Determination of Selenium in Human Milk by Hydride Cold-Trapping Atomic Absorption Spectrometry and Calculation of Daily Selenium Intake

Fangshi Li,*,† Erich Rossipal,‡ and Kurt J. Irgolic†

Institute for Analytical Chemistry and Department of Pediatrics, Karl-Franzens-University Graz, A-8010 Graz, Austria

A technique of hydride cold-trapping atomic absorption spectrometry following microwave digestion was developed and optimized for the determination of selenium in human milk. The method was validated by the analysis of two standard reference materials (CRM milk powder). The detection limit was 0.5 ng mL^{-1}. The method was then used to analyze 78 milk samples from 38 Austrian mothers throughout their first 10 months of lactation. The mean concentration of selenium in the mother's milk decreased with the days postpartum from 23.9 ± 12.0 $\mu g\ L^{-1}$ in colostrum to a plateau of 11.4 ± 3.0 $\mu g\ L^{-1}$ in mature milk. On the basis of the milk selenium concentrations, the selenium intakes of the fully breast-fed infants and the lactating mothers were calculated. The selenium intake of the infants during their first 3 months of life was >8.2 $\mu g\ day^{-1}$. The selenium intake of the lactating mothers was 48 $\mu g\ day^{-1}$. Compared to the recommended dietary allowance, the fully breast-fed infants received sufficient selenium but the lactating mothers obtained less than the recommended.

Keywords: *Selenium; human milk; daily intake; mothers; infants; Austria; microwave digestion; hydride generation atomic absorption spectrometry*

INTRODUCTION

After delivery, human infants have to develop and maintain their own regulatory systems including the antioxidant system. Because selenium, as part of the enzyme glutathione peroxidase, is essential for the proper function of a number of cellular defense mechanisms, a sufficient supply of selenium for the infant has to be guaranteed. It is generally assumed that the selenium concentration of human milk reflects the maternal selenium intake via food and that human milk, as the optimal source of nutrients, contains adequate amounts of trace elements including selenium to satisfy the growing demands of healthy infants. Consequently, the selenium status of the fully breast-fed infant depends entirely on the nutritional status of the nursing mother.

Accurate information is needed about selenium concentrations in human milk at different stages of lactation. This knowledge is important to establish the selenium status of newborns. Among the analytical procedures for the determination of selenium in body fluids described in the literature (Sanz Alaejos and Diaz Romero, 1995), the most often used methods are spectrofluorometry, atomic absorption spectrometry (AAS), and neutron activation analysis.

Because selenium concentration in human milk is low ($\sim$10 $\mu g\ L^{-1}$) and the sample volume available, especially the colostrum, is small, the analytical method for the determination of selenium in milk must have a low detection limit. In this study, a technique of hydride cold-trapping AAS following microwave digestion was developed and optimized for the determination of selenium in human milk. The method was used to investigate the selenium status in Austrian mothers' milk at different stages of lactation for calculation of selenium intake of the mothers and infants.

EXPERIMENTAL PROCEDURES

Reagents. All reagents were of analytical reagent grade or higher purity from Merck or Fluka. NANOpure water (18.0 MΩ cm) was obtained by double distillation in a quartz still (Destamat, Heraeus) and subsequent passage through an all-quartz cartridge system (Barnstead NANOpure, Boston, MA). All solutions were prepared with NANOpure water. Selenium stock solution (1000 mg L^{-1}) was prepared from sodium selenite pentahydrate with NANOpure water. The selenium working solutions for analysis were prepared daily by diluting the stock solution with NANOpure water. Sodium borohydride solution (0.5% m v^{-1}) was prepared daily by dissolving 2.50 g of sodium borohydride, 0.5 g of sodium hydroxide, and 0.5 g of barium hydroxide octahydrate in 500 mL of NANOpure water and filtered before use. Hydrochloric acid (32%) and nitric acid (65%) were purified by subboiling distillation. Hydrogen peroxide (30%) was suprapur grade. The two standard reference materials, CRM 063 milk powder (nature) and CRM 151 skim milk powder (spiked), were from the Commission of European Communities, Community Bureau of Reference, Brussels, Belgium.

Apparatus. A high-performance digestion system (MLS 1200 MEGA, Milestone, Leutkirch, Germany) with an EM-30 exhaust module was used for the digestion of milk samples. A Unicam VP90 continuous flow vapor system served as the continuous hydride generator and the gas−liquid separator.

* Address correspondence to this author at the Department of Applied Chemistry, Nanjing University of Chemical Technology, Nanjing 210009, People's Republic of China (fax +86-25-3418556; e-mail njfangli@jlonline.com).

† Institute for Analytical Chemistry.

‡ Department of Pediatrics.

10.1021/jf990268d CCC: $18.00
Published on Web 07/10/1999

Table 1. Instrumental Parameters for Determination of Selenium by Hydride Cold-Trapping AAS

wavelength (nm)	196.0
current of hollow cathode lamp (mA)	12
slit (nm)	1.3
temperature of quartz tube (°C)	900
flow rate of helium gas (mL min^{-1})	280
reductant	0.5% $NaBH_4$ in 0.1% NaOH
HCl (mol L^{-1})	1
flow rate of reductant (mL min^{-1})	3.5
flow rate of sample solution (mL min^{-1})	6
sampling time (min)	3

A Hitachi (San Jose, CA) Model Z-6100 atomic absorption spectrometer equipped with an electrically heated quartz tube served as the selenium-specific detector.

Cold Traps. Between the VP90 hydride generator and the AAS, there were a water trap and a hydride trap. The water trap was a U-tube with a spiral groove on one side immersed in methanol at −20 °C. The hydride trap was a quartz U-tube immersed in liquid nitrogen. The pump of the VP90 continuously delivered sodium borohydride solution and the sample solution to the mixing tube. The reaction mixture flowed into the gas−liquid separator. The separated gases diluted with helium as the carrier gas passed through the water trap, where the water was removed from the gas stream, and then into the hydride trap, where the H_2Se was collected. The hydride generation finished when the solenoid valve switched from the sample solution to the blank solution (1 mol L^{-1} HCl). After all of the H_2Se was condensed in the hydride trap, the coolant of liquid nitrogen was replaced with hot water (90 °C) to evaporate the H_2Se into the electrically heated quartz tube for determination of selenium.

Milk Samples. Seventy-eight milk samples were obtained from 38 healthy Austrian mothers at 1−293 days postpartum. The mothers had successfully given birth to mature babies after uneventful pregnancies. None of the mothers had received supplemental selenium during either pregnancy or lactation. Milk (20−30 mL) was collected for each sample at the University Maternity Clinic in Graz between March 1995 and February 1996. All sample collection equipment was acid washed to prevent contamination. The samples were transferred to polyethylene tubes and kept at −20 °C. Before analysis, the samples were warmed to room temperature and carefully mixed by shaking.

Procedure. Into a Teflon microwave digestion vessel was placed 1.0 mL of milk (weighed to 0.1 mg); 65% HNO_3 (1.0 mL) and 30% H_2O_2 (0.5 mL) were slowly added. The vessel was closed and fastened into the rotor. The rotor with 10 loaded vessels was placed into the microwave oven. The digestion program was performed as 250 W (1 min), 0 W (1 min), 250 W (5 min), 400 W (5 min), and 650 W (5 min). When the digestion was complete, the rotor with the vessels was transferred into a cold water bath and cooled to room temperature. The vessels were opened, and the content of each vessel was transferred into a 25-mL flask. The vessel was rinsed two times with 4 mL of NANOpure water, and the liquid was transferred to the flask. The flasks, each containing ∼10 mL of solution, were gently heated for ∼20 min in a 150 °C sand bath until 2−3 mL of solution remained. Then 2.0 mL of 32% HCl was added into the flask, and the solutions were heated with a water bath (90 °C) for 15 min. After cooling to room temperature, the solution was diluted to 20 mL with NANOpure water. Selenium was determined by the hydride cold-trapping AAS with optimized parameters (Table 1). The peak heights of the absorbance were used to calculate selenium concentrations.

RESULTS AND DISCUSSION

Microwave Digestion of Milk Samples. The amount of nitric acid required for the microwave digestion of selenium in milk was determined by digestion of commercial cow's milk and milk powder. For the microwave digestion of 1.0 mL of cow's milk or 0.2 g of milk powder, the optimal amount of HNO_3 was 1.0 mL combined with 0.50 mL of H_2O_2. The digests were clear and colorless. The excess of HNO_3 in the digests must be removed before hydride generation because the residual HNO_3 can suppress the generation of H_2Se (Dedina and Tsalev, 1995). In this study, all of the residual HNO_3 was removed by gently heating the digest for ∼20 min in a sand bath (150 °C). Care had to be taken not to evaporate the solution to dryness to avoid loss of selenium.

Generation of H_2Se. The flow rates of the sodium borohydride solution and the sample solution were controlled by the rotation speed of the pump head and the inner diameters of the pump tubing. In the optimal conditions, the flow rate of sodium borohydride solution was 3.5 mL min^{-1}, whereas the flow rate of the sample solution was 6 mL min^{-1}. The optimal concentration of sodium borohydride was 0.3−0.5% (w v^{-1}). For removing carbonate, which would be released as CO_2 in the step of hydride generation and interfere with the selenium determination (Oernemark et al., 1992), barium hydroxide was added to the borohydride solution and the solution was filtered before use.

The effect of the concentration of the hydrochloric acid in the sample solution on the selenium signal was studied. The measured absorbance increased with the increase of hydrochloric acid concentration in the range of 0.5−4 mol L^{-1}. Because the increase was only ∼20%, 1 mol L^{-1} hydrochloric acid was chosen to prolong the lifetime of the silanization on the tubes and reduce the corrosion of the system.

Cold Traps. The water trap, a U-tube with a spiral groove on one side immersed in methanol at − 20 °C, effectively removed water vapor from the gas stream. If this water trap was omitted, the hydride trap became quickly clogged with ice. A small plug of silanized quartz wool placed in the outlet side of the hydride trap was found to be satisfactory for collecting H_2Se.

The flow rate of the helium as carrier gas influenced the intensity of the absorbance and the rate of the volatilization of H_2Se. The flow rate of the carrier gas over the range 200−350 mL min^{-1} was investigated. The absorbance increased with the flow rate increase from 200 to 250 mL min^{-1}, stayed almost constant from 250 to 300 mL min^{-1}, and decreased above 300 mL min^{-1}. In the further experiment, the flow rate of helium used was 280 mL min^{-1}.

The absorbance diminished with the time elapsed between the generation of H_2Se and the volatilization of H_2Se into the quartz tube due to the depletion of hydrogen in the carrier gas. The hydride trap had to be heated at a fixed time after completion of H_2Se generation. In this study, the time from the hydride generation until the solenoid valve switched from the sample solution to the blank solution was set to 3 min. Then the Dewar with liquid nitrogen for cooling the H_2Se trap was replaced immediately with a Dewar of hot water (90 °C) to vaporize H_2Se. The U-tube was kept in the hot water bath until the selenium signal had returned to baseline.

Calibration Curve, Detection Limit, and Accuracy. The calibration curve for the determination of selenium was linear up to 10 ng. The equation for the calibration curve was $Abs = 0.0134x + 0.0003$ with the correlation coefficient $r^2 = 0.995$.

Table 2. Selenium Concentrations in Milk of Austrian Mothers at Different Lactation Stages

type of milk	lactation time (days postpartum)	no. of samples	Se concn (μg L^{-1} of milk) mean ± SD	median	range
clostrum	1−7	36	23.9 ± 12.0	20.2	9.2−53.4
transitional	9−12	3	18.2 ± 4.0	16.5	15.3−22.7
mature	15−60	24	12.2 ± 2.4	13.1	8.3−15.9
	66−79	9	10.9 ± 1.3	10.6	6.1−14.4
	97−150	3	7.0 ± 0.7	6.6	6.6−7.8
	224−293	3	13.5 ± 4.5	11.8	10.0−18.6

Table 3. Daily Selenium Intake of Fully Breast-Fed Infants

age of infants (days)	daily milk consumption (L)	Se concn (μg L^{-1} of milk)	intake of Se[a] (μg day^{-1})
1−7	0.75	23.9	17.9 ± 2.2
9−12	0.75	18.2	13.7 ± 1.7
15−60	0.75	12.2	9.8 ± 1.2
66−79	0.75	10.9	8.2 ± 1.0
97−150	0.75	7.0	5.2 ± 0.6

[a] With a coefficient of variation of 12.5%.

The detection limit was 0.3 ng of selenium, estimated from 3 times the standard deviation of the blank. The precision expressed as the relative standard deviation of five replicates was <10%.

Two types of milk powder reference materials were used to check the whole analytical procedure. The results, 84.0 ± 3.6 μg kg^{-1} (n = 5) for the CRM 063 skim milk powder (natural) and 123.5 ± 8.0 μg kg^{-1} (n = 5) for the CRM 151 skim milk powder (spiked), showed good agreement with the certified values, 88 and 125 μg kg^{-1}, respectively.

Selenium in Milk. The selenium concentrations in the 78 milk samples from 38 Austrian mothers on days 1−293 postpartum are shown in Table 2. The mean concentration of selenium in the milk decreased with days postpartum, from 23.9 μg L^{-1} in colostrum to a plateau of ~11.4 μg L^{-1} in mature milk. A decrease of selenium as a function of lactation time was also noted by other researchers (Benemariya et al., 1995; Robberecht et al., 1985; Roekens et al., 1985).

Daily Selenium Intake of Fully Breast-Fed Infants. For fully breast-fed infants, mother's milk is their only source of selenium. Selenium intake of fully breast-fed infants can be calculated by multiplying the volume of the milk by the concentration of selenium in the milk.

It is now accepted that the average milk consumption for infants born at term is 750 mL for the first 6 months and 600 mL during the next 6 months (U.S. Food and Nutrition Board, 1989).

On the basis of the concentration of selenium in the milk, the selenium intake of fully breast-fed infants was calculated (Table 3).

The status of human milk at ~3 months postpartum is important. At this stage of lactation, the milk is relatively mature and many of its constituents have reached fairly stable levels. Moreover, the third month is the time when many mothers start to wean their babies. After this age, therefore, the baby's intake of selenium no longer depends exclusively on the mother's milk.

According to this study, the selenium intake of infants during their first 3 months of life is >8.2 μg day^{-1}. The U.S. Food and Nutrition Board (1989) recommended a dietary allowance of 10 μg day^{-1} for infants of 0−6 months of age. The German Society of Nutrition (DGE, 1991) recommended 5−15 μg day^{-1} for infants of 0−4 months of age. Compared to the recommended, Austrian babies in their first 3 months of life get sufficient selenium from mother's milk.

Table 4. Dietary Selenium Intake and Selenium Concentration in Mature Milk

country	dietary Se intake (μg day^{-1})	Se concn (μg L^{-1} of milk)
China (Keshan)	8.8	2.6
New Zealand	25	7.6
Finland	30−50	7.6−11.8
Austria	48.2[a]	11.4[a]
Italy	43	13.8
Belgium	52	10.0
Germany (FRG)	59	15.4
Sweden	68	14
United States	80	20
Japan	88	18
Philippines	136	34
China (Hubei)	198	40
Venezuela	230−500	46−90

[a] This study.

Dietary Selenium Intake of the Mothers. Selenium in milk, as well as the selenium intake status of the newborns, is largely a function of the selenium intake of the mothers. Dietary intake of selenium can be obtained by different procedures including market basket studies, selective studies of individual foodstuffs, and duplicate portion technique. Comparing the selenium concentrations in mature milk with the dietary selenium intake of the population from a number of countries (Table 4), a linear relationship was reported (Braetter et al., 1991): selenium in milk (μg L^{-1}) = dietary selenium intake (μg day^{-1}) × 0.20 + 1.75 (r^2 = 0.989, n = 16, p < 0.001).

In this study the mean concentration of the selenium in mature milk (Table 1, 15−293 days postpartum, n = 39) was 11.4 ± 3.0 μg L^{-1}. Using the linear relationship, the average selenium intake of Austrian mothers was calculated as 48.2 μg day^{-1}. This appears to be in acceptable agreement with the result of food analysis that the estimated selenium intake of Austrian people was 45.5 μg day^{-1} (Pfannhauser, 1988). Compared with the recommended dietary allowances of 55 μg day^{-1} for adult females and 75 μg day^{-1} for lactating mothers by the U.S. Food and Nutrition Board (1989), the dietary selenium intake of Austrian mothers is below the recommended.

LITERATURE CITED

Benemariya, H.; Robberecht, H.; Deelstra, H. Copper, zinc and selenium concentrations in milk from middle-class women in Burundi (Africa) throughout the first 10 months of lactation. *Sci. Total Environ.* **1995**, *164*, 161−174.

Dedina, J.; Tsalev, D. L. *Hydride Generation Atomic Absorption Spectrometry*; Wiley: Chichester, U.K., 1995; Chapter 13, Selenium,

Deutsch Gesellschaft fuer Eraehrung (DGE). *Empfehlungen fuer die Naehrstoffzufuhr*; Ic Tab.25, 5; Ueberarbeitung: Frankfurt, Germany, 1991.

Oernemark, U.; Petterson, J.; Olin, A. Determination of total selenium in water by AAS after hydride generation and preconcentration in a cold trap system. *Talanta* **1992**, *39*, 1089−1096.

Pfannhauser, W. *Essentielle Spurenelemente in der Nahrung*; Springer-Verlag: Berlin, Germany, 1988.

Robberecht, H.; Roekens, E.; Caillie-Bertrand V.; Deelstra, H.; Clare, R. Longitudinal study of the selenium content in human breast milk in Belgium. *Acta Paediatr. Scand.* **1985**, *74*, 254−258.

Roekens, E.; Deelstra, H.; Robberecht, H. Trace elements in human milk, selenium, a case study. *Sci. Total. Environ.* **1985**, *42*, 91−108.

Sanz Alaejos, M.; Diaz Romero, C. Analysis of selenium in body fluids: a review. *Chem. Rev.* **1995**, *95*, 227−257.

U.S. Food and Nutrition Board, National Research Council. *Recommended Dietary Allowances*; National Academy of Sciences: Washington, DC, 1989.

Received for review March 10, 1999. Revised manuscript received May 12, 1999. Accepted May 26, 1999. F.L. thanks the Austrian Academic Exchange Services for awarding a scholarship to study at the University of Graz.

JF990268D