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Geographical, seasonal and formula-specific variations in the selenium levels of infant formulae

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Abstract

Fortification of infant formulae with selenium (Se) is currently under consideration by manufacturers and regulatory bodies. However, information on the endogenous Se levels of infant formulae is largely lacking. The objective of this study was to describe the seasonal-, geographic- and formula-specific variations in endogenous Se levels of infant formulae.

For most milk-based infant formulae and follow-on formulae, endogenous Se levels were from 4 to 10 μg/l and 5 to 14 μg/l, respectively. Higher Se levels were found for milk-based formulae from the USA (10–15 μg/l) and India (11–22 g/l). The lowest endogenous Se levels (2.5–4 μg/l) were observed for soy-based formulae. No differences were found between whey-adapted, casein-predominant or protein-hydrolysate formulae.

The protein source was the major determinant (>95%) of the endogenous formula Se content. The seasonal variations observed in the Se contents of formulae were due to corresponding variations in Se contents of the protein sources.

The present study indicated that the endogenous Se level of infant formulae is generally lower than that reported for human milk, and provides information that will need to be considered concerning the need for, and the level of, Se fortification.

Keywords: Selenium; Endogenous level; Variation; Infant formula; Follow-on formula

1. Introduction

Selenium (Se) is an essential trace element for man and its biological functions are carried out by selenoproteins, several of which are parts of oxidant defence enzymes (Institute of Medicine, 2000). Low Se status has been associated with a number of diseases (Institute of Medicine, 2000), and its role in the emergence of viral diseases (Beck, 1996) and in protection against certain types of cancer (Clark, Combs, & Turnbull, 1996) has recently added evidence to the importance of Se in human nutrition.

Se status is determined by its dietary intake and its bioavailability, which depends on the chemical form of dietary Se (Fairweather-Tait, 1997). Infants are reported to be at risk of poor Se status, due to the low levels of Se in human milk and infant formulae, their sole or major food source during early life (Litov & Combs, 1991). The Se content of infant formulae is reported to be lower than that of human milk (Foster & Sumar, 1996; Lombeck, Kasperek, Bonnermann, Feinendegen, & Bremer, 1978; Roekens, Robberecht, Van Caillie-Bertrand, Deelstra, & Clara, 1985; Sanz Alaejos & Diaz Romero, 1995; Zabel, Harland, Gormican, & Ganther, 1978). Breast-fed infants were also shown to have higher Se status than formula-fed infants, reflecting not only differences in dietary Se intake, but also, and more likely, better utilization of Se from human milk (Kumpulainen et al., 1987).

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Although overt signs of Se deficiency have not been observed in healthy term breast- and formula-fed infants at the lower intake levels, low dietary Se intake levels may be of concern during early life (Litov & Combs, 1991). Therefore, Se supplementation of lactating mothers and Se fortification of infant formulae were investigated and shown to effectively improve the Se status of breast- and formula-fed infants (Darlow et al., 1995; Johnson, Smith, Chan, & Moxeur-Mileur, 1993; Kumpulainen et al., 1987; McGuire et al., 1993). Recent data indicated that both selenite and selenate were equally well utilized by infants when added to a milk-based infant formula (Van Dael, Davidson, Ziegler, Fay, & Barclay, 2002).

Data on the endogenous Se levels in formulae are indispensable for evaluating the nutritional relevance of Se fortification of formulae. In the present study, we therefore investigated the variations of the endogenous Se levels of infant formulae differing in geographical origin and formulation. The survey comprised analyses of infant and follow-on formulae over a one-year period, which also allowed the description of longitudinal variations in formula Se content. Major infant formula ingredients were analysed in order to determine the sources of endogenous formula Se.

2. Materials and methods

2.1. Samples and materials

Samples of infant and follow-on formulae, differing in composition, were collected, monthly, in duplicate for one year, from major Nestlé infant formula production units in Europe (France, Germany, Spain and the Netherlands), the Americas (Brazil, Mexico and USA), Asia (China, India), Oceania (Australia) and Africa (S. Africa) (Table 1). Major protein and carbohydrate ingredients used in the selected formulae were also sampled (Table 2). All samples were sent, either in their original packaging or packed under vacuum, to the Nestlé Research Centre (Lausanne, Switzerland) for Se analysis. Samples were stored in the absence of light at ambient temperature.

Table 1
Infant formulas selected for Se analysis

Origin	Starter formula[a]	Follow-on formula
Africa		
S. Africa	X (CP)	X
Americas		
USA	X (PH)	X
Mexico	X (WA)	
Brazil	X (WA)	X
Asia		
India	X (CP)	X
China	X (CP)	X
Europe		
Germany	X (CP; PH)	X
France	X (CP)	X
Spain	X (WA)	X
The Netherlands	X (WA; SB)	
Oceania		
Australia	X (CP; WA)	

[a] CP: casein-predominant; WA: whey-adapted; SB: soy-based; PH: protein hydrolysate.

All chemicals used were of analytical reagent grade (Merck, Darmstadt, Germany) unless otherwise stated. Nitric and hydrochloric acid were further purified by sub-boiling in an all quartz distillation apparatus (Hans Kürner, Rosenheim, Germany). All glassware was acid-washed by soaking overnight in 2 M nitric acid. All dilutions were made with ultra pure water with a resistivity of $>18\ M\Omega\ cm^{-1}$ obtained from a Millipore Super-Q apparatus (Millipore, Milford, MA, USA).

Standard calibration solutions were prepared daily from a 1000 mg/l Se standard stock solution (Merck, Darmstadt, Germany) in 1.0 M hydrochloric acid. The reductant, a 0.2% w/v sodium tetrahydroborate solution, was prepared freshly before analysis by dissolving $NaBH_4$ (>97%, Fluka) in 0.5% w/v NaOH.

2.2. Se analysis

Selenium was determined by continuous flow hydride generation atomic spectrometry (HGAAS), using a Varian AA-400 instrument (Mulgrave, Australia) equipped with a PSC-56 autosampler and a VGA-57 hydride generation system, as reported previously (Van Dael, Van Cauwenbergh, Deelstra, & Calomme, 1995); the samples were acid digested with nitric/perchloric acid followed by a hydrochloric acid reduction prior to Se analysis. The accuracy of the Se analysis was verified against NIST 1549 Non-Fat Milk Powder (NIST, Gaithersburg, MD, USA) standard reference material.

3. Results and discussion

3.1. Accuracy and repeatability of Se analysis

The accuracy of the determination method was assessed during every run by analysing NIST 1549 Non-Fat Milk Powder ($n = 4$). The results obtained were always within the certified range of 0.110 ± 0.010 μg/kg of Se with an average of 0.107 ± 0.006 μg/kg ($n = 70$). The detection and determination limits of the HGAAS method were calculated at 0.19 and 0.37 μg/ml of Se, respectively. Under the experimental conditions (sample size: 500 mg powder), this corresponded to 3.8 and 7.4 μg/kg of Se for detection and determination limits, respectively. Similarly, for liquid formulae, the corresponding detection and determination limits were

Table 2
Se content of main protein and carbohydrate formula raw materials[a]

Origin	Ingredient	Se content (μg/kg dry matter)	
		Average	Range
Americas			
USA	Milk powder	254 ± 27 (n = 12)	212–248
	Whey protein concentrate	242 ± 19 (n = 12)	212–283
	Lactose powder	<4 (n = 4)	–
	Maltodextrin powder	<4 (n = 4)	–
Canada	Demineralised whey powder	55 ± 3 (n = 11)	51–60
Brazil	Milk powder	81 ± 11 (n = 12)	65–95
Europe			
France	Demineralised whey powder	33 ± 5 (n = 18)	26–42
The Netherlands	Milk powder	114 ± 15 (n = 10)	95–133
	Casein concentrate	386 ± 47(n = 11)	332–473
	Lactose powder	<4 (n = 5)	–
	Maltodextrin powder	<4 (n = 5)	–
Oceania			
Australia	Milk powder	99 ± 22 (n = 10)	77.4–135

[a] Number of samples between brackets.

0.95 and 1.8 μg/l, respectively (sample volume: 2 ml). The within- and between-run repeatabilities of the method, as determined for the reference material, were <3.5% and <7%, respectively. Thus, the analytical characteristics of the HGAAS analysis method selected allowed accurate and precise determination of formula Se content.

3.2. Selenium content of formula ingredients

The Se level of main protein and carbohydrate ingredients used in the formulae is shown in Table 2. The Se levels of lactose and maltodextrin powders were below the detection limit (<3.8 μg/kg), which indicates that the main carbohydrate formula ingredients do not contribute to the formula endogenous Se content.

The Se contents of milk and demineralised whey powders from North America were higher than those from Europe, South America or Oceania, which confirmed earlier reported geographical differences in milk Se (Sanz Alaejos & Diaz Romero, 1995). Higher Se levels have been associated with higher soil Se content, as well as Se supplementation of dairy cattle feed in North America. The Se content of protein ingredients also displayed considerable seasonal variation (Fig. 1), with higher Se levels found during the winter season, probably due to different feeding patterns of dairy cattle during winter (Koops, Klomp, & Westerbeek, 1989; Van Dael, Vlaemynck, Van Renterghem, & Deelstra, 1991).

Comparison between the measured endogenous Se levels and those calculated from the Se contents of the major formula ingredients indicated that protein sources contributed more than 93% of total endogenous formula Se (Table 3). Protein ingredients are thus the main, if not the sole, source of endogenous Se in infant formulae.

3.3. Selenium contents of formulae

The endogenous levels of Se in infant formulae, and follow-on formulae, differing in geographical origins and formulation, are presented in Tables 4 and 5. The Se levels were expressed as μg/l of reconstituted formula or ready-to-feed (RTF), calculated using the formula reconstitution guidelines on the labels. We assumed negligible contribution of water used for reconstitution, since drinking water Se levels are, in general, very low (<1 μg/l), although they can reach 5–10 μg/l in high Se regions (Robberecht & Van Grieken, 1983).

The mean endogenous Se levels of infant formulae varied between 3.4 and 13.6 μg/l. The lowest mean Se level (3.4 μg/l) corresponded to a soy-based formula from the Netherlands, confirming that soy formulae may be a cause for concern in relation to Se intake (Foster & Sumar, 1996). The highest mean Se level (13.6 g/l) was found for a casein-predominant infant formula from India.

The average endogenous Se levels of follow-on formulae varied between 6.4 and 17.8 μg/l, somewhat higher than those of infant formulae, due to their higher protein contents.

All Se levels were within the range of previously reported data, although observed variations were smaller than those reported previously (Foster & Sumar, 1996; Lombeck et al., 1978; Roekens et al., 1985; Sanz Alaejos & Diaz Romero, 1995; Zabel et al., 1978). No endogenous formula Se levels above 20 μg/l were found.

No difference in endogenous Se level was observed between milk-based formulae, i.e., casein-predominant, whey-adapted and protein-hydrolysate formulae. This finding is in agreement with the data of Van Dael et al. (1991) reporting that the Se content of casein

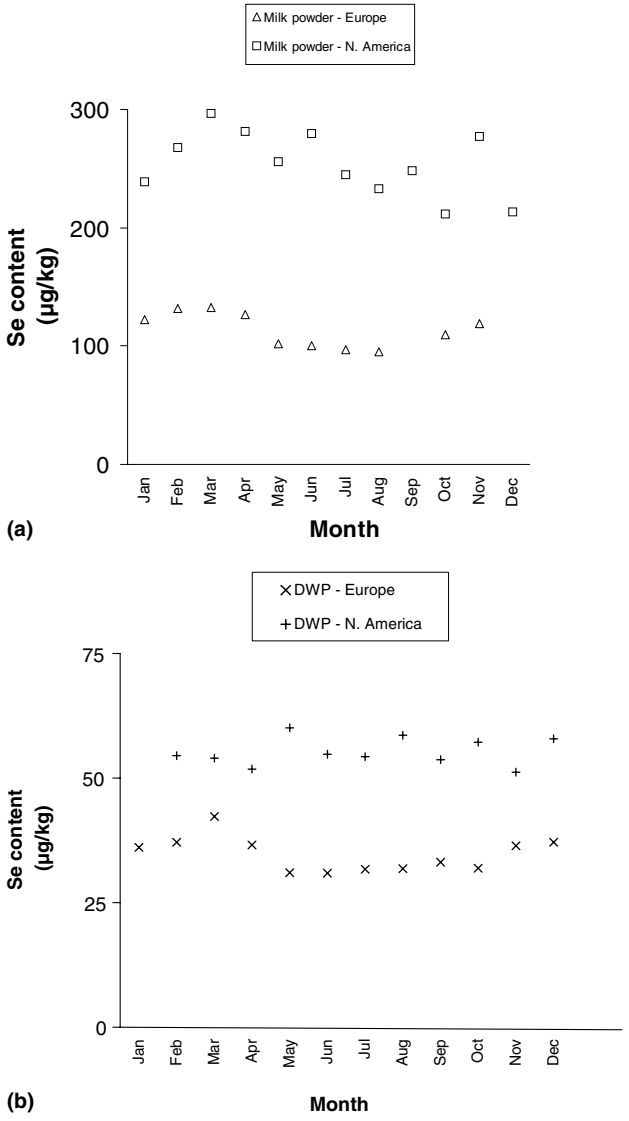

Fig. 1. Geographical and seasonal variation in Se contents of milk powder (a) and demineralised whey powder (DWP) (b) of North-American and European origin.

Table 3
Comparison of calculated and determined Se content of infant formulae[a]

Type of formula	Formula Se level (µg/kg powder)	
	Determined	Calculated[a]
Whey-adapted		
	39.3 ± 1.9	36.4 (93%)
	44.6 ± 1.3	45.5 (102%)
Casein predominant		
	43.9 ± 2.0	42.4 (97%)
	39.4 ± 1.2	38.6 (98%)
Follow-on		
	50.2 ± 2.4	49.2 (98%)
	88.6 ± 2.7	90.0 (102%)

[a] The % of calculated vs. determined is given between brackets.

Table 4
Se content of infant formulae[a]

Origin	Type of formula	Formula Se level (µg/l RTF)	
		Average	Range
Africa			
S. Africa	Casein predominant	5.3 ± 0.9 (n = 7)	4.3–6.7
	Whey-adapted	4.7 ± 0.5 (n = 7)	3.5–5.1
Americas			
USA	Protein hydrolysate	11.6 ± 0.7 (n = 12)	10.4–12.7
Mexico	Whey-adapted	6.7 ± 1.6 (n = 12)	4.5–9.9
Brazil	Casein predominant	7.0 ± 1.1 (n = 12)	5.5–8.8
	Whey-adapted	5.0 ± 0.9 (n = 14)	3.8–6.9
Asia			
India	Casein predominant	13.6 ± 2.0 (n = 11)	10.8–15.9
China	Casein predominant	4.5 ± 0.8 (n = 13)	3.8–6.8
Europe			
Germany	Casein predominant	6.8 ± 0.9 (n = 12)	5.6–8.0
	Protein hydrolysate	5.5 ± 0.5 (n = 8)	4.3–6.2
France	Casein predominant	5.1 ± 0.7 (n = 10)	4.2–6.2
Spain	Whey-adapted	6.5 ± 0.5 (n = 13)	5.6–7.2
The Netherlands	Whey-adapted	5.8 ± 0.9 (n = 13)	4.3–7.0
	Soy-based	3.4 ± 0.4 (n = 13)	2.7–4.0
Oceania			
Australia	Casein predominant	5.8 ± 0.9 (n = 10)	4.5–7.0
	Whey-adapted	5.8 ± 0.8 (n = 10)	4.8–7.0

[a] Se levels are expressed as µg/l ready-to-feed (RTF). Number of samples between brackets.

and whey protein isolates, expressed in relation to protein level, are similar.

The formula Se content showed geographical and seasonal differences, in agreement with corresponding variations in Se content of protein ingredients. Our data also confirmed earlier findings showing that formula Se content is generally lower than that of human milk, taking into consideration, however, that the latter depends

Table 5
Se content of follow-on formulae[a]

Origin	Formula Se level (µg/l RTF)	
	Average	Range
Africa		
S. Africa	11.8 ± 0.9 (n = 7)	10.6–13.1
Americas		
USA	12.5 ± 1.4 (n = 11)	9.5–14.5
Mexico	9.1 ± 4.9 (n = 12)	5.3–18.2
Brazil	6.4 ± 1.0 (n = 14)	4.8–8.1
Asia		
India	17.8 ± 3.0 (n = 11)	14.6–31.4
China	7.4 ± 1.4 (n = 13)	4.7–11.1
Europe		
Germany	10.3 ± 1.9 (n = 10)	7.2–13.0
France	6.4 ± 1.1 (n = 10)	5.2–7.9
Spain	6.7 ± 0.5 (n = 13)	5.5–7.6

[a] Se levels are expressed as µg/l ready-to-feed (RTF). Number of samples between brackets.

on the geographical origin and the stage of lactation, as shown in Table 6.

The endogenous Se levels of milk-based infant formulae from the USA (11.6 μg/l) and India (13.6 μg/l) were higher than those of formulae from other geographical origins (4.5–7.0 μg/l). Similar differences were also found for follow-on formulae. These geographical differences in endogenous Se formula content can be associated with the variations in Se content of protein ingredients described above.

Similarly, the observed longitudinal variations in the Se content of most formulae, over the one-year survey period, are most likely due to the seasonal fluctuations in protein ingredient Se contents as described above.

The Se content of infant formulae was found to be independent of the form of commercialisation, namely powder, RTF or concentrated liquid. Indeed our data showed comparable Se contents for reconstituted powder (11.6 ± 0.7 μg/l), reconstituted formula concentrate (11.2 ± 0.6 μg/l) and RTF protein-hydrolysate formulae (11.3 ± 0.4 μg/l) produced in the USA. Likewise, the Se levels in different follow-on formulae commercialised in the USA were similar: reconstituted powder, 12.5 ± 1.4 μg/l and RTF formulae, 12.9 ± 2.0 μg /l.

As mentioned above, Se bioavailability depends on the chemical form of dietary Se. Previous studies demonstrated that cow's milk Se was mainly associated with milk proteins (Debski, Picciano, & Milner, 1987; Van Dael et al., 1991), which is in agreement with the findings of the present study. However, neither of the studies did evaluate the chemical form of Se associated with the milk proteins. Although human milk Se was also mainly associated with proteins, Debski et al. (1987) demonstrated that the Se distribution was considerably different between human milk and cow's milk. These differences in Se distribution and thus dietary forms of Se were suggested to be likely associated with the better utilisation of human milk Se and thus the higher Se status of breast-fed infants than of formula-fed infants (Milner, Sherman, & Picciano, 1987).

Estimations for Se requirements during infancy have taken into consideration differences in both dietary Se intake of breast- and formula-fed infants and differences in bioavailability (Institute of Medicine, 2000; World Health Organization, 1996). Based on our findings and assuming an average formula consumption of 750 ml/day during the first four months of life, the average Se intake of formula-fed infants for most formulae would be between 3.8 and 5.3 μg/day. Infants fed non-fortified soy-based formula have the lowest average Se dietary intake, at 2.5 μg/day. The highest calculated Se intake values were for infants fed US protein hydrolysate and Indian casein-predominant formula (8.7 and 10.2 g/day, respectively). Se intake from follow-on formulae is likely to vary in a manner similar to that for infant formulae. However, since the contribution of follow-on formulae to total daily food intake shows substantial geographical variation, its contribution to the

Table 6
Human milk Se content in function of geographical location and lactational stage (mean ± SD)

Country	Se level (μg/l)	Lactation stage	Reference
Europe			
Austria	8.3 ± 3.0	Mature milk	Tiran et al. (1993)
Belgium	15.3 ± 5.1	Colostrum	Deschuytere et al. (1987)
	12.7 ± 4.5	Transitional milk	
	9.7 ± 2.1	Mature milk	
Finland	16.4 ± 3.2	Mature milk (1987)	Kantola and Vartiainen (2001)[a]
	18.9 ± 3.0	Mature milk (1995–7)	
Poland	25.5 ± 16.6	Colostrum	Trafikowska et al. (1997)
	11.0 ± 3.3	Transitional milk	
	9.1 ± 3.0	Mature milk	
Americas			
USA	15 ± 1	Mature milk	Moser et al. (1987)
Oceania			
Australia	11.9 ± 3.5	Mature milk	Cumming et al. (1991)
Asia			
Japan	29.7 ± 10.5	Colostrum	Yuzo and Mohri (1991)
	18.9 ± 9.4	Transitional milk	
	10.8 ± 2.7	Mature milk	
Nepal	10.1 ± 1.0	Mature milk	Moser et al. (1987)

[a] Supplementation of all fertilizers with Se-selenate was implemented in 1984 in Finland.

daily intake is less easy to estimate. Our data indicate that formula-fed infants have a lower dietary intake than those reported for breast-fed infants (Foster & Sumar, 1996; Lombeck et al., 1978; Roekens et al., 1985; Sanz Alaejos & Diaz Romero, 1995; Zabel et al., 1978). The low Se levels of a number of the formulae analysed may correspond to Se intakes below the current estimated requirements during the first months of life (Institute of Medicine, 2000; World Health Organization, 1996). Indeed more than three quarters of the formulae analysed do not cover the daily Se requirement of 6 μg (World Health Organization, 1996). The US Institute of Medicine estimated the adequate intake for Se at 15 μg/day during the first six months of life (2000), a level that cannot be covered by these infant formulae. Although our data, in agreement with previous findings, clearly confirm the lower Se intake of formula-fed infants compared to breast-fed infants, overt clinical signs of Se deficiency have never been reported for healthy term formula- and breast-fed infants (Institute of Medicine, 2000).

In conclusion, the present study indicates that the endogenous Se levels of infant formulae are lower than that of human milk, and may not cover the requirements of growing infants. Thus Se fortification of infant and follow-on formulae may need to be considered as a means to provide adequate Se nutrition during infancy.

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