Selenate fortification of infant formulas improves the selenium status of preterm infants1,2

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ABSTRACT The purpose of this study was to determine whether selenate fortification of infant formula would improve the selenium status of relatively well, growing, preterm infants during the first 12 wk of enteral feeding. A high-selenium group (n = 7, mean body weight = 1312 g) received selenate-fortified preterm and full-term infant formulas containing 0.36 and 0.22 μmol Se/L, respectively, and a low-selenium group (n = 10, mean body weight = 1262 g) received non-selenium-fortified preterm and full-term infant formulas containing 0.12 and 0.11 μmol Se/L, respectively. There were no significant differences in growth between the two groups throughout the study. The high-selenium group had significantly greater mean selenium intakes than did the low-selenium group from weeks 2 to 12. Plasma selenium concentrations decreased over the study period in the low-selenium group. Plasma selenium-dependent glutathione peroxidase activity was greater in the high-selenium group at week 12 only. Red blood cell selenium concentrations decreased over time in both groups and were significantly greater in the high-selenium group at weeks 4, 8, and 12. Plasma selenium concentrations were significantly correlated with plasma glutathione peroxidase activity for all infants on study day 1 and at weeks 4 and 12. Selenium intake of all infants was significantly correlated with plasma glutathione peroxidase activity at 12 wk. Selenate fortification of infant formulas can improve the selenium status of preterm infants. Current selenium contents of infant formulas and recommendations for dietary intakes of selenium for some preterm infants may be inadequate. Am J Clin Nutr 1996;64:860–5.

INTRODUCTION

Although the essential nature of selenium in animal diets was first documented in 1957, it was not until 1979 that selenium was shown to be essential in human nutrition (1–3). Selenium-dependent glutathione peroxidase, which requires selenium for its structure and function (4), has been shown to play a crucial role in the prevention of free radical formation by conversion of lipid and hydrogen peroxides to harmless oxygen species via the oxidation of glutathione. In 1989 selenium was determined to be an essential nutrient for infants by the National Academy of Sciences; a recommended dietary allowance (RDA) of 10 μg Se/d for infants between birth and 6 mo was declared (5).

The mean selenium content of mature human milk in the United States ranges from 0.19 to 0.25 μmol/L (15–20 μg/L) (6, 7). Unfortified 2789.9-kJ/L (20-kcal/oz) milk- and soy-based infant formulas contain ≈0.02–0.16 μmol/L (2–13 μg Se/L), with most having inherent selenium concentrations of 0.02–0.10 μmol/L (2–8 μg/L). Selenium intakes of full-term infants fed non-selenium-fortified, milk-based formula have been shown to be near or below the RDA whereas infants in the United States fed human milk generally have selenium intakes meeting or exceeding the RDA (8). Although selenium deficiency has not been reported in full-term infants fed non-selenium-fortified formulas, selenium fortification of milk- and soy-based formulas has been shown to result in significant increases in plasma glutathione peroxidase activity in infants fed these formulas (9, 10). Increasing the selenium intake of preterm infants may also be beneficial for a variety of reasons. Preterm infants have reduced fetal accretion of selenium (11) and decreased plasma selenium and glutathione peroxidase concentrations at birth (12), experience rapid postnatal growth, and are at increased risk for exposure to oxidative stress. Selenium deficiency in preterm infants is therefore an important nutritional goal. The optimal chemical form for selenium fortification in infant formulas has not been determined, however. Inorganic forms such as selenite and selenate are considered to be more appropriate than the organic forms selenocysteine and selenomethionine found in human milk because of decreased retention and less concern about toxicity (13, 14). Most studies on selenium supplementation of infant formulas have been conducted in full-term infants and have used selenite as the source of supplementation. Selenite, however, is relatively unstable and may be reduced in the presence of copper or iron to elemental selenium, a nonbioavailable form. Selenate is chemically more stable and may be more bioavailable when used to fortify infant formulas. The purpose of this study was to determine whether sodium selenate fortification of formula fed to preterm infants over the first 12 wk of enteral feedings would improve the selenium status of these infants.

SUBJECTS AND METHODS

Preterm infants who were patients in the intensive care nursery at Temple University Hospital and who met the fol-

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lowing criteria were eligible to be recruited for study: had a gestational age at birth of \( \leq 33 \) wk; had a birth weight \( < 2000 \) g; had received less than three packed red blood cell (RBC) transfusions; had no congenital anomalies; had no evidence of any significant disease process; could receive enteral feedings within 14 d of birth; had no history of maternal drug use, diabetes, or congenital syphilis; and had a parent who agreed to have formula be the sole source of energy (\( \geq 90\% \) of energy intake) during the post–hospital discharge part of the study. The protocol was approved by the Institutional Review Board of Temple University Hospital. Informed, written consent was obtained from the mothers of all study infants.

A controlled, randomized, and blinded study was conducted in which study infants received one of the following feeding regimens. The low-selenium group received non-selenium-fortified, low-iron premature infant formula (Similac Special Care 24; Ross Products Division, Abbott Laboratories, Columbus, OH); mean inherent selenium content = 0.12 \( \mu \)mol/L, or 10.0 \( \mu \)g/L) in the hospital followed by non-selenium-fortified infant formula with iron (Similac 20 With Iron 20; Ross Products Division; mean inherent selenium content = 0.11 \( \mu \)mol/L, or 8.6 \( \mu \)g/L) after discharge. The high-selenium group received the low-iron premature infant formula fortified with sodium selenate (mean total selenium content = 0.36 \( \mu \)mol/L, or 28.4 \( \mu \)g/L) in the hospital followed by the infant formula with iron fortified with sodium selenate (mean total selenium content = 0.22 \( \mu \)mol/L, or 17.6 \( \mu \)g/L) after hospital discharge. All study formulas were clinically labeled and manufactured by Ross Products Division.

Study day one was defined as the first day of enteral feedings. Non-selenium supplemented intravenous alimentation fluids were administered as clinically indicated. The low-iron premature infant study formula was started when the infant had achieved full enteral feeds defined as 120 mL \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\).

**Dietary tolerance and stool assessment**

While in the hospital, the volume of study formula consumed by the infants was recorded daily. After the infants were discharged, stool patterns, tolerance, and formula intake for a 3-d period before the 4-, 8-, and 12-wk study visits were recorded by the parents or obtained by parental interview. Selenium intake was calculated from the selenium content of the formula as determined by Ross Products Division and the intakes were recorded.

**Anthropometric measurements**

Weight, crown–heel length, and head circumference measurements at birth were obtained from hospital records. While the infants were in the hospital, weights were recorded daily; length and head circumference were measured and recorded weekly by the study nurse. After infants were discharged, weight, length, and head circumference data were obtained at the 4-, 8-, and 12-wk study visits. Length was measured with a premature infant length board (Ellard Instrumentation Ltd, Seattle). Head circumference was measured across the frontal bone area and at the point of maximum circumference with a paper tape measure.

**Blood and urine collection and analysis**

Two milliliters of heparin-treated blood was obtained on study day 1 and at 4, 8, and 12 wk. After centrifugation at 4 °C for 10 min at 2000 \( \times \) g and separation of the plasma and erythrocyte fractions, the samples were stored at \(-70^\circ \)C until analyzed. Plasma samples were analyzed for selenium, glutathione peroxidase, and total protein. RBC specimens were analyzed for selenium and hemoglobin concentrations. Urine samples were obtained on study day 1, and at 4, 8, and 12 wk and were stored at \(-20^\circ \)C until analyzed. Urine samples were analyzed for creatinine and selenium.

All samples were analyzed in the laboratory of Phillip Whanger at Oregon State University. Corvallis, OR. Selenium contents of erythrocytes and urine were measured with an AlpChem II autoanalyzer (AlpChem Corp, Clackamas, OR) with a model 306 fluorometer and 310 strip recorder (AlpChem Corp) according to the automated fluorimetric method of Brown and Watsonson (15). Plasma selenium content was analyzed with an atomic absorption instrument with Zeeman background corrector (model 3030; Perkin-Elmer, Norwalk, CT). A nickel-magnesium solution was used as a matrix modifier and was aspirated twice for analysis. Glutathione peroxidase activity of plasma was determined through use of the coupled assay of Paglia and Valentine (16) as modified by Whanger et al (17) with a spectrophotometer (DU-64 ultraviolet-visible; Beckman, Fullerton, CA). The substrate used for this assay was \( \nu \)-butyl hydroperoxide at a concentration of 25 mmol/L. Plasma total protein concentration was measured by the method of Lowry et al (18). Hemoglobin concentration of the erythrocyte fractions was measured by the cyanmethemoglobin method as described by Eilers (19). Urine creatinine concentration was measured colorimetrically with a creatinine test kit (#555-A; Sigma Chemical Co, St Louis).

**Statistical methods**

Differences in categorical variables such as overall tolerance, sex, gestational age, birth weight, length, and head circumference between the two study groups were assessed with Fischer’s exact test; continuous variables were compared with Student’s \( t \) test. Ranked data were used in the absence of normality or in the presence of outliers. The hospital information was analyzed only to week 4 because the number of observations beyond this point declined considerably. Pearson’s correlation coefficient was used to assess the relation between selenium intake and glutathione peroxidase activity at each time point. All hypothesis testing was two-sided; the level of significance was set at \( \alpha = 0.05 \). Statistical analyses were done with SAS, version 6.08 (SAS Institute Inc, Cary, NC).

**RESULTS**

**Subjects**

A total of 25 subjects were enrolled in the study from December 1991 through August 1993. All subjects were representative of the urban, inner-city community that Temple University Hospital serves. Seventeen infants (15 blacks and 2 Hispanics) successfully completed the protocol; 7 in the high-selenium group and 10 in the low-selenium group. Eight subjects (seven in the high-selenium group and one in the low-selenium group) dropped out of the study for reasons unrelated to formula tolerance and were classified as protocol failures. There were no treatment failures.
There were no significant differences between groups in gestational age, birth weight, or length (Table 1). Head circumference at birth in the high-selenium group was slightly greater compared with that in the low-selenium group. One infant in the low-selenium group was classified as being small for gestational age. The mean ages of infants in the high-selenium and low-selenium groups on study day 1 and at weeks 4 and 8 were not significantly different. The low-selenium group was slightly older at week 12 (93 compared with 89 d).

**Anthropometric analysis**

Mean weights, lengths, and head circumferences throughout the study and the gains in these measures from 8 to 12 wk were not significantly different between the high-selenium and low-selenium groups (data not shown). The mean (± SEM) weights at hospital discharge were also not significantly different between the groups (2077 ± 77 and 2151 ± 30 g, respectively).

**Formula intake**

Most infants received enteral feedings by 5–10 d of age and were started on diluted concentrations of Pregestimil, a hypoallergenic iron-fortified protein hydrolysate formula containing medium-chain triacylglycerols (Mead Johnson Nutritional, Evansville, IN); advanced to commercially labeled 2789.9-kJ/L (20-kcal/fluid oz) nonstudy preterm formula; and switched to the assigned study formula when they achieved full feeds. Some of the larger infants were started initially on nonstudy preterm formula and were then advanced to the assigned study formula. By week 4, all infants in the study were consuming study formula exclusively. Formula and energy intakes between groups were not different throughout the study period.

**Selenium intake**

Infants in the high-selenium group had significantly greater mean selenium intakes than did infants in the low-selenium group at weeks 2, 3, 4, 8, and 12 (P < 0.01). Mean selenium intakes from week 2 to 12 ranged from 4.86 to 12.77 μg Se/d (0.06–0.159 μmol · kg⁻¹ · d⁻¹) in the high-selenium group and from 1.15 to 6.63 μg Se/d (0.014–0.08 μmol · kg⁻¹ · d⁻¹) in the low-selenium group. The mean adjusted selenium intakes from week 2 to 12 ranged from 3.24 to 4.75 μg Se · kg⁻¹ · d⁻¹ (0.04–0.06 μmol · kg⁻¹ · d⁻¹) in the high-selenium group and from 1.38 to 1.82 μg Se · kg⁻¹ · d⁻¹ (0.017–0.23 μmol · kg⁻¹ · d⁻¹) in the low-selenium group. The mean selenium intake (μg · kg⁻¹ · d⁻¹) decreased at week 12 in both groups because although total intake (μg/d) remained relatively constant, body weight increased significantly.

**Formula tolerance and stool patterns**

There were no differences in formula tolerance between the two groups as determined by episodes of spitting or vomiting. The mean number of daily stools for both groups was similar and ranged from 1.0 to 2.7 stools/d at weeks 8 and 12. All infants were assessed by one of the investigators (EET) for ability to tolerate the study formulas at study exit.

**Blood and urine chemistry indexes**

Plasma selenium concentrations were 0.38 and 0.48 μmol/L (29.8 and 38.2 μg/L) on study day 1 in the high-selenium and low-selenium groups, respectively, and were not significantly different (Figure 1). Plasma selenium concentrations decreased significantly over the study period in the low-selenium group (P < 0.05). Plasma selenium concentrations of the high-selenium group were significantly higher at weeks 4 and 8 compared with those in the low-selenium group. Plasma glutathione peroxidase was significantly greater in the high-selenium group at week 12 only (P < 0.05) (Figure 1). RBC selenium concentrations (expressed as μg Se/g hemoglobin) decreased over time in both groups but were significantly greater in the high-selenium group at weeks 4, 8, and 12 compared with the low-selenium group (P < 0.05) (Figure 1).

Plasma selenium concentrations were significantly correlated with plasma glutathione peroxidase for infants in both groups on study day 1 and at weeks 4 and 12 (r = 0.66, r = 0.69, r = 0.54, P < 0.05, respectively). Selenium intake among all infants was significantly correlated with plasma glutathione peroxidase activity at 12 wk (expressed per g protein) (r = 0.74, P < 0.002) (Figure 2).

Urine selenium concentrations were significantly greater in the high-selenium group compared with the low-selenium group (P < 0.01) at 4 and 12 wk (insufficient data were available at 8 wk). Urine selenium concentrations were 132 and 114 μmol Se/mol creatinine (128 and 91 μg Se/g creatinine) on study day 1 in the high- and low-selenium groups, respectively. Urinary selenium concentrations were 197 and 32 μmol/mol creatinine (201 and 27 μg/g creatinine) at 12 wk in the high- and low-selenium groups, respectively.

**DISCUSSION**

The purpose of this study was to compare the selenium status of preterm infants who were fed selenate-fortified formulas with those fed non-selenium-fortified formulas over a 12-wk period of enteral feedings. The results of this study confirmed that preterm infants fed selenate-fortified formula had improved selenium status compared with infants who received non-fortified formula.

Total liver selenium content has been shown to be lower in preterm than in full-term infants (11). Therefore, preterm infants born with smaller selenium stores may be at risk for deficiency states or have suboptimal selenium status if adequate selenium is not provided in the diet. At birth, preterm infants typically have lower plasma selenium concentrations and lower plasma glutathione peroxidase activity than do full-term infants when comparisons are made under similar study conditions.
conditions (12). Comparisons of data from different centers are
difficult, however, because of geographic differences in the
selenium content of soil and therefore of maternal dietary
intake and because of differences in the analytical methods
used to measure selenium and glutathione peroxidase activity.
Mean plasma selenium concentrations of preterm infants
within the early days of life have been reported to range from
as low as 0.34–0.54 μmol Se/L (27–43 μg Se/L) (20, 21) to as
high as 1.10–1.57 μmol Se/L (87–124 μg Se/L) (22, 23).

In our study, mean plasma selenium concentrations on study
day 1 were 0.38 μmol Se/L (29.8 μg Se/L) and 0.48 μmol Se/L
(38.2 μg Se/L) for the high-selenium and low-selenium groups,
respectively, suggesting that the selenium status of infants
in the present study was low compared with that of infants in
other US studies (22, 24). The infants in our study appear to

FIGURE 1. Plasma selenium concentrations, red blood cell (RBC)
selenium concentrations, and plasma glutathione peroxidase activity
of preterm infants fed high-selenium (○; n = 7) and low-selenium (□; n =
10) infant formulas on study day 1 and at 4, 8, and 12 wk. ± SEM, Hb,
hemoglobin. * and ** Group means are significantly different: * P < 0.05,
** P < 0.01.

FIGURE 2. Correlation of selenium intake with plasma glutathione
peroxidase activity at 12 wk. High-selenium group, n = 5; low-selenium
group, n = 10. r = 0.74; P < 0.002.

resemble more closely infants from New Zealand (20) and
Ireland (21), areas of the world with endemic low soil concen-
trations of selenium. The apparent low concentrations seen in
our infants compared with other US-based populations is un-
explained and merits further investigation. The age of the
infants on study day 1, when the first blood sample was
obtained, was greater (8 ± 2 d and 11 ± 1 d for the high- and
the low-selenium groups, respectively) than that of infants in
other studies (20, 21, 22, 24). A significant decline in plasma
or RBC selenium concentrations may have occurred in these
infants over the first week of life because non-selenium-forti-
fied parenteral and enteral feedings were not given.

Lombeck et al (25) from Germany showed decreasing serum
centrationsof selenium over the first 4 mo of life in full-
term infants fed low-selenium commercial infant formulas. The
median serum cord blood concentration of selenium in this
group was 0.63 μmol/L (50 μg Se/L) and the estimated mean
selenium intake over the early months of life was 3.5 μg/d.
Rudolph and Wong (26) reported cord plasma concentrations
of selenium in full-term US infants of 1.78 μmol/L (140 μg/L),
considerably higher than the value reported by their German
counterparts.

Plasma selenium concentrations < 0.17 μmol Se/L (13.4 μg
Se/L) have been associated with Keshan disease, an endemic,
selenium-responsive disease in China (27) that is associated
with myocarditis. Vinton et al (28) described a selenium defi-
ciency state in four children receiving long-term, non-
selenium-supplemented parenteral nutrition for an average of
26 mo. With a mean serum selenium concentration of 0.48
μmol Se/L (38 μg/L) at the time of diagnosis, a selenium-
responsive syndrome of erythrocyte macrocytosis, loss of pig-
mentation of hair and skin, elevated transaminase and creatine
kinase, and profound muscle weakness was described. One
infant in the low-selenium group in our study had a plasma
selenium concentration of 0.09 μmol Se/L (7 μg Se/L) on
study day 1 and no subsequent concentration that exceeded
0.32 μmol Se/L (25 μg Se/L). Additionally, all 10 infants in
the low-selenium group had at least one plasma concentration
< 0.32 μmol Se/L (25 μg Se/L) at sometime during the study,
whereas only two infants in the high-selenium group had a
single concentration in this category. Note, however, that no
clinical symptoms of selenium deficiency were evident in the
study infants at any time. Many authors have suggested that
selenium deficiency and associated decreased concentrations of glutathione peroxidase, an important antioxidant, may be a predisposing factor to the development of bronchopulmonary dysplasia and other forms of oxidative damage in preterm infants (12, 22, 29).

We observed significant differences in plasma and RBC selenium concentrations between the high- and low-selenium groups 4 wk after the initiation of study formula. The differences in plasma selenium concentrations between the two groups occurred not because there was an increase in concentrations in the supplemented group but because infants in the low-selenium group showed decreasing plasma selenium concentrations over the 12-wk study period. Although RBC concentrations of selenium declined in both groups, there was less of a decline in the high-selenium group. Sluis et al (20) reported dramatic declines in plasma selenium concentrations over the first 2 mo of life in non-selenium-supplemented preterm infants fed either breast milk or preterm infant formula, which is similar to our results for the low-selenium group. Smith et al (30) could not show any differences or changes in selenium status in preterm infants fed either a non-selenium-fortified or selenite-fortified (0.44 μmol Se/L, or 34.8 μg Se/L) preterm infant formula over a relatively short, 3-wk study period. These infants also had received parenteral fluids supplemented with 0.04 μmol Se·kg⁻¹·d⁻¹ (3 μg Se·kg⁻¹·d⁻¹) before enteral feedings, which may have influenced the results of the study as well.

In adults, correlations between plasma selenium and glutathione peroxidase activity and between RBC selenium and glutathione peroxidase activity occur when selenium retention results in enzyme saturation that is below the maximal point of the capacity of the tissues to synthesize glutathione peroxidase. We did not see any significant differences between the high- and the low-selenium groups regarding plasma glutathione peroxidase activity until the 12th week of supplementation. This may suggest that insufficient retention had occurred even in the high-selenium group as a result of either the low selenium status of infants at birth or the low selenium intakes early in the study. Measurements of plasma glutathione peroxidase activities beyond the 12th week would have been required to determine when enzyme saturation was reached. Plasma glutathione peroxidase activity was unlikely to be saturated at 12 wk because plasma selenium concentrations were still low. A minimum enteral intake of 0.016–0.038 μmol Se·kg⁻¹·d⁻¹ (1.3–3.0 μg Se·kg⁻¹·d⁻¹) has been recommended for preterm infants (31). The results of this study suggest that the current recommendations for selenium intake may be too low for meeting the nutritional needs of some preterm infants because infants in the high-selenium group had selenium intakes ranging from 0.04 to 0.06 μmol·kg⁻¹·d⁻¹ (3.24 to 4.75 μg·kg⁻¹·d⁻¹).

The greater urinary selenium concentrations observed in the high-selenium group compared with the low-selenium group suggest that the selenate added to the formulas was absorbed. Retention of organic selenomethionine, the primary form of selenium found in human milk, is significantly greater in body tissue than that of either inorganic selenite or selenate (13, 32–34). Selenomethionine may be incorporated into body proteins as methionine and has the potential to accumulate in significant amounts, particularly in rapidly growing infants. Although this may be desirable if selenium stores are depleted, long-term supplementation with selenomethionine could alter the function of some proteins. It is for this reason that selenomethionine is not considered to be the preferred form of selenium for infant formula supplementation. Selenate, the form of selenium used to fortify formulas in our study, is chemically more stable than selenite and has been shown to have increased absorption and retention compared with selenite (32, 34). Studies in full-term infants fed a selenate-fortified soy formula (10) showed higher plasma and erythrocyte glutathione peroxidase activity after 16 wk compared with that in full-term infants fed either a selenite-fortified soy formula or human milk (35). This suggests that selenate may be more available for synthesis of glutathione peroxidase than either selenite or selenomethionine (36).

Our study confirms that selenate is a safe and biologically available form of selenium that may be used to supplement infant formula. Current selenium content of infant formulas and recommendations for dietary intake of selenium for preterm infants may be inadequate for meeting the nutritional needs of some populations of these infants.

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