



## Vitamin D content and variability in fluid milks from a US Department of Agriculture nationwide sampling to update values in the National Nutrient Database for Standard Reference

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### ABSTRACT

This study determined the vitamin D<sub>3</sub> content and variability of retail milk in the United States having a declared fortification level of 400 IU (10 µg) per quart (qt; 1 qt = 946.4 mL), which is 25% daily value per 8 fluid ounce (236.6 mL) serving. In 2007, vitamin D<sub>3</sub> fortified milk (skim, 1%, 2%, whole, and 1% fat chocolate milk) was collected from 24 statistically selected supermarkets in the United States. Additionally, 2% milk samples from an earlier 2001 USDA nationwide collection were reanalyzed. Vitamin D<sub>3</sub> was determined using a specifically validated method involving HPLC with UV spectroscopic detection and vitamin D<sub>2</sub> as an internal standard. Quality control materials were analyzed with the samples. Of the 120 milk samples procured in 2007, 49% had vitamin D<sub>3</sub> within 100 to 125% of 400 IU (10 µg)/qt (label value), 28% had 501 to 600 IU (12.5–15 µg)/qt, 16% had a level below the label amount, and 7% had greater than 600 IU (15 µg)/qt (>150% of label). Even though the mean vitamin D<sub>3</sub> content did not differ statistically between milk types, a wide range in values was found among individual samples, from nondetectable [ $<20$  IU (0.5 µg)/qt] for one sample to almost 800 IU (20 µg)/qt, with a trend toward more samples of whole milk having greater than 150% of the labeled content. On average, vitamin D<sub>3</sub> in 2% milk was higher in 2007 compared with in 2001 [473 vs. 426 IU (11.8 vs. 10.6 µg)/qt].

**Key words:** fluid milk, vitamin D, food composition, nationwide sampling

### INTRODUCTION

The importance of adequate vitamin D intake for bone health is well recognized. In addition, vitamin D may have other roles in promoting oral health and preventing colon cancer (Bischoff-Ferrari et al., 2006). Its

requirement for optimal health, such as neuromuscular and immune function, has received increasing attention in recent years (Stroud et al., 2008). Sunlight induces cutaneous vitamin D synthesis, but many factors may reduce an individual's sun exposure (Calvo et al., 2005; Holick, 2007); therefore, vitamin D from the diet and from supplements plays a critical role in achieving optimal physiological levels (Chen et al., 2007). Natural vitamin D in dietary sources is primarily cholecalciferol (vitamin D<sub>3</sub>), found in foods such as oily fish (Moore et al., 2004). Ergosterol, which occurs in mushrooms, is converted to ergocalciferol (vitamin D<sub>2</sub>) on exposure to UV light (Mattila et al., 1994). Smaller amounts of vitamin D<sub>3</sub> and its metabolite, 25-hydroxyvitamin D<sub>3</sub>, are found in meats and eggs (Mattila et al., 1995; Jakobsen, 2007). Vitamin D is added to most fluid retail milk in the United States, generally as vitamin D<sub>3</sub>. Vitamin D<sub>2</sub> is occasionally used to fortify nondairy beverages (e.g., soy milk).

In 1997, the Food and Nutrition Board of the Institute of Medicine (Washington, DC) established the adequate intake, representing the recommended average daily nutrient intake of vitamin D that has been deemed adequate for apparently healthy adults, at 5 µg for those  $\leq 50$  yr of age, 10 µg for those 51 to 70 yr of age, and 15 µg for those  $\geq 70$  yr of age (Institute of Medicine of the National Academies, 1997). However, a committee of the Institute of Medicine is currently reviewing these 1997 Dietary Reference Intakes and considering more recent research and is expected to issue updated recommendations in 2010 (Office of Dietary Supplements, 2008).

In the United States, vitamin D fortification of milk and milk products began in the 1930s. Almost all retail milk in the United States is now fortified with vitamin D (primarily vitamin D<sub>3</sub>) at the targeted final concentration of 400 IU (10 µg) per quart (qt; 1 qt = 946.4 mL; FDA, 2007). Because of its high consumption, milk is a major source of dietary vitamin D in the absence of supplements (Moore et al., 2004; Fulgoni et al., 2007).

Received April 21, 2010.

Accepted July 2, 2010.

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Food and Drug Administration regulations require the actual amount of vitamin D to be at least equal to the value declared on the label, and although no upper limit is technically specified, the acceptable range within limits of good manufacturing practices is 100 to 150% of the declared content (FDA, 2007). This wide tolerance means the actual vitamin D content of an individual carton of milk could vary appreciably from the labeled value, either within a specific product or among products from different sources or production lots. Also, because regulatory guidelines specify no upper limit for the actual content relative to the label but do not allow an amount under the declared level, manufacturers are likely to overshoot the declared content to ensure compliance with regulatory guidelines.

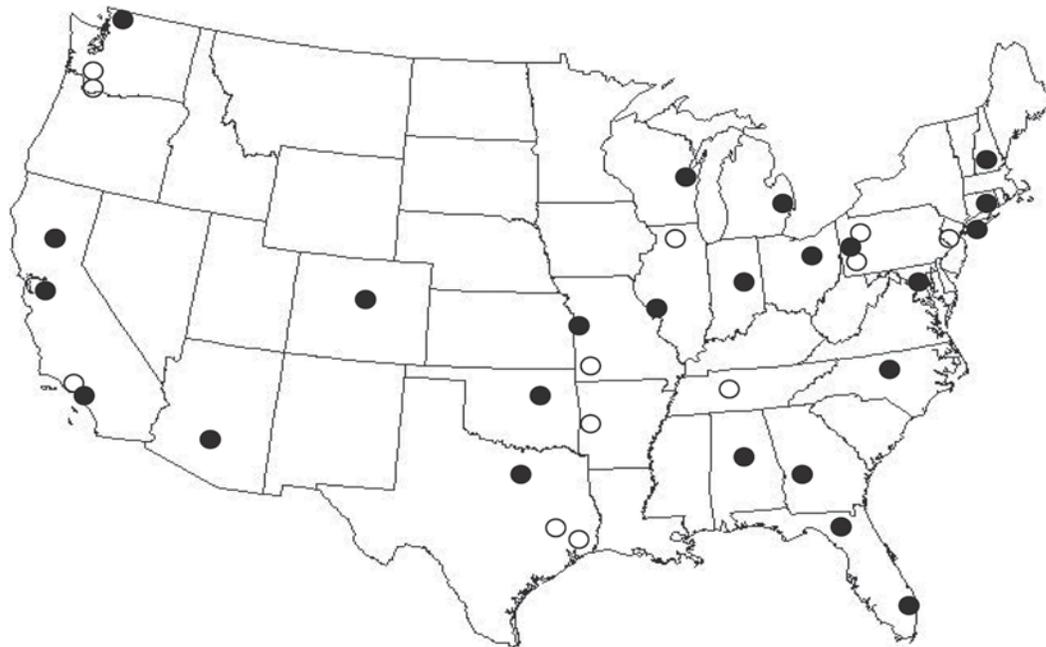
Previous studies show a consistent history of variability in the vitamin D content of retail milk. In 1988, Tanner et al. (1988) analyzed skim, 2%, and whole milk as well as high fat whipping cream and half-and-half. In general, vitamin D in low fat milk was less than the label value, and it was concluded that in some cases the vitamin may have been added to the raw milk before processing, resulting in underfortification of lower fat products and overfortification of higher fat products. Another report (Holick et al., 1992), on the content in 42 samples of milk representing 13 brands procured from local supermarkets in 5 Eastern states, showed vitamin D within 20% of the labeled content in only 12 of the 42 samples, with 3 of 14 skim milk samples containing no detectable vitamin D. Murphy et al. (2001) sampled milk in retail packaging from various producers in New York State from 1997 to 2000 and analyzed vitamin D using a validated HPLC method. Only about half of the samples contained 100 to 150% of the labeled content. Similarly, in a Canadian study (Faulkner et al., 2000) in which whole, skim, and 2% fat milk from a random sampling of producers in Ontario were assayed using a validated HPLC methodology, only 29% of 45 milk samples were in compliance; 36% were above and 36% were below the allowable Canadian range (31.7–51.6 IU/100 mL), and 4 samples had no detectable vitamin D. Overall, these studies suggest that the actual vitamin D content of individual samples could be significantly different from the label value, with some containing a large excess and others much less than the declared content.

Accurate food composition data for vitamin D are critical for reliable assessment of dietary intake, including epidemiological studies of the health effects of vitamin D (e.g., Moore et al., 2004; Affenito et al., 2007; Gilmore et al., 2008), estimation of intake by individuals, and characterization of diets used in clinical feeding trials. Yetley (2008) recently reviewed the assessment of vitamin D status based on food intake. In the United

States, population estimates of dietary intake are often based on the “What We Eat in America” dietary component of the National Health and Nutrition Examination Survey which uses the Food and Nutrient Database for Dietary Surveys (USDA, 2008a). The source of those food composition data is the USDA National Nutrient Database for Standard Reference (SR; Gebhardt et al., 2009), which is maintained by Agricultural Research Service’s Nutrient Data Laboratory (NDL). The 2009 version of the SR (SR22; Gebhardt et al., 2009) contains data for more than 7,500 foods with more than 4,000 values for vitamin D, some of which are zero for those foods that do not contain vitamin D. Approximately 2,700 foods that have vitamin D values are used in the Food and Nutrient Database for Dietary Surveys either as single food items or as ingredients for items prepared by recipe techniques.

Prior to 2009, vitamin D values in the SR for dairy products were imputed from federal fortification standards (USDA, 1980). In 2001, as part of the USDA’s National Food and Nutrient Analysis Program aimed at updating and improving nutrient data in the SR (Haytowitz et al., 2008), milk was sampled from 12 statistically selected supermarkets in the United States. Although targeted for analyses of other nutrients, these milk samples were also analyzed for vitamin D at a commercial laboratory using an existing standard method (method 995.05; AOAC, 2003). The high relative standard deviations for vitamin D assayed in the skim, 1%, and 2% milk samples (>25%), and the lack of reference materials and quality control data at that time, left questions about what portion of the variability could be attributed to analytical uncertainty (Holden et al., 2008).

Because of the variability in the vitamin D content of milk shown in studies published by other researchers and analytical uncertainty, combined with the growing interest in the possibility of the wider physiological importance of vitamin D, NDL collaborated with other scientists in USDA’s Food Composition and Methods Development Laboratory (FCMDL; Beltsville, MD) and other groups to develop and characterize control materials and to validate an HPLC method for analysis of vitamin D in milk and other key food sources. These steps were necessary to support generation of reliable vitamin D data for release in SR (Phillips et al., 2008). With quality control measures in place, skim (nonfat), 1% total fat, 2% total fat, and whole (approximately 3.5% total fat) white milk and 1% total fat chocolate milk were statistically sampled in 2007 from 24 locations in the United States. The purpose of this report is to present the vitamin D results for those milk samples; those data have been incorporated into SR, providing nationally representative values.



**Figure 1.** Statistically determined sampling locations in 2001 (open circles) and 2006–2007 (filled circles).

## MATERIALS AND METHODS

### Samples

Between November 2006 and February 2007 sample units of skim, 1%, 2%, and whole white milk and 1% chocolate fluid milk were procured from 24 different statistically selected retail outlets (hereafter referred to as 2007 samples). Most of the products purchased were in half-gallon (1.9 L) plastic cartons, some were in quart-size cardboard or plastic containers, and 1 chocolate milk sample unit was in an individual serving plastic container. The 2% milk products sampled in November 2001 (before the initiation of the current vitamin D study) were obtained from 12 statistically selected (Pehrsson et al., 2000; Perry et al., 2003) supermarkets in the United States. Analytical samples, prepared as described below, were stored at  $-60^{\circ}\text{C}$ . Vitamin D has been shown to be quite stable under both heating and freezing as long as it is protected from UV light (Renken and Warthesen, 2006; Wielders and Wijnberg, 2009).

Figure 1 shows the distribution of sampling locations. The 2007 retail outlets were different from those procured in 2001 because they were chosen using an updated sampling plan based on the 2000 census (Pehrsson et al., 2000; Perry et al., 2003). Also, the number of locations was expanded to 24 because of the large variability observed in the vitamin D content of the 2001 samples. Each location was given a code including the state abbreviation and a site number. The loca-

tions are given only to identify where the samples were obtained. Where sample units were not available at the designated retail outlets, local samples were substituted, either from Blacksburg, Virginia (for AZ1 whole milk and FL2, IN1, OK1, and NY1 chocolate milk) or Bluefield, West Virginia (for WA1 chocolate milk).

Additionally, sample units were obtained at 4 of the original sites approximately 1 yr (2008) after the initial procurement as a preliminary follow-up to the initial findings. These locations and products were re-sampled because at least 1 type of milk sampled from the location had either very high or low or nondetectable vitamin D content or the level was consistently low among products. The sample units were obtained from the same store (CA3 and CO1) or from a nearby outlet supplied by the same dairy (FL2 and MD1), as determined from the plant code found on the package, or the dairy name on the label, or both (USDA, 2008b), and in all cases were produced by the same dairy as the 2007 samples.

### Sample Preparation

Milk was shipped in the original containers in coolers, maintained at refrigerated temperature with ice packs, to the Food Analysis Laboratory Control Center (FALCC) at Virginia Tech (Blacksburg, VA) using methods reported elsewhere (D. Trainer, NDL, Beltsville, MD; unpublished data). Upon receipt at FALCC, sample units were inspected to ensure the integrity of

the packaging and were stored refrigerated (2–5°C) before subsampling.

Subsampling was performed within 6 d of receipt and always before the labeled expiration date. The milk carton was inverted 5 to 10 times to mix and was opened immediately; using a glass liquid measuring cup, 3 cups (709.76 mL) of milk were removed and poured into a stainless steel bowl. The milk was stirred for 2 min with a stainless steel spoon. Subsamples (75–100 mL) were dispensed with tricorned polypropylene beakers into 125-mL straight-sided glass jars with polytetrafluoroethylene-lined lids. The jars were sealed under nitrogen, surrounded with aluminum foil, and stored at –60°C until analyzed.

Frozen subsamples were sent from FALCC in coolers on dry ice, via express overnight delivery, to the analytical laboratory (Heartland Laboratories, Ames, IA). This facility was a participant and one of the validated laboratories in the previously reported method validation study (Phillips et al., 2008). The sample identities were blinded, with only a general description of the milk type included for each sample (e.g., skim, 1%). A blinded sample of the previously developed skim milk control composite (Phillips et al., 2008) was included in each designated assay batch of about 15 milk samples, and the laboratory was instructed to analyze the samples by batch.

### Vitamin D Analysis

The 2007 and 2008 samples and the 12 2% fat milk samples from 2001 were analyzed for vitamin D using a previously validated HPLC method with UV detection (Phillips et al., 2008; Byrdwell, 2009). Briefly, internal standard (vitamin D<sub>2</sub>, 60 ng) was added to a 5-mL portion of milk, which was then saponified with methanolic potassium hydroxide and extracted with hexane. The hexane layer was washed with water, collected, and dried under vacuum. The dried extract was resuspended in 1.0 mL of hexane/methylene chloride (90/10 vol/vol), applied to a hand-packed solid-phase extraction cartridge (0.5 g of 10–40 µm silica; Varian, Palo Alto, CA; part no. A8501, equipped with a stainless steel frit), eluted with methylene chloride/2-propanol (99.8/0.2, vol/vol), and dried. The residue was resuspended in hexane/methylene chloride/alcohols (85/15/0.2, vol/vol/vol; the alcohols consisted of 2/1 vol/vol isopropanol/methanol). The sample was applied to a Zorbax SIL column (25 cm × 9 mm, 5µm; Agilent Technologies, Santa Clara, CA), and the vitamin D fraction was collected and dried. The residue was resuspended and eluted in hexane/alcohols (99.5/0.5, vol/vol) using a Zorbax NH2 column (25 cm × 4.6 mm, 5µm; Agilent Technologies), and the vitamin D fraction was collected

and dried for final purification. The final purification was achieved using a Vydac ODS column (Vydac part no. 201TP54; Chrom Tech Inc., Apple Valley, MN) with a mobile phase of acetonitrile/methylene chloride (65/35, vol/vol) and UV detection at 265 nm. Vitamin D<sub>3</sub> was quantified by comparison of the UV peak areas at 265 nm with authentic standards, using the vitamin D<sub>3</sub> to vitamin D<sub>2</sub> internal standard area ratio.

### Quality Control

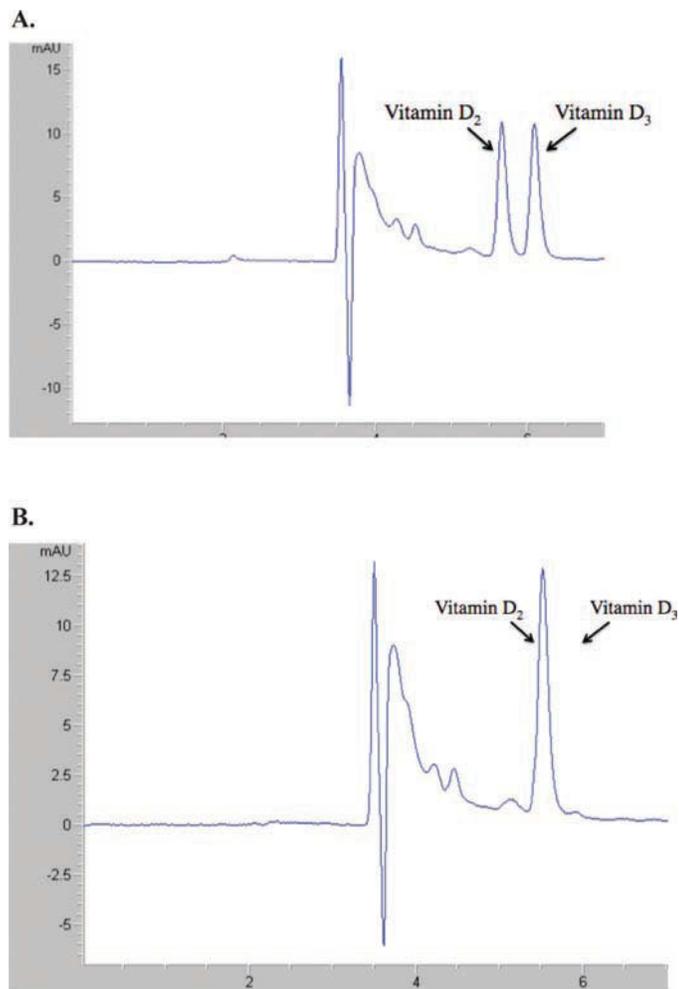
A blinded sample of the skim milk control composite was included in each assay batch with at least 1 sample in each analytical batch of 13 to 15 samples run in duplicate, for a total of 11 replicate analyses, 1 per batch. Additionally, a blinded sample of the certified reference material Community Bureau of Reference (BCR) 421 milk powder (Institute of Reference Materials and Methods, Geel, Belgium) was assayed in duplicate, with the weighed analytical portion being reconstituted 1:10 with water before analysis. Three samples, including some that had unexpected high or low results, were also analyzed at FCMDL, which was the reference laboratory for the previous method validation study (Phillips et al., 2008).

Acceptable precision was considered to be <5% difference between within-laboratory and between-laboratory duplicate analyses. The value for the control sample was required to fall within the established tolerance limits (Phillips et al., 2008) to validate the accuracy of results from each assay batch. Agreement within 6% was expected for samples run at both laboratories. Also, although vitamin D<sub>3</sub> was expected to be the fortificant in all milk samples, the vitamin D<sub>2</sub> internal standard peak area was monitored in all chromatograms to confirm the absence of vitamin D<sub>2</sub> in each sample.

### Data Analysis

The analytical results were measured in nanograms per gram and reported as IU / 100 g using a factor 40 IU/µg. These values were further converted from grams to quarts using a density of 243.7 g/8 fluid ounces (236.6 mL). For the dry milk reference material, the certified limits are given on a dry mass basis; therefore, results were adjusted to dry mass basis using the ambient moisture content (2.45 g/100 g) that was determined as specified in the certificate of analysis (BCR, 1998).

A Z-score for the reference material results was calculated as described by Jorhem et al. (2001; method 1.3). The expected ratio of the actual relative standard deviation (RSD) to the expected RSD for replicates was calculated as described by Horwitz et al. (1980). Means and standard deviations for the various milk



**Figure 2.** Representative chromatograms for A) BCR 421 milk powder (Institute of Reference Materials and Methods, Geel, Belgium) and B) 2% milk found to contain no vitamin D. Vitamin D<sub>2</sub> is the internal standard (60 ng/5-mL analytical sample). Color version available in online PDF.

types were calculated using Microsoft Excel (Professional Edition 2003; Microsoft Corporation, Redmond, WA), and ANOVA ( $\alpha = 0.05$ ) was performed using Quattro Pro (version 14.0.0.603; Corel Corporation, Ottawa, Ontario, Canada).

## RESULTS

### Quality Control

As previously reported, recovery was 96 to 106% for vitamin D<sub>3</sub> spiked into skim milk (Phillips et al., 2008). The percentage difference in the assayed values for the 11 within-laboratory duplicates ranged from 0.1 to 5.7% (median = 1.3%). The between-laboratory differences were 0.2, 0.4, and 4.2% for the 3 samples that were assayed at both facilities. The expected ratio of

the actual RSD to the expected RSD was low for both the within-laboratory (0.1–0.6) and between-laboratory (0.03–0.5) replicates.

Representative chromatograms for BCR 421 and a 2% milk sample found to contain no vitamin D are shown in Figure 2. The vitamin D<sub>3</sub> peak from the BCR sample was well resolved from the vitamin D<sub>2</sub> internal standard. The assayed vitamin D<sub>3</sub> concentrations of BCR 421 milk powder were 15.6 and 15.8  $\mu\text{g}/100\text{ g}$ , giving an acceptable Z-score of 1.3 relative to the certified mean of  $14.3 \pm 0.8\ \mu\text{g}/100\text{ g}$  (BCR, 1998).

All values for the skim milk control composite assayed with each analytical set of samples fell within the tolerance limits of 0.92 to 1.19  $\mu\text{g}/100\text{ g}$  that were established during method validation (Phillips et al., 2008; Figure 3F). The RSD for the total of 13 analyses of the control material across 13 assay batches was 4.1%, with a mean of 1.11  $\mu\text{g}/100\text{ g}$ . In total, the quality control results suggested that a  $\pm 5\%$  interval around any value for a sample assayed in singlicate would be a reasonable and conservative estimate of the analytical uncertainty.

### Vitamin D<sub>3</sub> Content of Nationwide Sampling of Milk

Table 1 and Figure 3 summarize the results for the assayed vitamin D<sub>3</sub> content of the samples of skim, 1%, 2%, whole, and 1% chocolate milk procured in 2006–2007. The mean assayed vitamin D<sub>3</sub> ranged from nondetectable [ $<20\text{ IU}$  ( $0.05\ \mu\text{g}$ )/qt] for one sample to 797 IU ( $19.9\ \mu\text{g}$ )/qt across the 5 types of milk, with no statistically significant difference among skim, 1%, 2%, whole, and 1% chocolate milk. Overall, 59 of 120 samples (49%) contained 100 to 125% of the labeled vitamin D content [ $400\text{--}500\text{ IU}$  ( $10\text{--}12.5\ \mu\text{g}$ )/qt] and 34 (28%) contained 125 to 150% [ $501\text{--}600\text{ IU}$  ( $12.5\text{--}15\ \mu\text{g}$ )/qt]. A large variability was found among individual samples of all types of milk (Figure 3). Eight samples (7%) had vitamin D<sub>3</sub> exceeding 150% of the declared content [ $>600\text{ IU}$  ( $15\ \mu\text{g}$ )/qt] and 19 (16%) had less than the labeled content. Whereas on average the vitamin D<sub>3</sub> content did not differ significantly among the 5 types of milk, more of the individual whole milk samples (4/24) exceeded 150% of the label compared with only 1 for each of the 4 other lower fat products. Comparing 2% milk sampled in 2007 and 2001, the mean vitamin D<sub>3</sub> content was higher ( $P < 0.0001$ ) in 2007 than in 2001 [ $473\text{ vs. }426\text{ IU}$  ( $11.8\text{ vs. }10.6\ \mu\text{g}$ )/qt], but variability was similar in both years (27 and 30% RSD, respectively). Figure 4 gives the distribution of samples for 2% milk in 2001 and in 2006–2007.

The resampled products showed a large difference in vitamin D content in most cases, ranging from  $-558$  to  $+632\text{ IU}$  ( $-14.0$  to  $+15.8\ \mu\text{g}$ )/qt compared with

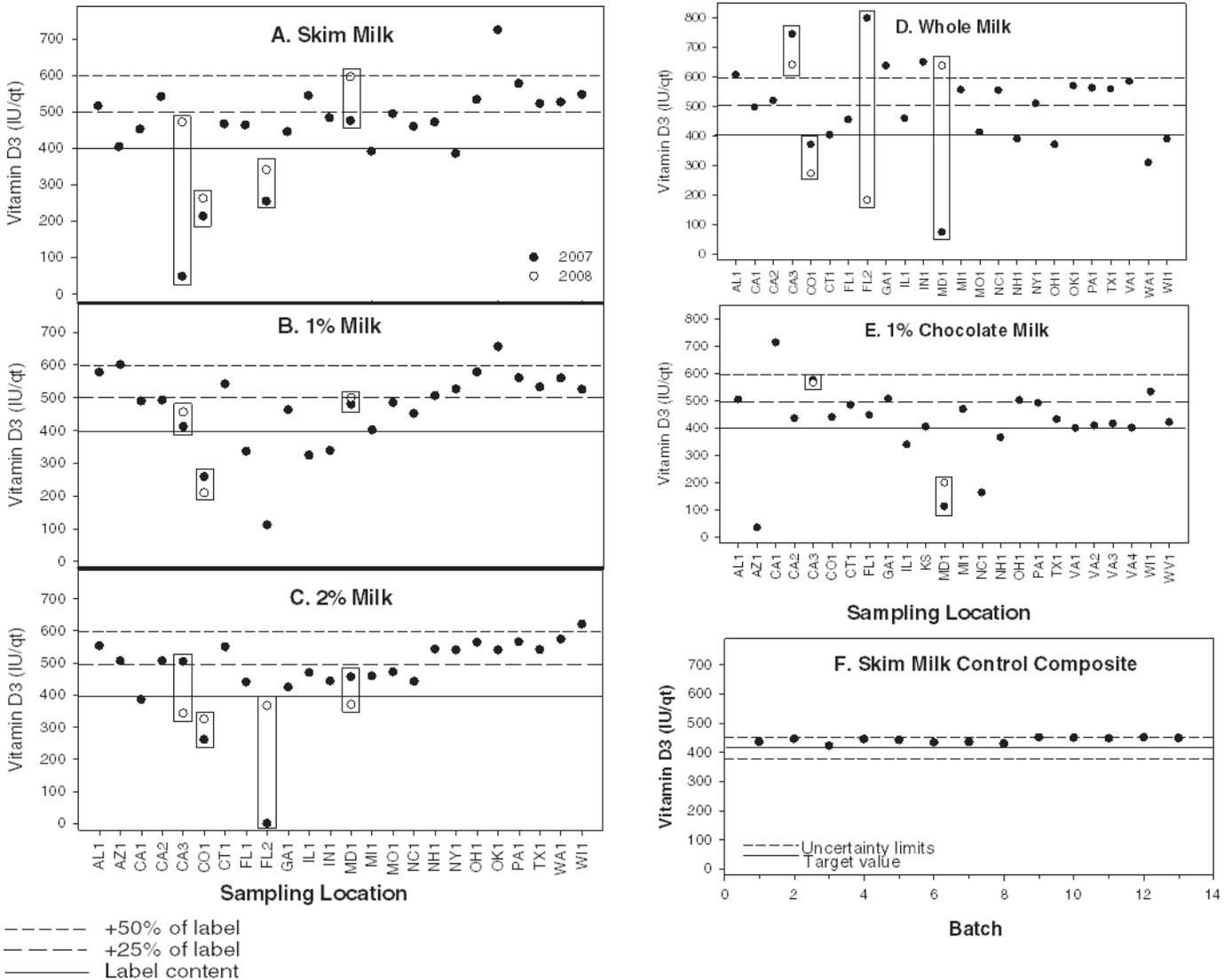
the original sample (Figure 3; open vs. filled markers). The most extreme differences were found for samples of whole milk, for which the results of the 2007 sampling from the 4 sampled locations fell above or below the fortification range.

**DISCUSSION**

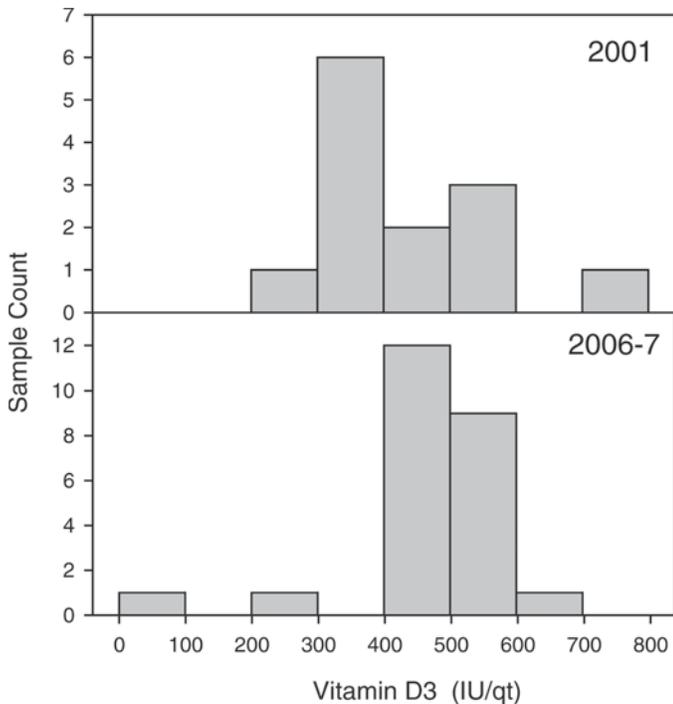
If vitamin D is added to milk, FDA regulations require that "...each quart of the food contains 400 International Units thereof within limits of good manufacturing practice" (FDA, 2007) and that the acceptable range is from 100 to 150% of the label amount. Therefore, it would be expected that manufacturers might add vitamin D at a rate exceeding the declared

content to ensure compliance. In 1992, FDA guidelines specified 800 IU (20 µg)/qt as the upper threshold for safety, calculated based on the recommended intake at that time (FDA, 1992). However, this concern may be mitigated by recent research that reports that higher levels of vitamin D may be beneficial for optimal health (Holick, 2007). In addition, Dietary Reference Intakes for vitamin D are under review (Institute of Medicine of the National Academies, 2010).

Whereas the goal of this USDA study was to update the milk vitamin D values in SR, not to monitor compliance, the results do suggest some trends and appear to support conclusions of previous researchers who reported high variability and low vitamin D content in some samples of retail milk (Tanner et al., 1988; Faulkner et



**Figure 3.** Assayed vitamin D<sub>3</sub> content of retail milk sampled in 2006–2007 (shown with filled circles). Open circles represent repeat samples obtained in 2008. Original and repeat samples by location are shown in boxes.



**Figure 4.** The distribution of vitamin D in 2% milk sampled in 2001 ( $n = 12$ ) and in 2006–2007 ( $n = 24$ ).

al., 2000; Murphy et al., 2001). However, the instances of low fortification in the sampling reported here appear to be less frequent than in the previous studies.

More cases of overfortification were found for whole milk compared with reduced fat products, although lower than declared concentrations of vitamin D occurred in all types of milk. In the 2001 samples, 7 out of 12 samples of 2% milk were below 400 IU (10  $\mu$ g)/qt; in 2007, only 2 of 24 samples were below 400 IU (10  $\mu$ g)/qt. Holick et al. (1992) found that 3 of 14 retail skim milk samples contained no vitamin D in their 1992

study; in this current study only 1 of the 120 samples had no detectable vitamin D.

Nine different points exist at which vitamin D might be added in the processing of milk, including widely divergent locations such as the raw milk tanker and the homogenizer (Hicks et al., 1996). In one method vitamin D is added in a lipid soluble form before separation of the cream, which could explain underfortification or the absence of vitamin D in low fat and skim milk and overfortification in higher fat products, as suggested previously by other researchers (Tanner et al., 1988). In the Pasteurized Milk Ordinance the FDA provided guidance, but not a requirement, on the process for fortification of milk, suggesting that vitamin D be added with a metering system after separation of the fat and before homogenization (FDA, 2007). However, a manufacturer may decide exactly how the fortification is accomplished as long as the required vitamin D content is achieved. The results from the 2008 resampling illustrated that the fortification of milk products within a given dairy or differences among processors are not necessarily consistent. Whole milk from one location was significantly underfortified in 2006–2007 and overfortified in 2008, with the reverse being true for another sampling location. On the other hand, although all the white milk products from the CO1 location failed to meet the label requirements in either 2006–2007 or 2008, they were generally consistent. Values for the statistical sampling are valid only when the entire United States is considered, and results obtained for any location are not to be taken as indicative of the vitamin D content of milk for either the state or location. The variability in vitamin D content among individual milk samples emphasizes the importance of a carefully designed, broad-based statistical sampling plan for this type of product, and the validated methodology and quality control measures implemented support the reliability

**Table 1.** Summary of results for vitamin D in milk from 2007 determined by validated HPLC-UV analysis

Item	Samples (n)	Mean <sup>1</sup>	Median	Low	High	SD	% Relative SD	Total samples in each value range <sup>2</sup>			
								<400 IU/qt	400–500 IU/qt	501–600 IU/qt	>600 IU/qt
Skim	24	456 (1.17)	473	48	724	133	29	3 (13)	13 (54)	7 (29)	1 (4)
1%	24	466 (1.19)	491	111	655	123	26	5 (21)	9 (38)	9 (38)	1 (4)
2%	24	473 (1.21)	505	ND <sup>3</sup>	621	126	27	2 (8)	12 (50)	9 (38)	1 (4)
Whole	24	498 (1.28)	513	73	797	151	30	4 (17)	9 (38)	7 (29)	4 (17)
1% Chocolate	24	417 (1.07)	433	34	714	144	35	5 (21)	16 (67)	2 (8)	1 (4)
All samples	120	462 (1.18)	481	ND	797	136	29	19 (16)	59 (49)	34 (28)	8 (7)

<sup>1</sup>Values are given in IU/quart. Means are given in IU/quart ( $\mu$ g/100 g). Mean values for the 5 types of milk did not differ significantly ( $P > 0.05$ ).

<sup>2</sup>Total samples in each value range are given as number (percentage). If a sample value was within 5% less than 400 IU (10  $\mu$ g)/quart, it was counted in the 400 to 500 range. If it was within 5% more than 600 IU (15  $\mu$ g)/quart, it was counted within the 500 to 600 range. This allowed for  $\pm 5\%$  analytical uncertainty.

<sup>3</sup>ND = not detected [ $<20$  IU (0.05  $\mu$ g)/quart].

of the data. Because on average the actual vitamin D content exceeded the labeled value, and because food labeling regulations stipulate that the content of a fortified nutrient must at least equal the declared amount, addition of a reasonable excess is acceptable.

Researchers should be cognizant of the likely deviations from the labeled vitamin D content of individual lots of milk products and sample-to-sample variability and consider the potential effect of using mean food composition values in a particular application. For example, in feeding trials that may rely on a single source of milk, average or label values could result in inaccurate assumptions about dietary intake, and analytical determination of vitamin D concentrations should be considered in such studies. Similarly, if milk from a particular producer is routinely purchased by the same consumer, any systematic underfortification could have a negative effect on health over time. For the present work, the sampling plan was based on obtaining products marketed at retail sites with sales greater than \$2 million/yr (Pehrsson et al., 2000). Certainly, deviations in the vitamin D content of milk from other brands and suppliers would be no less important to the individuals consuming those particular products.

The values for the 2007 samples have been incorporated into SR beginning with the 2009 version (Gebhardt et al., 2009). These data are statistically supported estimations of vitamin D in fluid milk in the US food supply. It is expected that, in future years, additional sampling and analysis may be done to follow any changes that may occur in the products, given the importance of vitamin D for human health.

### ACKNOWLEDGMENTS

This work was supported by the USDA Agricultural Research Service as part of the National Food and Nutrient Analysis Program, including cooperative agreement #Y1-HV-8116-11 between the USDA Nutrient Data Laboratory and Virginia Polytechnic Institute and State University. Partial support was received from the Office of Dietary Supplements and other offices and institutes of the National Institutes of Health, agreement #Y1CN5010, and from the Beverage Institute for Health & Wellness, an affiliate of The Coca-Cola Company. The detailed work of Amy Rasor and Nancy Conley (FALCC) on coordination of sample preparation is greatly appreciated.

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