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Retinol and α-tocopherol in infant formulas produced in the EEC

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Abstract

The level of compliance in preterm and term infant formulas between levels of vitamin A (retinol), vitamin E (α-tocopherol), and the vitamin E/long chain polyunsaturated fatty acids (LCPUFA) ratio with the label declarations and the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) recommendations was studied in 30 commercially available infant formulas in Spain, France, Germany and England. The mean values ± standard deviation were 89.5 ± 34.5 μg/100 kcal for analysed vitamin A (VA), 2.01 ± 1.17 mg/100 kcal for vitamin E (VE) and 2.44 ± 1.46 mg VE/g PUFA. VE levels were higher ($p < 0.05$) in preterm infant formulas (3.24 ± 0.48 μg/100 kcal) than in term infant formulas (2.13 ± 0.29 μg/100 kcal). These differences were not found in VA levels. When the mean VE levels of formulas produced by the various firms were compared on the basis of percentage of label declaration, significant differences ($p < 0.05$) were found. Analysed VA ranged from 50 to 236% of the label declaration concentration. Mean analysed VE levels ranged from 68 to 256% of declared levels in infant formulas. While 85% of the formulas showed analysed vitamin E levels above the label declaration, 60% of analysed vitamin A levels were lower. Twenty per cent of infant formulas presented VA levels lower than recommendations. All infant formulas observed ESPGAN recommendations on their labels except one, in which the labelled VE concentration was lower.

Keywords: Vitamin A; Vitamin E; Infant formula

1. Introduction

Infant formulas are the most highly regulated consumer food products on the market today because they are often the only source of nutrients for preterm and term infants. In Europe, nutrient levels in infant formulas are primarily based on the recommendations of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN). Since human milk contains the pattern of nutrients best suited to meeting the physiological requirements of the infant, many of the recommendations are based on the composition of mature milk.

Currently, it is recommended that preterm infant formulas should contain LCPUFA (BNF, 1992; EEC, 1996; ESPGAN, 1991; FAO/WHO, 1994; ISSFAL, 1994). Although the recommendation to add LCPUFA is only for preterm infant formulas, some studies indicate that term infants may require these compounds in their diet (Jorgensen, Hernell, Lund, Holmer, & Michaelsen, 1996; Makrides, Neumann, Simmer, & Gibson, 1995) and some manufacturers produce term infant formulas with LCPUFA and added vitamin E (VE).

Fatty acid unsaturation exponentially increases the rate of oxidation and, although the amount of LCPUFA in the supplemented formulas is low, the formula needs substantial antioxidant protection. ESPGAN recommend a minimum of 0.9 mg vitamin E/g PUFA for all formulas, which is higher than the figure recommended by the American Academy of Paediatrics for term infants of 0.7 mg (AAP, 1985) and the Recommended Dietary Allowances (RDA) of the National Research Council of 0.5 mg (NRC, 1989). Some studies suggest that the serum vitamin E levels are normal in preterm infants fed on LCPUFA infant formula only when an additional vitamin E supplement is included (Bendich & Brock, 1997; Koletzko, Decsi, & Sawatzki, 1995; Uauy et al., 1994). As a result, some of the available infant formulas have increased their vitamin E concentration. This enrichment of LCPUFA and vitamin

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E may indirectly affect the bioavailability of other micronutrients in infant formulas such as retinol (VA) (Nair et al., 1993; Willet, Stampfer, Underwood, Taylor, & Hennekens, 1983; Wolmarans, Labadarios, Benade, Kotze, & Louw, 1993), which are essential for growth and directly related to the visual capacity. The manufacturer may add extra vitamin E to the infant formula indicating the increase on the label, and offering an adequate vitamin A level, but the real concentration of these vitamins after storage and manufacture needs to be checked in order to ensure correct intake and the accuracy of the label statements.

This paper presents data on vitamins A and E levels of a broad spectrum of infant formulas produced in the CEE, and compares the real level with label declarations and international recommendations.

2. Materials and methods

2.1. Samples

Samples were collected as 23 commercial brands from Spain, two from France, three from Germany and two from England. Two samples of different lots of each brand were analysed. Between one and five different brands of each manufacturer were analysed. Samples from nine different manufacturers were collected. No samples had exceeded expiry date at the time of analysis. All the formulas (five preterm infant formulas and 25 term infant formulas for infants between 0 and 4–6 months of age) were manufactured in powder form. Analyses were initiated immediately after reconstitution and each sample was analysed in duplicate.

2.2. Analytical procedure

The lipid-soluble components were extracted from the aqueous phase by homogenising in a solvent mixture of dichloromethane–methanol (2:1 v/v) with sodium sulphate added to remove water, following a modified version of Folch et al.'s procedure (Folch, Less, & Stanley, 1951). The organic phase was evaporated and the residue dissolved in diethyl ether in order to eliminate non-lipid substances retained by methanol, and then filtered and evaporated. Fat was kept in a dark vial, flushed with nitrogen and stored at −20°C until analysis.

The vitamins were extracted by the method of Stancher and Zonta (1982) with modifications. To the 1 g of fat, 0.1 g ascorbic acid, 5 ml distilled water, 5 ml of methanol and 5 ml of potassium hydroxide solution (80%, p/v) were added. Flasks were flushed by nitrogen and the mixture was refluxed for 12–14 h at room temperature without light. The contents of the flask were quantitatively transferred to a 250 ml separator funnel. The flask was rinsed with 60 ml of methanol:water (20:40, v/v), and the rinses were added to the funnel. A 50-ml portion of diethyl ether was added to the separator funnel, and then shaken with occasional venting, and the layers were allowed to separate. The process was repeated twice. The three ether extracts were combined in the first separator funnel. Then, 50 ml of potassium hydroxide (0.5 M) was added to the ether extract and the aqueous layer was discarded. The ether extract was repeatedly washed with distilled water until the water was neutral to 1% phenolphthalein solution (no visible pink colour). The extracted ether was filtered through anhydrous sodium sulphate into a round-bottom flask, and the funnel was rinsed with an additional millilitre of diethyl ether, 0.05 ml of an ethanolic retinol acetate solution (0.5 mg/ml) was added to the extracted ether. The mixture was evaporated to dryness, first on a rotary evaporator and then on a nitrogen jet. The residue was immediately dissolved in 1 ml ethanol by vigorous shaking. Two extractions were performed for each sample and injected twice.

Foods such as milk or infant formula are most commonly fortified with vitamin A in the form of retinyl palmitate and vitamin E in the form of α-tocopherol acetate. Both of these esters are converted to free alcohols by alkaline digestion.

Retinol and α-tocopherol levels were determined by reverse-phase high-performance liquid chromatography (HP-1050, Hewlett–Packard, Palo Alto, CA) using a Spherisorb ODS-2 (250×4.6 mm i.d., 5 μm particle size) (Tracer, Barcelona) column protected by a guard cartridge (C18, 5 μm) system and maintained at 50°C. Isocratic elution was performed with methanol:water (95:5) at a flow rate of 1 ml/min. Ten microlitres of sample was injected. Detection was performed with an ultraviolet photodiode array detector (HP-1040 M, Hewlett–Packard, Palo Alto, CA) at 292 nm for vitamin E and at 325 nm for vitamin A.

The coefficient of variation (CV) for 10 replicates of the sample was 8.9% for retinol and 8% for α-tocopherol, according to the limit for intralaboratory variability analysis (Horwitz, 1982). The mean recovery for the method at three levels of vitamin concentration was 89.5% for retinol and 91.6% for α-tocopherol. The method was lineal for concentrations of retinol from 3.92 μg/ml to 90.7 μg/ml and concentrations of tocopherol from 21.7 to 502 μg/ml. The coefficients of correlation and determination were positive with a probability above 99.9% for both lineal regression equations.

Polyunsaturated fatty acid composition was performed by preparing fatty acid methyl esters with methanolic BF_3 and dissolving in hexane following the method of Morrison and Smith (1964). Two methyl esters were prepared for each sample and injected twice. Separation was performed on a Model 5890 A gas chromatograph (Hewlett–Packard, Palo Alto, CA)

equipped with a flame ionization detector and split-splitless injector. A 60 m×0.25 mm i.d. fused-silica capillary column SP-2380 (Supelco, Bellofonte, PA) was used. The conditions for the infant formula analysis were as follows: initial oven temperature was 90°C with a hold of 5 min and then a rise of 10°C/min to 185°C with a hold of 10 min, followed by a rise of 7°C/min, to 235°C with a hold of 20 min. Fatty acid quantification was calculated by internal normalization. The coefficients of variation (CV) for 10 replicates of the same sample ranged between 2.26 and 5.22%, according to the limit for intralaboratory variability analysis (Horwitz, 1982).

Statistical analyses were performed using Statgraphics Plus for Windows (release 1.4). MANOVA analysis was used to detect the influence of type of infant formula (LCPUFA-supplemented or not, preterm or term infant formula) and the manufacturer in VA and VE levels, VE/PUFA ratio and percentage deviation of vitamin levels analysed according to label declaration.

3. Results and discussion

Mean ± standard deviation of vitamin E contents of the 30 infant formula samples was 2.01 ± 1.17 mg/100 kcal, with range from 0.8 to 5.5 mg/100 kcal. Mean vitamin E/PUFA ratio was 2.44 ± 1.46 mg/g, ranging from 1.04 to 7.05 mg/g.

Only one infant formula stated lower vitamin E levels (0.5 mg/100 kcal) on its label than ESPGAN recommendations; all analysed VE levels followed VE recommendations, containing more than 0.6 mg/100 kcal of vitamin E and 0.9 mg/g of vitamin E/PUFA ratio. No infant formulas were above the maximum allowable vitamin E level of 10 mg/100 kcal (ESPGAN, 1991).

Vitamin E is the major physiological fat-soluble antioxidant. It is an important factor preventing oxidative damage of neonates after birth, when the neonate is suddenly exposed to higher oxygen levels than those in the intrauterine environment (Crawford, Ghebremeskel, & Phylactos, 1995).

Peroxidative damage of tissue due to reactive oxygen radicals plays a role in the pathogenesis of necrotizing enterocolitis and bronchopulmonary dysplasia of neonates (Saugstad, 1990) and may contribute to the pathogenesis of other reactive oxygen radical-induced diseases in immature babies with poorly developed antioxidant defence systems (Van Zoeren-Grobben, Moison, Ester, & Berger, 1993). Increased production of reactive oxygen radicals can occur in stored human milk due to the presence of macrophages (Speer, Gahr, & Pabst, 1986) and in stored infant formula feeds due to their iron content and high polyunsaturated fatty acids with insufficient antioxidant protection.

We found little basic information to give guidance for appropriate levels of fat-soluble vitamins in infant feeds. Nutritional Commitees based their recommendations on the vitamin A and E concentrations of human milk. The composition of fat-soluble vitamins changes as the lactation period progresses. Colostrum contains almost five times the vitamin E concentration of mature milk (Boersma, Offringa, Muskiet, Chase, & Simmons, 1991; Chappel, Francis, & Clandinin, 1985; Tamai et al., 1996).

As shown in Table 1, vitamin E levels were found to be higher in preterm formulas than in term formulas ($p < 0.01$). The vitamin levels of LCPUFA supplemented infant formulas did not differ from those of the unsupplemented infant formulas. Analysed vitamin E levels and vitamin E/PUFA ratio levels differed ($p < 0.05$ and $p < 0.01$, respectively) according to the manufacturer: manufacturer C showed the lowest levels and manufacturer D the highest. On comparing the labelled vitamin E of different manufacturer there were also differences ($p < 0.01$) (Table 2).

Cow's milk, traditionally the basis for infant formulas in industrialized countries, has a less than ideal composition for human babies. The fat composition of infant formulas is now based on vegetable oils, which are more similar to human milk fat (Gurr, 1997). In recent years, other fats, such as marine, egg or seaweed oils, have changed the fat composition of infant formulas which now include LCPUFA and resemble human milk more closely. Some studies report deterioration in visual acuity, not only in preterm infants, but also in term infants fed unfortified LCPUFA-formula (Makrides et al., 1995; Jorgensen et al., 1996). Therefore, some manufacturers produce term infant formulas with LCPUFA and additional vitamin E. On the whole, the vitamin E contents and vitamin E/PUFA ratios of infant formulas are now adequate, and a supplement of tocopherol is used as an ingredient by many manufacturers.

The mean percentage of analysed levels in relation to label declaration levels (100%) is shown in Table 3. The

Table 1
Vitamin A, vitamin E and the vitamin E/PUFA ratio of LCPUFA-supplemented formula (LCPUFA-F), unsupplemented-formula (F), preterm infant formula (PF) and term infant formula (TF)

	LCPUFA-F ($n=8$)[b]	F[a] ($n=22$)[b]	PF[a] ($n=5$)[b]	TF[a] ($n=25$)[b]
Vitamin A (μg/100 kcal)	120 ± 15.0	104 ± 14.3	126 ± 18.42	98.2 ± 11.25
Vitamin E (mg/100 kcal)	3.01 ± 0.39	2.36 ± 0.37	3.24 ± 0.48[a]	2.12 ± 0.29 [a]
Vitamin E/PUFA (mg/g)	3.39 ± 0.49	2.39 ± 0.47	2.99 ± 0.61	2.8 ± 0.37

[a] Pairs of values showing identical superscript indicate significant differences ($p < 0.01$).

[b] Mean ± standard deviation (n = number of samples).

Table 2
Vitamin A and vitamin E analysed on infant formulas produced by different manufacturers

			Vitamin A		Vitamin E	
Mfr	No. Brands	No. Samples	μg/100 kcal	% of label declaration	μg/100 kcal[a]	% of label declaration
A	5	10	131 ± 15.5	144 ± 15.53	2.48 ± 0.41[a]	125 ± 15.3
B	2	4	102 ± 24.7	96.5 ± 24.73	2.70 ± 0.64	103 ± 24.3
C	5	10	108 ± 15.1	106 ± 15.15	1.37 ± 0.401[b,c,d]	170 ± 14.9
D	4	8	81 ± 17.3	80.8 ± 17.33	4.00 ± 0.45[a,b,e,f,g]	122.61 ± 17.0
E	4	8	90.0 ± 17.3	98.1 ± 17.33	1.95 ± 0.45[e]	131 ± 17.0
F	3	6	122 ± 20.9	122 ± 20.87	2.38 ± 0.54[f]	142 ± 20.5
G	1	2	160 ± 33.7	132 ± 33.75	3.72 ± 0.88[e]	169 ± 33.2
H	3	6	111 ± 20.8	111 ± 20.87	3.03 ± 0.54[d]	142 ± 20.5
I	3	6	105 ± 20.9	92.7 ± 20.87	2.54 ± 0.54[g]	86.5 ± 20.5

[a] Mean ± standard deviation.
[b] Pairs of values in the same column showing identical superscript indicate significant differences ($p < 0.05$).

Table 3
Percentage of label declaration of analysed vitamin A and vitamin E in supplemented formula (LCPUFA-F), unsupplemented formula (F), preterm infant formula (PF) and term infant formula (TF)[a]

	LCPUFA-F	F	PF	TF
	($n = 8$)[b]	($n = 22$)[b]	($n = 5$)[b]	($n = 25$)[b]
Vitamin A	122 ± 15.0	97.0 ± 14.3	118 ± 18.4	100 ± 11.3
Vitamin E	105 ± 14.7*	160 ± 14.1*	151 ± 18.1	114 ± 11.1

[a] Mean ± standard deviation (n = number of samples).
[b] Pairs of values showing identical superscript indicate significant differences ($p < 0.05$).

mean percentage of analysed vitamin E levels in relation to the label declaration for the 30 samples was 133 ± 37.5%. Vitamin E percentage of the label declaration of preterm formulas was 152 ± 18.1% and 114 ± 11.1% for term infant formulas. Mean vitamin E levels ranged from 113% to 189% of those declared in premature infant formulas, and from 90.4% to 137.71% in infant formulas for term infants. Using percentage of label declaration for vitamin E, statistical differences appear between LCPUFA-supplemented infant formula (105 ± 14.7%) and unsupplemented infant formula (160 ± 14.1%) ($p < 0.05$), indicating greater accuracy in the manufacturing process of these modern infant formulas.

The level of vitamin E required in LCPUFA-supplemented infant formulas should ensure equivalent serum and tissue vitamin E levels in formula- and breast-fed infants. The high vitamin E concentration in colostrum results in a rapid increase in the blood concentration of tocopherol in breast-fed infants; this is not the case in term infants bottle-fed, with formula, 6.5 mg/l of tocopherol (Mino, Nakagawa, Tamai, & Miki, 1982; Paredes et al., 1990). While it has been reported that preterm neonates fed infant formula containing 20 mg/l of vitamin E and 0.8 mg/l of iron had a lower serum peroxyl radical-trapping capacity (Van Zoeren-Grobben et al., 1994), α-tocopherol contributes considerably to the total radical-trapping antioxidant potential of the organism (Wayner, Burton, Ingold, Barclay, & Locke, 1987). These amounts of vitamin E are above that the minimum limit set by ESPGAN, AAP, and RDA. This may be due to the decrease in the level of vitamin E in the commercialized product. We checked that infant formulas normally contain similar amounts of vitamin E to those declared on the label, or on occasion more.

Vitamin A is an essential nutrient for all animal species for normal vision, growth and cellular differentiation. These roles are critical during periods of proliferative growth and tissue development, such as infancy (Underwood, 1994). Human milk provides sufficient vitamin A concentration to the neonate. This amount decreases during lactation; in the first few days, the concentration is twice the vitamin A concentration of mature milk (Underwood, 1994).

Mean ± standard deviations of vitamin A in the 30 infant formula samples were 95.5 ± 34.5 μg/100 kcal and ranged from 46.5 to 210 μg/100 kcal. Although all term infant formulas showed vitamin A levels above ESPGAN recommendations (ESPGAN, 1991) on their labels, seven infant formulas contained less than the 75 μg/100 kcal of vitamin A, the minimum level of vitamin A specified by the ESPGAN recommendations for term infants (ESPGAN, 1991). Four of these contained below 80% of the VA level recommended; two samples contained less than 70% and one sample contained 62%. In low birth weight infants, vitamin A levels above 90 μg/100 kcal are recommended. All preterm infant formulas analysed contain this amount, or higher.

No infant formulas were above the maximum allowable vitamin A level of 150 μg/100 kcal (ESPGAN, 1991).

No statistical differences in vitamin A contents were found between preterm infant formulas and term infant formulas ($p > 0.05$) or between formulas supplemented with LCPUFA and those that were not (Table 1). Nor

were there statistical differences according to the manufacturer (Table 2).

The mean percentage of analysed levels of vitamin A in relation to label declaration for the 30 samples was 97.4 ± 35.9%. As shown in Table 3, label declaration value of preterm formulas was 118 ± 18.4% and the value of term formulas 100 ± 11.3%. Vitamin A analysed in the preterm infant formulas ranged from 79.5% to 157% of the level stated on the label and from 76.8 to 124% in infant formulas for term infants.

Twenty per cent of infant formulas presented vitamin A levels below ESPGAN recommendations, despite label declarations. While 85% of formulas showed analysed vitamin E level above the label declaration, 60% of analysed vitamin A values were below the level stated on the label.

The higher amount of tocopherol may be due to attempts to prevent losses due to its lability, and thus ensure a correct level at the time of commercialization. In vitamin A the philosophy of the manufacturer is different: the range of recommended levels is narrower. An excess of this vitamin can cause problems of toxicity and consequently the amount added tends not to be particularly large.

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