THE VITAMIN D CONTENT OF FORTIFIED MILK AND INFANT FORMULA

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Abstract *Background.* The fortification of milk and infant formula with vitamin D has had an important role in eliminating rickets in children and osteomalacia in adults. A recent outbreak of vitamin D intoxication caused by drinking milk fortified with excess vitamin D has led to questions about the level of vitamin D in milk from other producers.

Methods. We used high-performance liquid chromatography to measure vitamin D in samples of 13 brands of milk with various fat contents and 5 brands of infant formula purchased at random from local supermarkets in five Eastern states.

Results. Only 12 (29 percent) of the 42 samples of the 13 brands of milk and none of the 10 samples of the 5 brands of infant formula contained 80 to 120 percent of the amount of vitamin D stated on the label. Twenty-six of the 42 milk samples (62 percent) contained less than 80 percent of the amount claimed on the label. No vitamin D was detected in 3 of the 14 samples of skim milk tested (lower limit of assay, 4.7 IU per quart [5.0 IU per liter]). One milk sample labeled as containing vitamin D_2 (ergocalciferol) contained vitamin D_3 (cholecalciferol).

Seven of the 10 samples of infant formula contained more than 200 percent of the amount stated on the label; the sample with the highest concentration contained 419 percent of the stated amount. None of the samples of infant formula contained less than the amount stated.

Conclusions. Milk and infant-formula preparations rarely contain the amount of vitamin D stated on the label and may be either underfortified or overfortified. Since both underfortification and overfortification are hazardous, better monitoring of the fortification process is needed. (N Engl J Med 1992;326:1178-81.)

RICKETS plagued the children who lived in the industrial cities of North America and Europe from the 17th through the 19th century. At the beginning of this century, over 85 percent of the children living in these areas had rickets.[1] Although cod-liver oil had been used by some physicians to cure this crippling disease, it was not until the early 1900s that Huldschinsky showed that exposure to ultraviolet radiation could cure rickets[2] and Hess and Unger demonstrated that exposure to sunlight could cure the disease.[3] These observations provided the crucial link between exposure to sunlight and normal skeletal development. In 1924, Steenbock and Black[4] and Hess and Weinstock[5] independently found that ultraviolet irradiation of human and animal food substances such as corn oil, egg yolk, milk, and rat chow could impart antirachitic activity to them.

These seminal observations were the impetus for the addition of provitamin D_2 (ergosterol) to milk, followed by ultraviolet irradiation. Once a simple method of producing vitamin D was developed, vitamin D was added directly to milk. The fortification of milk in Europe and North America resulted in the eradication of rickets as a major health problem in children. In the 1940s and 1950s, however, there were several sporadic outbreaks of vitamin D intoxication in Europe that led to the prohibition of the fortification of foods with vitamin D and a resurgence of rickets in Europe.[6,7]

In 1957, the American Medical Association's Council on Foods and Nutrition[8] reaffirmed the importance of vitamin D–fortified milk as the chief means of preventing rickets in children. The council recommended that milk contain 400 IU (10 μg) per quart and that the vitamin D content be measured at least twice yearly by an independent laboratory. The recent report of high concentrations of vitamin D — more than 500 times the amount stated on the label — in milk from a dairy in Massachusetts[9] prompted us to determine the vitamin D concentration of randomly selected samples of vitamin D–fortified milk and infant formula.

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METHODS

We purchased 42 containers of milk and 10 cans of infant formula from supermarkets in five Eastern states. They included 14 samples of whole milk, 10 samples of milk with a fat content of 2 percent, 4 samples of milk with a fat content of 1 percent, and 14 samples of skim milk from 13 milk processors and 10 samples of different types of infant formula from 5 manufacturers. All the samples were purchased before the expiration date and analyzed within 24 hours after purchase. The concentration of vitamin D was determined in 10 ml of each milk sample according to modifications of the procedures of Hollis[10] and Chen et al.[11]

Tritiated vitamin D_3 (cholecaliferol) (10,000 cpm; specific activity, 10 to 20 Ci per millimole; Amersham, Arlington Heights, Ill.) in 100 μl of ethanol was added to each 10-ml sample. The samples were equilibrated at room temperature, after which 40 ml of ethyl acetate was added. The samples were shaken and centrifuged, and the organic (upper) phase was removed. The aqueous (lower) phase was extracted twice with 40 ml of ethyl acetate. The organic phases were combined, dried under nitrogen, redissolved in 6 ml of methanol, placed at $-80°C$ for 10 minutes, and then centrifuged. The precipitate was reextracted with methanol and again centrifuged. The supernatants were combined, dried under nitrogen, and redissolved in methylene chloride. Five milliliters of 0.2 N disodium phosphate (at pH 10.5) was added, and the mixture was centrifuged. The organic (lower) phase was removed, and the aqueous phase was reextracted with methylene chloride. The methylene chloride fractions were dried and dissolved in 2 ml of 50 percent methanol in water.

The sample was applied to a C-18 cartridge (Analytichem International, Harbor City, Calif.). The column was eluted with distilled water, 70 percent methanol in distilled water, and then acetonitrile, after which the vitamin D was eluted with methanol. The methanol fraction was dried under nitrogen and redissolved in 1 percent isopropyl alcohol in *n*-hexane, after which it was fractionated by normal-phase high-performance liquid chromatography with use of an Econosphere silica column (5-μm particle size, 250 by 4.6 mm; Alltech Associates, Deerfield, Ill.) at 1.1 ml per minute. The fractions containing vitamin D_2 (ergocalciferol) and vitamin D_3 were collected, dried under nitrogen, and dissolved in 25 percent methanol in acetonitrile. The vitamin D_2 and vitamin D_3 were separated by high-performance liquid chromatography on a reverse-phase Vydac C-18 column (250 by 4.6 mm; 5-μm particle size; Rainin Instruments, Woburn, Mass.).

The concentrations of vitamin D_2 and vitamin D_3 were determined by comparison of the areas under the peaks for each vitamin with the areas of peaks obtained by high-performance liquid chromatography of samples containing known amounts of vitamin D_2 or vitamin D_3 (Fig. 1). The amount of radioactivity recovered was determined in order to measure the overall recovery for both vitamin D_2 and vitamin D_3, and the results were corrected for this recovery. The recovery for vitamin D_2 and vitamin D_3 from the reverse-phase column was the same; the typical amount recovered was 50 to 70 percent, and the intraassay and interassay coefficients of variation for the vitamin D assay were 8 percent and 10 percent, respectively.

To determine the concentration of vitamin D in infant formula, 5 ml of liquid formula or 0.5 g of powdered formula was saponified and extracted as described previously[12]; we then followed the two-step chromatographic procedure, as described for the milk samples.

Results

Figure 1 shows a representative reverse-phase high-performance liquid chromatographic profile of standard vitamin D_2 and vitamin D_3 and a lipid extract of milk. There was clear separation of vitamin D_2 and vitamin D_3 from ultraviolet-absorbing lipid contaminants. To be certain that the vitamin D_3 in the milk was stable during storage, we measured, in triplicate, the concentration of vitamin D_3 in samples of whole and skim milk on the day of purchase and after storage in a refrigerator (at 4°C) for seven days (until the expiration date). The mean (±SD) vitamin D_3 concentrations were similar on both occasions (whole milk, 193±13 vs. 185±5 IU per quart [205±14 vs. 196±5 IU per liter]; skim milk, 356±33 vs. 333±6 IU per quart [377±35 vs. 353±6 IU per liter]).

The results of the measurements of vitamin D in the milk and infant-formula samples are shown in Tables 1 and 2. Although each quart of milk was labeled as containing 400 IU (10 μg) of either vitamin D_2 or vitamin D_3, there was wide variation in the amount of vitamin D measured in the milk samples. For example, the brand A whole milk and milk with 2 percent fat purchased on July 19, 1991, contained 276 IU and 11 IU of vitamin D_3 per quart (293 and 12 IU per liter), respectively. The same brand of skim milk purchased at the same time contained no detectable vitamin D_3 (lower limit of assay, 4.7 IU per quart [5.0 IU per liter]), but skim-milk samples purchased on August 30, 1991, contained as much vitamin D_3 as whole milk or more. Brand B milk with 1 percent fat that was fortified with *Lactobacillus acidophilus* (Lactaid) or with calcium (Calcimilk) contained 496 IU and 515 IU of vitamin D_2 per quart (526 and 546 IU per liter), respectively, whereas brand B skim milk purchased at the same time contained only 155 IU per quart (164 IU per liter), or 39 percent of the amount stated on the label. Brand B skim milk purchased on June 18, 1991, had even less vitamin D_2 (87 IU per quart [92 IU per liter]). In brand C milk, not only the content but also the type of vitamin D varied with the date of purchase; one brand J milk sample labeled as containing vitamin D_2 contained vitamin D_3.

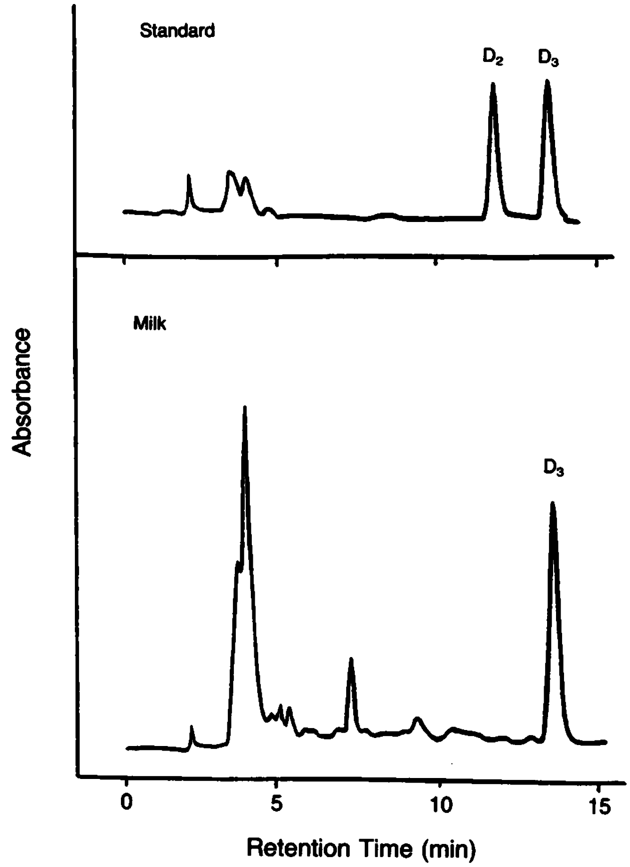

Figure 1. Reverse-Phase High-Performance Liquid Chromatograms of Samples Containing Known Amounts of Vitamin D_2 and Vitamin D_3 (Upper Panel) and a Lipid Extract of Milk Containing Vitamin D_3 (Lower Panel).

The lipid extract was prepared from brand E whole milk, purchased on July 10, 1991. Absorbance was measured at a wavelength of 254 nm.

Only 12 (29 percent) of the 42 milk samples from the 13 milk processors that we analyzed contained 80 to 120 percent of the amount stated on the label. Twenty-six (62 percent) of the milk samples contained less than 80 percent and 4 (10 percent) contained more than 120 percent of the amount stated on the label.

The vitamin D concentrations of the 10 samples of five brands of infant formula contained from 715 to 1608 IU of vitamin D per quart (756 to 1700 IU per liter) (Table 2). Seven of the 10 samples (70 percent) contained more than 200 percent of the amount stated on the label. That with the highest concentration was the iron-fortified brand Q, which contained 419 percent of the amount of vitamin D stated.

Discussion

In the United States, the chief food that is fortified with vitamin D is milk. Indeed, the fortification of milk and infant formula with vitamin D during the

Table 1. Type of Vitamin D and Vitamin D Concentration in Milk Samples.*

Brand and Type	Date of Purchase	Expiration Date	Form of Vitamin on Label	Concentration on Label	Actual Concentration†	
					vitamin D_2	vitamin D_3
				IU/qt	IU/qt	
Brand A						
Whole	7/19/91	7/26/91	D_3	400	ND	276±11 (3)
	8/12/91	8/18/91	D_3	400	ND	231±6 (3)
	8/22/91	8/28/91	D_3	400	ND	201±11 (3)
	8/30/91	9/6/91	D_3	400	ND	261±26 (2)
2%	7/19/91	7/25/91	D_3	400	ND	11±5 (2)
	8/6/91	8/12/91	D_3	400	ND	276±53 (4)
	8/22/91	8/27/91	D_3	400	ND	81±3 (2)
1%	8/12/91	8/18/91	D_3	400	ND	159±15 (3)
Skim	7/10/91	7/16/91	D_3	400	ND	<4.7
	7/19/91	7/25/91	D_3	400	ND	<4.7
	8/22/91	8/28/91	D_3	400	ND	269±13 (3)
	8/30/91	9/6/91	D_3	400	ND	333±25 (3)
Brand B						
Whole	6/18/91	6/18/91	D_2	400	286±17 (5)	ND
Skim	6/18/91	6/18/91	D_2	400	87±16 (2)	ND
	8/8/91	8/17/91	D_2	400	155±4 (3)	ND
Lactaid (1%)‡	8/8/91	8/14/91	D_2	400	496±35 (3)	ND
Calcimilk (1%)§	8/8/91	8/14/91	D_2	400	515±21 (3)	ND
Brand C						
Whole	7/10/91	7/16/91	D_2	400	384±3 (2)	ND
	8/26/91	9/2/91	D_3	400	ND	272±27 (3)
2%	7/10/91	7/16/91	D_2	400	195±3 (2)	ND
Skim	7/10/91	7/16/91	D_2	400	352±6 (2)	ND
	8/26/91	8/31/91	D_3	400	ND	344±33 (3)
Brand D						
Whole	7/10/91	7/16/91	D_3	400	ND	405±17 (3)
2%	7/10/91	7/16/91	D_3	400	ND	250±14 (3)
Skim	7/10/91	7/16/91	D_3	400	ND	242±11 (2)
Brand E						
Whole	8/12/91	8/18/91	D_3	400	ND	492±30 (3)
Brand F						
Whole	12/2/91	12/4/91	D_3	400	ND	207±7 (3)
2%	12/2/91	12/4/91	D_3	400	ND	410±10 (3)
Skim	12/2/91	12/11/91	D_3	400	ND	340±16 (3)
Brand G						
Whole	12/2/91	12/9/91	D_3	400	ND	379±36 (3)
2%	12/2/91	12/9/91	D_3	400	ND	355±20 (3)
Skim	12/2/91	12/9/91	D_3	400	ND	201±16 (3)
Brand H						
Whole	11/25/91	11/30/91	D_3	400	ND	343±9 (3)
Brand I						
Skim	11/25/91	12/2/91	D_3	400	ND	<4.7
Brand J						
1%	11/25/91	12/1/91	D_2	400	ND	544±27 (3)
Brand K						
2%	11/25/91	1/29/91	D_3	400	ND	350±17 (3)
Brand L						
Whole	11/30/91	12/8/91	D_2	400	117±26 (3)	ND
2%	11/30/91	12/8/91	D_2	400	68±4 (3)	ND
Skim	11/30/91	12/8/91	D_2	400	42±8 (3)	ND
Brand M						
Whole	11/29/91	12/4/91	D_2	400	<4.7	ND
2%	11/29/91	12/9/91	D_2	400	302±8 (3)	ND
Skim	11/29/91	12/11/91	D_2	400	355±41 (3)	ND

*Plus–minus values are means ±SD. Brands A through E were purchased in Massachusetts, brands F and G in Virginia, brands H through K in New Jersey, brand L in Vermont, and brand M in New Hampshire. To convert vitamin D values to nanomoles per liter, multiply by 0.073. To convert to international units (IU) per liter, multiply by 1.06.

†Numbers in parentheses indicate the number of samples analyzed. ND denotes not detectable in the vitamin D assay used.

‡Milk fortified with *Lactobacillus acidophilus*.

§Milk fortified with calcium.

past 60 years has eradicated rickets as a major health problem in the United States. Until the development of sophisticated chromatographic assays for vitamin D, vitamin D activity in milk was measured by bioassay.[13] The Food and Drug Administration Standard of Identity for Milk states that if vitamin D is added, the milk should contain 400 IU per quart and the fortification process should follow standard manufacturing practices. The federal government has given the states the responsibility for monitoring the vitamin D content of milk.

In 1988, a survey of the vitamin D concentrations of fortified milk by the FDA[14] revealed that only 26 percent of 669 samples obtained in Oregon, Rhode Is-

Table 2. Vitamin D Concentrations in Infant Formula.*

Brand and Type	Form of Vitamin D on Label	Concentration on Label	Actual Vitamin D_3 Concentration†
		IU/qt	
Brand N			
Newborn formula	D_3	384	1351±87 (3)
6–12 month, liquid	D_3	416	757±38 (3)
6–12 month, powder	D_3	416	715±25 (6)
Brand O			
Low iron	D_3	403	1298±76 (3)
Milk-free, soy protein	D_3	403	810±42 (5)
Brand P			
Low iron	D_3	384	738±40 (6)
Hypoallergenic	D_3	288	897±64 (3)
Brand Q			
Nonfat milk	D_3	384	1608±15 (3)
Milk-free, soy protein	D_3	384	1143±72 (4)
Brand R			
Low iron	D_3	384	1112±38 (5)

*Plus–minus values are means ±SD. To convert vitamin D values to nanomoles per liter, multiply by 0.073. To convert to international units (IU) per liter, multiply by 1.06.

†Numbers in parentheses indicate the number of samples analyzed.

land, and Ohio contained 80 to 120 percent of the amount of vitamin D stated on the label. In general, the vitamin D concentration tended to be lower in samples with lower fat content. The FDA speculated that this might result from the method of adding vitamin D, in which the fat is removed from whole milk after the addition of vitamin D, which is soluble in fat. The report urged that this problem be corrected; otherwise, the FDA would institute regulatory programs to ensure compliance. Our results clearly demonstrate that this warning has not been heeded, since only 29 percent of the 42 milk samples we analyzed contained 80 to 120 percent of the amount of vitamin D stated on the labels. Ten percent of the milk samples and all the samples of infant formula contained excessive amounts of vitamin D, but some of the samples of skim milk contained no detectable vitamin D.

The lack of a national program to monitor the vitamin D content of milk has serious and costly consequences. Because the Massachusetts Department of Public Health did not monitor the vitamin D content of the state's milk supply twice a year, as required by law, the fortification of milk with very large amounts of vitamin D in one dairy was not recognized. Nearly 12,000 households were, therefore, at risk of excessive vitamin D intake, and several cases of vitamin D intoxication were recently identified.[9]

Conversely, the lack of vitamin D in milk can eventually have devastating consequences in both children and elderly persons, who depend on this food source for their vitamin D requirement. If children are not exposed to sunlight, they are at risk for rickets. Elderly people not only have decreased capacity to produce vitamin D in their skin,[15] but they are also more likely to have little exposure to sunlight. Therefore, they depend on multivitamins and milk as their principal sources of vitamin D. Several studies in the United States have suggested that 30 to 40 percent of patients with hip fractures are deficient in vitamin D.[16-18] Thus, elderly people who drink milk that contains no vitamin D or is underfortified with vitamin D are likely to be more prone to have vitamin D deficiency and, therefore, to have an increased risk of hip fracture. This possibility is supported by the recent observation that healthy postmenopausal women with daily vitamin D intakes of only 100 IU can reduce wintertime bone loss by increasing their vitamin D intake.[19]

Particularly when considered along with the findings of Jacobus et al.,[9] the results of this study demonstrate the need for greatly increased testing of all food products containing vitamin D. Although in the past such testing had to be done with cumbersome bioassays, the availability of relatively simple and much more precise chromatographic methods, such as that used in our study, makes frequent testing much easier. There is no longer any excuse for the failure to monitor vitamin D fortification regularly.

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