Vitamin D and Selected Fatty Acids in U.S. Infant Formulas

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Abstract

Vitamin D, a nutrient critical to normal calcium absorption and bone health, plays a significant role in preventing rickets in infants and very young children. The 1980 Infant Formula Act, and subsequent legislation, mandated fortification of all infant formulas with at least 40 IU but not more than 100 IU vitamin D/100 kilocalories; manufacturers are required to assure these levels in their products. Many manufacturers have also voluntarily fortified formulas with two fatty acids found in breast milk, docosahexaenoic acid (DHA, C22:6,n-3) and arachidonic acid (ARA, C20:4,n-6). Some studies suggest these nutrients may play a role in visual and mental development of infants. USDA has analyzed selected infant formula sampled at 12 locations to provide the first analytical dataset for these nutrients. Vitamin D, fatty acids, proximates, minerals, and some vitamins were analyzed in 13 highly consumed milk-based and soy-based, ready-to-feed (RTF) or reconstituted infant formulas; the formulas were analyzed unheated and heated to determine vitamin D retention. Samples were analyzed for vitamin D by HPLC with ultraviolet detection, using vitamin D_2 as the internal standard; fatty acids were analyzed by gas chromatography. These values were compared to standard values reported by the industry and values currently included in USDA Nutrient Data Base for Standard Reference (SR). Analytical values for vitamin D, DHA, and ARA (mean ± SEM) are being reviewed for release in SR22 (2009) and compared with existing industry-provided data. Preliminary review shows that vitamin D values are variable and meet or exceed label claims. For non-breastfed infants, infant formulas are consumed during a relatively short but critical growth period; for at least part of this time, formula is the sole source of nutrition. This is the first USDA-generated analytical dataset for nutrients in infant formulas.

Introduction

Fifty percent or more of infants in the U.S. have been fed infant formula by six months of age, according to the Ross Laboratories Mothers Survey (Ryan et al., 2002). Vitamin D, the omega-6 (n-6) fatty acid ARA and the n-3 fatty acid DHA are nutrients determined to be important to infant development (bones, brain, and eyes, IOM, 1997, 2002). Regulations for nutrient concentrations in infant formulas are mandated in the Infant Formula Act of 1980 and subsequent amendments (CFR, 2002).

Vitamin D - The current recommendation for normal infants is 5 µg (200 IU)/day for prevention of vitamin D deficiency in healthy infants (IOM, 1997); infant formulas are required to contain 40-100 IU/100 kcal as vitamin D_3 (CFR, 2002), or about 25-71 IU/100 g (26-73 IU/100 ml). Early research by Holick et al. (1992) found that of 10 popular infant formulas analyzed, all met or exceeded the amount on the label; three formulas analyzed contained 200% of the label declaration of vitamin D. Data were later reported as a range of 23.8-43.8 IU/100 mL for standard formulas (2002 manufacturers' data, IOM, 2004).

Fatty Acids – Voluntary DHA and ARA fortification began in 2002 to support brain development and visual acuity in young formula-fed infants (CFR, 2002; IOM, 2004); many brands in the U.S. are currently fortified with these fatty acids in the approved ratio (see below for approved ratio; IFC, 2008). FDA requires manufacturers to continue post-marketing surveillance of their fortified products; to date, scientific evidence of observed benefits to formula-fed infants with supplements of ARA and DHA or fortified formulas are mixed (IOM, 2002; Koletzko et al., 2008). If fortified, FDA has permitted the following levels: DHA must be 0.2% to 0.5% by of weight of total fatty acids; the amount of ARA must be ≥ DHA (CFR, 2002).

General - This is the first USDA-generated analytical dataset for these nutrients in U.S. infant formulas.

Methods

Sampling

A nationally representative sampling frame was developed for collection of infant formulas under the USDA National Food and Nutrient Analysis Program (NFNAP, Fig. 1, Pehrsson et al., 2003). The stratified, probability proportional-to-size (PPS) design was based on a three-stage approach using 2000 Census data (U.S. Bureau of the Census): Stage 1, 48 geographically dispersed counties; Stage 2, grocery store outlets in 12 of the 48 counties; and Stage 3, 13 high consumption infant formulas. Four major manufacturers and several types of formula (both soy- and cow milk-based) were represented in the sampling; 2 toddler formulas were included for vitamin D analysis because they presented similar label claims; they were separated into a 14th group for DHA/ARA analysis because the amount wasn't declared on the label. Formulas were combined a priori into six random pairs based on location but because they were not found in each retail location, the number of samples ranged from two to six by brand/type. When the RTF form of the formula was not found, the powder or liquid concentrate form was selected and the data adjusted to the "as-fed" form (reconstituted).

Vitamin D analysis

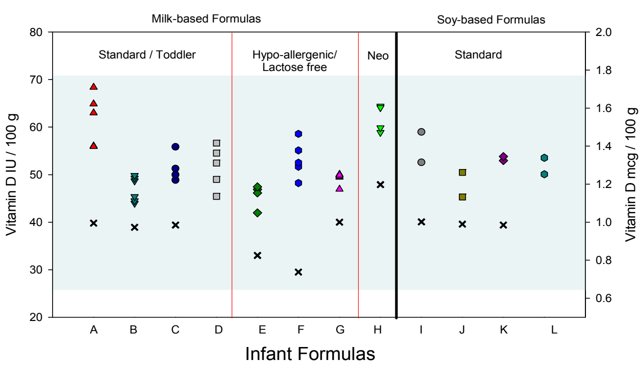
- 5.0 mL of sample spiked with internal standard (D_2); saponified in methanolic KOH for 20 minutes at 60ºC and extracted with hexane
- Hexane extract washed with dilute methanol and dried
- Sample resuspended (three times)
  - hexane/methylene chloride, applied to silica SPE cartridge, eluted with methylene chloride/2-propanol (99.8/0.2) and dried
  - hexane/methylene chloride/alcohol (85/15/0.2), applied to HPLC ZORBAX SIL column (5ų), vitamin D fraction collected and dried
  - hexane/2-propanol (99.5/0.5), applied to a HPLC ZORBAX SIL column, vitamin D fraction collected and dried
- Sample applied to Vydac ODS column in acetonitrile/methylene chloride (75/25)
- Vitamin D_3 quantified by comparison of UV peak areas with standards and corrected for recovery. Vitamins D_2 and D_3 are baseline resolved

Fatty acid analysis

ARA and DHA were measured by a USDA-contracted lab using AOAC Method 996.06 (41.1.28A) for Fat (Total, Saturated and Monounsaturated; AOAC, 2001) with some modifications (e.g., 100m column). The method was optimized to enhance resolution.

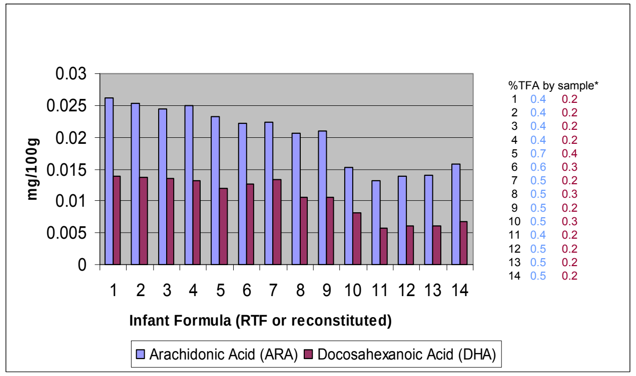
Figure 1. NFNAP Sampled Counties

Figure 2. Vitamin D in Select Infant Formulas



***FDA permitted variance given in blue shaded area: 40-100 IU/100 kcal required (~ 25-71 IU/100 g, depending on formula)(CFR, 2002). Store brands were combined and no label value is presented.**

Figure 3. DHA and ARA in Select Infant Formulas: mg/100g and %Total Fatty Acids (TFA)



***Added DHA must range between 0.2-0.5% of the total fatty acids and added ARA must equal or exceed that of DHA (CFR, 2002). Human breast milk values are: DHA 0.32% and ARA 0.47% of total fatty acids (Brenna et al., 2007)**

Results and Conclusions

Preliminary results for the representative sampling and analysis of 13 U.S.-produced infant formulas collected as part of the NFNAP project are shown in Figs. 2 and 3.

- Vitamin D values were consistently higher than the label values for all formulas. For brand names, they ranged from 107-199%, with a mean 137% and all were within the range required under the Infant Formula Act (Fig. 2). Analytical values in this study, although higher than label claims, were not as high as those reported in earlier research (Holick et al., 1992). Soy-based formulas were slightly closer (131%) to the amount reported on the label than milk-based formulas (138%).
- DHA and ARA results were variable among formulas and averaged 0.24% and 0.49%, respectively, of total fatty acids (all were within the requirement of 0.2-0.5% of total fatty acids); the ARA content always exceeded the DHA content (range 1.75-2.50 times higher; Fig. 3).
- When compared to global averages for human breast milk, 0.32% (DHA) and 0.47% (ARA) as % of total fatty acids (Brenna et al., 2007), average DHA in tested infant formulas was lower and ARA about the same. Values were consistent with concentrations found specifically in ranges for breast milk of U.S. and Canadian women (IOM, 2002): 0.1-0.4% (DHA) and 0.1-0.6% (ARA).
- Since infant formulas often report nutrients on a 100-mL basis, these values would be approximately 1.03 (average) higher than on a 100-gram basis, depending on the specific gravity of the infant formula.
- These U.S. infant formulas met or exceeded recommendations for added DHA and ARA (when fortified) and mandated amounts for vitamin D. These new data for high consumption U.S. infant formulas will be evaluated for inclusion in the USDA National Nutrient Database for Standard Reference (USDA, 2008).

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Acknowledgement

This project was supported by ARS, USDA. Partial support was received from the National Institutes of Health, Agreement # Y1-HV-8116-15.