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Absorption of Selenium From Milk Protein and Isolated Soy Protein Formulas in Preschool Children: Studies Using Stable Isotope Tracer ^{74}Se

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Summary: Absorption of selenium as the stable isotopic tracer [^{74}Se]selenite was measured in four preschool children who were receiving liquid formula diets based on casein, isolated soy protein, and a 50:50 combination of the two protein sources. The children were in continuous ambulatory balance studies within the Clinical Research Center during three consecutive 11-day collection periods. The enrichment of the $^{74}Se/^{76}Se$ ratio in feces was measured by radiochemical neutron activation analysis, with fractional absorption estimated therefrom. Mean fractional absorption of selenium (± SD) from the formulas based on milk, isolated soy protein, and milk-soy were 64.2 ± 14.6, 73.4 ± 19.0, and 45.0 ± 10.9%, respectively, with the combined formula having a significantly lower intestinal uptake for added selenite than the casein formula. Stable isotopes of selenium are safe and potentially useful tools for examining its bioavailability in the diets of young children. **Key Words:** Selenium—Selenite—Stable isotopes—Milk—Isolated soy protein—Intestinal absorption—Infant feeding.

Selenium has been confirmed as an essential nutrient for humans (1), but large gaps in our understanding of its nutritional metabolism remain to be filled. On the basis of studies in animals (2–4), there was reason to suspect that dietary factors might influence the biological availability of selenium for humans. Experimental approaches to the investigation of selenium bioavailability have involved metabolic balance (5–9), radioisotopic studies (6,10–14), and more recently, stable (nonradioactive) isotope tracers (15–17), and repletion of glutathione peroxidase in blood (18) or platelets (19). Except for the report by Heinrich et al. (13) of juvenile cystic fibrosis patients, all of these studies involved adult volunteers.

The stable isotopic tracer methodology has a specific appeal and potential applicability for pediatric investigation insofar as "true" intestinal absorption of selenium can be measured in a setting that does not involve hazardous radiation exposure or invasive procedures. Moreover, in children, selenium bioavailability may be of more than academic concern, given the documentation of selenium deficiency in preschool children with protein-energy malnutrition (20–22). In order to explore the use of stable isotope tracer techniques in young children and to develop information about selenium absorption from common infant foods, we evaluated absorption of ^{74}Se from liquid formula diets based on milk solids, isolated soy protein, or a combination of the two foods.

MATERIALS AND METHODS

Subjects

Four male preschool children were enrolled in the study, ranging in age from 23 to 27 months. They had been admitted to the Clinical Research Center of the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City for the treatment of protein-

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energy malnutrition. Each had undergone the standard rehabilitation regimen of our center (23) and had achieved full nutritional recovery, as defined by normal weight-for-height and creatinine-height indices (24), before enrollment in the study. The studies were conducted in the Clinical Research Center during an additional 33 days of hospitalization. The protocol was approved by the Committee on the Use of Humans as Experimental Subjects of MIT and the Human Rights Committee of INCAP. The parents gave their informed consent to the participation of their child after the nature and purpose of the study had been explained.

Diet

Three basal formula diets, provided by the Ralston Purina Co., St. Louis, MO, were used. They differed only with respect to their protein source: formula C was based on casein; formula S on isolated soy protein; and formula CS on a 50:50 (wt/wt) mixture of casein and isolated soy protein. Each child consumed five liquid meals of identical volume daily; the daily ration provided the necessary nutrients to satisfy each child's nutritional requirements. The composition of the dry mixes for each formula diet are shown in Table 1. After reconstitution with water, the diets provided 2 g of protein and 90 kcal of energy per 100 g of liquid. In addition, three other formulas, identical in composition to the basal diets, were enriched with ^{74}Se by adding $^{74}SeO_3^{2-}$ (Union Carbide, Oak Ridge, TN) during processing. By analysis, the isotopic tracer content of the respective diets, expressed as nanograms of ^{75}Se/g liquid formula, were: 4.51 (formula C); 4.38 (formula S); and 2.90 (formula SC). The total selenium content was not measured directly, but we have used the median values from published food composition tables for selenium (25,26). Thus, we estimated that the intrinsic selenium per gram of the respective dry powder bases used to prepare the liquid beverages was 18.14, 14.45, and 16.35 ng[1]. When prepared as the liquid formula diets (242 g dry base/1,000 g beverage), the respective contributions of intrinsic selenium were 4.4, 3.5, and 4.0 ng/g of formula[1].

Absorption Studies

The study lasted 33 days and was divided into three 11-day periods, one with each protein source. The order of presentation of the diets was randomly assigned. On day 1, a child began receiving the first assigned diet. The ^{74}Se-enriched form of this formula was fed on days 5 and 6, and the child resumed eating the nonenriched diet for 5 additional days. The same sequence of unenriched–enriched–unenriched diets was repeated on days 12–22 and 23–33, with the other two formulas. Each stool passed throughout the 33-day period was weighed, transferred to a plastic container, and frozen at $-20°C$ until analyzed.

Isotopic Analyses

All stools collected from the day on which the ^{74}Se-enriched meals were introduced until the end of that specific diet period were pooled and homogenized (i.e., days 5–11, 16–22, and 27–33). Weighed aliquots were later analyzed for ^{74}Se and ^{76}Se at the Nuclear Reactor Laboratory of MIT by radiochemical neutron activation analysis using methods previously developed in this laboratory (27). Aliquots of three unenriched and three enriched diets were also analyzed for their isotopic contents. The estimation of fractional absorption of tracer selenium was made using a mathematical equation previously published (27) (see Appendix).

Statistical Analyses

Differences between treatments were determined using Student's t test for paired data (28).

RESULTS

The volume of formula containing isotopic enrichment consumed on the days of dosing, along with the measured amounts of ^{74}Se tracer and the estimated total selenium intake, are shown in Table 2. The fractional absorption of ^{74}Se for each formula in each child and the average absorption per participant and per treatment are provided in Table 3. There were no significant intersubject differences when all treatments were considered. The absorption of selenium for formula SC was significantly less than that for formula C ($p < 0.05$). There were large intertreatment differences as well between formulas S and SC in two subjects, but minimal differences in the other two, and the overall paired Student's t statistic failed to reveal significance.

DISCUSSION

The present experience demonstrates that stable isotopes of selenium can be applied in young children to estimate the true absorption of the nutrient. Barbezat et al. (29) have summarized the published data on metabolic balance and isotopic tracer studies used to

[1]The food composition tables did not provide a value for casein, so the median value of selenium in dry powdered milk has been used for this estimation.

TABLE 1. *Composition of formula diets*[a]

Ingredient	100% Soy protein	50% Soy protein and 50% casein	100% Casein
Corn syrup solids 24 DE	34.04535	34.12743	34.20956
Sucrose	31.77257	31.84917	31.92583
Soy protein isolate d.b.	11.17010	5.58500	—
Casein d.b.	—	5.42642	10.85263
Modified corn starch	4.04563	4.04563	4.04563
Carageenan	0.06068	0.06068	0.06068
Potassium citrate, H_2O	0.76867	0.76867	0.76867
Calcium phosphate tribasic	1.61825	1.61825	1.61825
Potassium chloride	0.56639	0.56639	0.56639
Magnesium chloride	0.11351	0.11351	0.11351
Sodium chloride	0.04855	0.04855	0.04855
Ferrous sulfate	0.02427	0.02427	0.02427
Cupric sulfate, 5 H_2O	0.00150	0.00150	0.00150
Potassium iodide	0.00015	0.00015	0.00015
Coconut oil	9.07462	9.07462	9.07462
Soy oil	6.12538	6.12538	6.12538
Atmos 150 (mono and diglycerides)	0.1520	0.1520	0.1520
Sta-Sol lecithin	0.1520	0.1520	0.1520
γ-Tocopherol acetate (905 IU/g)	0.01141	0.01141	0.01141
Vitamin A palmitate (250,000 IU/g)	0.00763	0.00763	0.00763
Vitamin D_3 (400,000 IU/g)	0.00076	0.00076	0.00076
Choline chloride (50)	0.0445	0.0445	0.0445
L-Methionine	0.0355	0.0355	0.0355
Ascorbic acid, USP	0.14160	0.14160	0.14160
Niacinamide, USP	0.00686	0.00686	0.00686
Calcium pantothenate, USP	0.00413	0.00413	0.00413
Riboflavin, USP	0.00046	0.00046	0.00046
Pyridoxine HCl, USP	0.000367	0.000367	0.000367
Thiamine HCl, USP	0.000342	0.000342	0.000342
Phytonadione	0.000114	0.000114	0.000114
Biotin	0.000114	0.000114	0.000114
Folic acid	0.000076	0.000026	0.000076
Cyanocobalamin	0.002287	0.002287	0.002287
Total calories	463.9	463.9	463.9
Percent calories from fat	30.07	30.07	30.07
Total protein	10.31	10.31	10.31
Calories/2 g protein	90.0	90.0	90.0

DE, dextrose equivalent; d. b., dry base.
[a]Values are in grams per 100 grams of powder.

assess selenium absorption. The only isotopic selenium studies performed previously in children involved pork meat labeled bioorganically in vivo by administering [^{75}Se]selenomethionine to the pigs. The children studied were suffering from cystic fibrosis and showed impaired selenium absorption (13); the controls for that study were normal adults who absorbed an average of 87% of the isotope from the meat. Estimates of apparent absorption of selenium in metabolic balance studies in adults ranged from 35 to 75%; "true" absorption, determined with selenium isotopes provided as selenite to adults, ranged from 44 to 95%. Our results for preschool children in the present study fell in the middle of the range of fractional absorption observed with isotopic techniques in adult subjects, with the global mean for the children being 63%. The average absorption of the isolated soy-casein formula (SC) was at the lower limit of the range reported for adults (29).

The small sample size in the present study would caution against generalization of these absorptive values to the preschool population at large. Similarly, only tentative conclusions can be made regarding the biological implications of the differential bioavailability of selenium from the different protein sources. It appears, however, that neither isolated soy protein alone nor casein alone differ from one another, nor do they inhibit the absorption of selenium tracers to a greater extent than other meal situations in adults (29). The 50:50 mixture of the protein sources (formula SC) significantly reduced selenium absorption as compared to the milk protein-based (formula C)

TABLE 2. *Individual intakes of ^{74}Se, total selenium, and total formula diet during the 2 days of consuming the isotope tracer*

Subject	Casein	Soy	Casein/soy
PC448	9.18[a]/18.14[b] (2,036)[c]	8.95/15.26 (2,044)	5.96/14.10 (2,054)
PC459	8.37/16.53 (1,856)	8.13/14.63 (1,856)	5.38/12.74 (1,856)
PC462	7.08/13.99 (1,570)	6.96/12.25 (1,590)	4.59/10.86 (1,582)
PC468	8.64/17.19 (1,916)	8.30/14.94 (1,896)	5.52/13.08 (1,906)

[a]Intake of ^{74}Se during 2 days based on the measured amounts of the tracer in the respective formulas (see text) and the volume of ingested diet. Intake is expressed in μg/2 days.

[b]The total selenium intake calculated from the measured amount of ^{74}Se plus the *estimated* contribution of intrinsic selenium from food composition tables (25,26). Intake is expressed in μg/2 days.

[c]Measured volume of intake of the formula in grams during the 2-day period.

diet. There is no clear explanation for this apparent interaction. One could postulate that the lesser amount of tracer selenium ($^{74}SeO_3^{2-}$) added to the combination diet might have been a factor. Conventionally, a lesser amount of mineral would be expected to be absorbed more efficiently from the intestine, if calcium, zinc, or iron are comparable examples. In fact, in adults studied with 54 and 108 μg of ^{74}Se as the tracer dose, no differences in fractional absorption were observed (15). Moreover, if we consider the total amount of selenium in the intestinal lumen after the ^{74}Se-enriched meals (Table 2), we would observe that the background intrinsic selenium explained as much of variance as the amount of selenite tracer added to the formulas. Thus, it is unlikely that the lesser quantity of tracer in the combined protein diet played any role in the lower fractional absorption.

The effect of interactions between protein sources on mineral absorption are not unique to the present study, however. We observed a similar result in our studies with a stable tracer of zinc (^{70}Zn) in adult men consuming a beef meal or a texturized soy protein meal (30). In an additional experimental treatment from that study (Solomons NW, Janghorbani M, Young VR, et al., unpublished observations), the same subjects consumed a 50:50 mixture of soy and beef and showed a tendency toward a lower absorption of zinc isotope; but this did not achieve statistically significant proportions. However, there was no interaction on fractional zinc absorption of a milk:soy mixture in a liquid formula diet tested in the same study (30), and mixtures of chicken meat and isolated soy protein had a similar zinc bioavailability to chicken meat alone in another human experiment with adult subjects from our laboratory (31).

Recent advances in the technology of stable isotope tracers of selenium for human bioavailability studies have been encouraging (32–34). Our present experience proves that administration of the tracer is safe in young children and that fecal monitoring of changes in isotopic ratios of ^{74}Se/^{76}Se provides consistent results. Numerous biological issues regarding selenium in diets of infants and children remain to be resolved, and the stable isotope tracers provide a promising experimental approach for their investigation.

APPENDIX

Mathematical Considerations on the Calculation of Fractional Absorption of Selenium by Fecal Monitoring

We use the concept of single enrichment of the diet with ^{74}Se, which is the naturally occurring stable isotope that has the lowest natural abundance (0.87%). Both this isotope (^{74}Se) and ^{76}Se, with a natural abundance of 9.0%, are measured in the fecal pool. The fractional absorption of ^{74}Se is calculated as follows:

$$F = \frac{A_o{*}\ {}^{74}\text{Se} - A_f\ {}^{74}\text{Se} + R_{74/76} \cdot A_f\ {}^{76}\text{Se}}{A_o{*}\ {}^{74}\text{Se}}$$

where F is the fractional absorption of the tracer; $A_o{*}$ ^{74}Se is the measured quantity of ^{74}Se given as tracer; A_f ^{74}Se is the measured quantity of ^{74}Se in the fecal pool; A_f ^{76}Se is the measured quantity of ^{76}Se in the fecal pool; and $R_{74/76}$ is the natural mass isotope ratio of ^{74}Se to ^{76}Se, in this case, 0.097.

TABLE 3. *Absorption of ^{74}Se by children fed different formula diets (% of oral dose)*

Subject	Casein	Soy	Casein/soy	Mean ± SD
PC448	83.5	59.2	53.8	65.5 ± 15.8
PC459	48.8	96.4	41.9	62.4 ± 29.7
PC462	58.9	81.4	30.9	57.1 ± 25.3
PC468	65.7	56.4	53.5	58.5 ± 6.4
Mean ± SD	64.2 ± 14.6	73.4 ± 19.0	45.0 ± 10.9	63.4 ± 19.8

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