol Hb}$ (range: 0.239–0.534); plasma GPx, $10.1 \pm 1.4 \text{ nmol NADPH}/\text{min}$ (range: 8.0–11.8); and erythrocyte GPx, $1.25 \pm 0.18 \text{ nmol NADPH}/\text{min g Hb}$ (range: 1.00–1.50).

Isotope balance data. During the 72h balance period, the mean (\pm SD) total intake of infant formula (labeled plus unlabeled) was $3133 \pm 403 \text{ g}$. Intake of labeled formula was completed in $17.9 \pm 5.1 \text{ h}$ (^{74}Se -selenite) and $18.8 \pm 5.4 \text{ h}$ (^{76}Se -selenate). Mean fecal and urine fresh weight was $173 \pm 46 \text{ g}$ and $1701 \pm 271 \text{ g}$, respectively.

Data on apparent absorption, urinary excretion, and apparent retention for ^{74}Se -selenite and ^{76}Se -selenate are summarized in Table 1. Mean apparent absorption was 97.1% for ^{76}Se -selenate and 73.4% for ^{74}Se -selenite. The mean difference was 23.7% (range: 13.8%–35.7%; SD 6.8%; $p < 0.001$). The mean urinary excretion (% of ingested dose) was 36.4% for ^{76}Se -selenate and 9.7% for ^{74}Se -selenite, with a mean difference of 26.7% (range: 13.9%–36.5%; SD 5.9%; $p < 0.001$). The mean urinary excretion during the first 24h (% of total urinary excretion) was 82.3% for ^{76}Se and 47.8% for ^{74}Se , with a mean

difference of 34.5% (range: 23.1%–44.9%; SD 6.8%; $p < 0.001$). The mean apparent retention of the two isotopes was not significantly different: 60.7% (^{76}Se -selenate) versus 63.7% (^{74}Se -selenite). The mean difference was -3.01% (range: -14.0% – 12.0% ; SD 9.4%;, $p = 0.36$). Isotope enrichment of postbalance fecal samples was negligible, representing $1.1 \pm 0.9\%$ and $2.0 \pm 1.8\%$, respectively, of the total 72h fecal excretion of ^{74}Se and ^{76}Se .

Classical chemical balance data. The data for the classical chemical balance technique are summarized in Table 2. During the 72h balance period, total selenium intake from the formula (native plus added stable isotope selenium) was $60.8 \pm 4.6 \mu\text{g}$. Apparent selenium absorption was $45.6 \pm 5.2 \mu\text{g Se}$, corresponding to $75.7 \pm 5.4\%$ of total selenium intake. Total urinary Se excretion was $21.0 \pm 11.4 \mu\text{g Se}$, representing $46.6 \pm 11.4\%$ of apparently absorbed selenium or $34.9 \pm 7.0\%$ of total selenium intake. Mean apparent selenium retention was $24.7 \pm 7.0 \mu\text{g Se}$, corresponding to $40.8 \pm 11.1\%$ of total selenium intake.

DISCUSSION

In the present study, $10 \mu\text{g}$ of each selenium stable isotope were administered. The dose was based on both nutritional and analytical considerations, *i.e.* to evaluate selenium apparent absorption and retention at levels used for food fortification of infant formulas (14, 15, 17, 26) and to enable accurate determination of isotope enrichments in fecal and urine samples. Consumption of the labeled formulas approximately doubled the infants' daily selenium intake during the first 24h of the study. The results from this study are thus relevant to apparent selenium retention and absorption from selenium-fortified infant formulas consumed by healthy term infants with plasma selenium in the same range as reported for formula-fed North American infants (14, 15, 17).

The infants in this study retained approximately 60% of selenium added as selenite or selenate to a milk-based infant formula. Although apparent retention was similar for selenate and selenite, large differences were found in their apparent absorption and urinary excretion. These results are similar to our previous findings in adults, also using stable isotope methodology (27). In the previous study, mean retention of 41% and 46% from selenite and selenate, respectively, added to the same milk-based formula was found. Fractional selenium retention from both compounds was higher in infants than in adults, possibly because of the higher selenium requirements and lower dietary selenium intake during infancy.

Table 1. Mean apparent selenium absorption, urinary excretion and apparent retention of ^{74}Se -selenite and ^{76}Se -selenate in nine term infants fed labeled milk-based infant formula. Results are given relative to the total doses of ^{74}Se -selenite and ^{76}Se -selenate

	^{74}Se -selenite	^{76}Se -selenate
Apparent absorption (%)	$73.4 \pm 5.8^*$ (63.0–81.4)	$97.1 \pm 1.3^\dagger$ (65.2–98.7)
Urinary excretion (%)	$9.7 \pm 2.8^*$ (6.1–15.0)	$36.4 \pm 5.5^\dagger$ (30.7–47.2)
Apparent retention (%)	$63.7 \pm 6.0^*$ (54.9–70.6)	$60.7 \pm 5.6^*$ (50.6–67.9)

Values are mean \pm SD (range). Values in the same row not sharing a common superscript symbol are significantly different, $p < 0.001$.

Table 2. Results based on chemical balance data: Apparent selenium absorption, urinary excretion and apparent retention of total selenium from a milk based infant formula labeled with ^{74}Se -selenite and ^{76}Se -selenate fed to nine full term infants

Total Se intake (μg)	60.8 ± 4.6 (53.4–67.0)
Apparent Se absorption (μg)	45.6 ± 5.2 (36.2–53.3)
Apparent Se absorption (%)	75.7 ± 5.4 (67.8–84.1)
Total urinary Se excretion (μg)	21.0 ± 4.2 (14.7–26.3)
Urinary Se excretion (% of intake)	34.9 ± 7.0 (25.1–42.8)
Apparent Se retention (μg)	24.7 ± 7.0 (13.7–34.5)
Apparent Se retention (%)	40.8 ± 11.1 (25.7–59.0)

Values are mean \pm SD (range).

In the present study, apparent absorption of selenate (91%) was almost double that of selenite (50%). The intraindividual variation in selenium absorption was larger for selenite (CV 8%) than for selenate (CV 1%), which is in agreement with earlier data in adults (27). Higher absorption of selenate as compared with selenite from infant formula has been reported in suckling rats, presumably owing to more efficient absorption of selenate in the small intestine (28). Selenate has been demonstrated to be absorbed by a saturable transport mechanism of the ileal mucosa (29), facilitated by the transmembrane Na^+ gradient across the intestinal brush border (30). Thus, a rapid transport across the brush border by a carrier-mediated mechanism (29, 31) was found for selenate, whereas selenite was absorbed by passive diffusion and showed a strong tendency to bind to the brush border (31).

Renal clearance is the major pathway for excretion of absorbed selenium (32, 33). Our data in term infants showed that urinary selenium excretion of the absorbed dose was 3-fold higher for selenate (38%) than for selenite (13%). Urinary excretion of selenium absorbed as selenate was very rapid; 80% of total urinary selenium excretion was excreted within 24h after administration, compared with 48h for selenite. Elevated and rapid urinary excretion of absorbed selenate has been reported in adults (27, 34) and is most likely related to the faster absorption and metabolism of selenate compared with selenite. Very limited information is available on the metabolic handling of absorbed selenate and selenite. Selenite has been shown to be rapidly taken up by erythrocytes, metabolized, and effluxed into plasma bound to albumin (35). To our knowledge, no information is available on the metabolism of newly absorbed selenate.

Since approximately 60% of selenium was apparently retained by term infants from formula fortified with selenate or selenite, it can be assumed that the effect of either compound on selenium nutritional status would be similar. This assumption is supported by the finding that selenite and selenate supplementation resulted in similar increases in platelet GPx activity in Finnish men (36). Although no direct comparison has been performed on the effect of these two selenium compounds on selenium status in infants, feeding infant formulas fortified with selenite (13–16) or selenate (17) improved plasma selenium level and plasma GPx activity in term infants.

Mean apparent selenium absorption measured by classical chemical balance technique was 76% of total selenium ingested during 72h. Urinary selenium excretion was high and represented almost half of the apparently absorbed selenium, resulting in a mean apparent selenium retention of 41%. The intraindividual variation in apparent retention was high (range: 26%–59%), reflecting the large variation in urinary selenium excretion (CV 20%). For comparison, Ehrenkranz *et al.* (19) reported mean apparent absorption of 68% and mean apparent retention of 53% of total selenium from preterm formula fortified with selenite in very low birthweight infants.

A selenium compound used for fortification of foods consumed by population groups at risk of low selenium intake, such as infants and patients with chronic or acute diseases, should ensure adequate absorption and retention of selenium. Therefore, fortification of nutritional products with selenate

may be considered to be more efficient than selenite for these population groups. A number of aspects of selenate absorption and utilization favor the use of selenate *versus* selenite, including the higher fractional absorption, as compared with selenite, observed in the present study as well as in previous human studies (27, 34) and in suckling rats (28). In addition, the high and rapid urinary excretion of selenium absorbed from selenate can be considered an advantage over selenite for maintaining selenium homeostasis. However, selenate may be less suitable for infants with renal dysfunction or preterm infants with immature renal function, because urinary excretion of absorbed selenium is essential for homeostatic control of body selenium. In addition, concerns have been raised about the potential pro-oxidative properties of selenite and its lower stability compared with selenate when added to foods (37). Although these concerns need to be further investigated, they are additional factors potentially in favor of the use of selenate for selenium fortification of foods.

In conclusion, our results indicate that selenite and selenate were equally well retained by healthy term infants, although large differences in apparent absorption and urinary excretion were observed. Thus, the nutritional impact of infant formulas fortified with either of these inorganic selenium compounds can be expected to be similar in healthy term infants. Differences in the metabolism of these two inorganic selenium compounds may favor the use of selenate.

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