Vitamin D in foods: development of the US Department of Agriculture database[1–4]

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ABSTRACT

Specific data on vitamin D_2 and vitamin D_3 are needed to enable the assessment of vitamin D dietary intake. These forms of the vitamin can occur in foods, both naturally or from fortification. The Nutrient Data Laboratory at the Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture collaborated with vitamin D experts in an analytic project with 2 major goals: *1*) to review and develop methods for analyzing a variety of food items for vitamin D content and *2*) to sample and analyze foods considered to be major contributors of vitamin D. During 2007, analysts from up to 6 laboratories compared methods, made modifications in some cases, and validated results with quality-control samples of similar food types in preparation for the analysis of sampled foods. The Nutrient Data Laboratory has prioritized foods for analysis and has identified the following as important contributors of vitamin D: finfish and shellfish, naturally occurring sources, and fortified foods such as milk, calcium-fortified orange juice, breakfast cereals, American cheese, margarines, and yogurt. A nationwide multistage sampling plan was designed and conducted to select and procure representative sample units of all such foods. After analysis of these food samples and review of the results, acceptable values for vitamin D_2 and D_3 will be disseminated in the National Nutrient Database for Standard Reference (Internet: http://www.ars.usda.gov/nutrientdata). *Am J Clin Nutr* 2008; 87(suppl):1092S–6S.

INTRODUCTION

Recently, the scientific community has focused increased attention on the need to assess the intake of vitamin D. Because multiple factors can impair sunlight-induced cutaneous vitamin D synthesis, many persons must rely on dietary sources to satisfy their vitamin D requirements (1). To support intake research, current and accurate vitamin D_2 and D_3 data for foods and dietary supplements must be determined and the variability of vitamin D contents investigated. The purpose of this report is to describe the US Department of Agriculture (USDA)/Agricultural Research Service efforts to plan and initiate a program to generate original analytic data for specific foods considered to be contributors of dietary vitamin D.

On the basis of recommendations of participants in a December 2005 vitamin D technical meeting, a working group was formed to develop a project plan to address methodology and data needs. This project plan included the following tasks: *1*) reviewing the status of existing vitamin D data, *2*) reviewing and validating existing analytic methods, *3*) developing and characterizing analytic reference and control materials, *4*) identifying foods of concern, *5*) developing nationwide sampling plans, *6*) conducting sampling and analysis, and *7*) compiling and disseminating the final approved data on the vitamin D content of foods.

THE USDA NATIONAL NUTRIENT DATABASE FOR STANDARD REFERENCE

The Nutrient Data Laboratory (NDL) develops and maintains the USDA Standard Reference (SR) and related data products (2). These products are the basis of numerous other applications that address nutrients in foods. The SR is considered to be the authoritative source of food-composition data for the United States. The database contains data for agricultural commodities, eg, wheat flour, beef, and raw carrots, as well as data for formulated and processed foods, eg, cookies, beverages, and breakfast cereals. Currently, the database contains values for up to 140 different components [including nitrogen (protein), total fat, carbohydrate, and moisture, as well as individual fatty acids, amino acids, vitamins, and minerals] in >7500 foods. A subset of the SR provides the foundation for the Food and Nutrient Database for Dietary Studies (FNDDS), which is used to support the continuous federal dietary survey, What We Eat in America, NHANES (3).

VITAMIN D VALUES IN THE USDA NATIONAL NUTRIENT DATABASE FOR STANDARD REFERENCE

Release 20 of the USDA SR (SR20) contains vitamin D values, expressed as IU/100 g, for ≈600 foods. To date, values for specific forms of vitamin D, ie, D_2 and D_3, have not been available in the database. Furthermore, the availability of data for only 600 foods limits the use and application of the vitamin D dataset for dietary intake studies. The source of vitamin D values and

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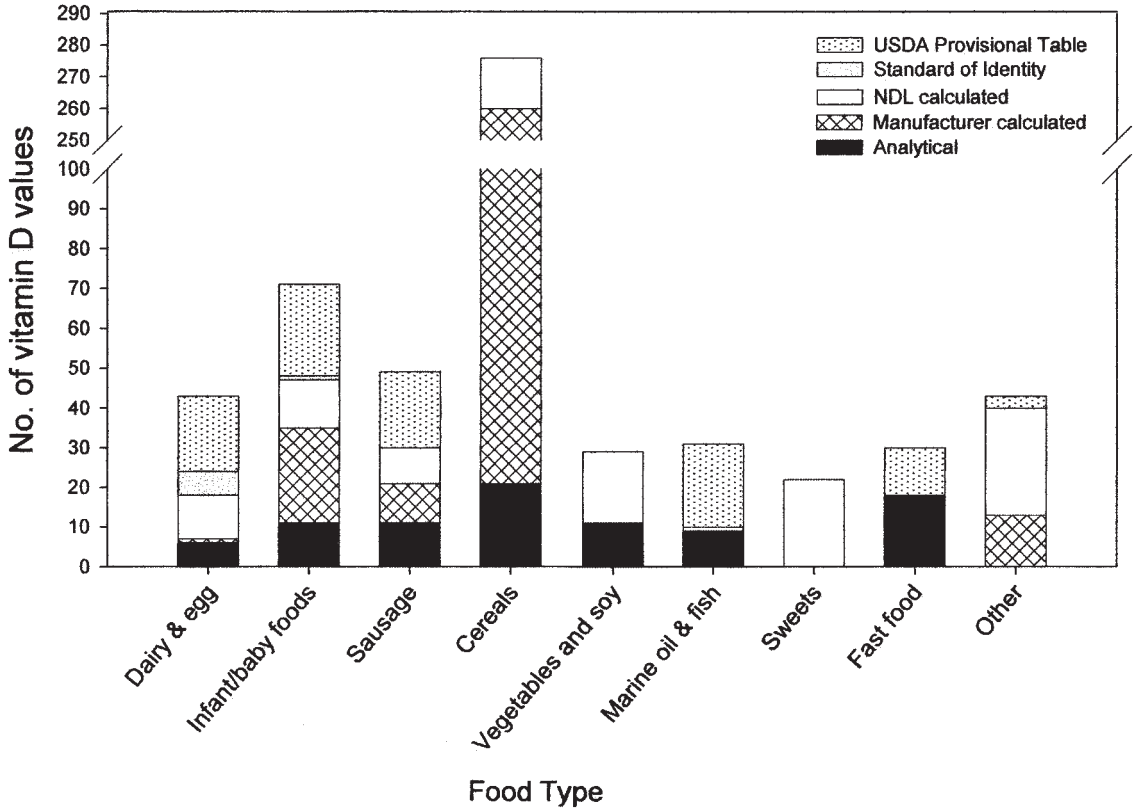

FIGURE 1. Number of vitamin D values in various food groups of the US Department of Agriculture (USDA) National Nutrient Database for Standard Reference, release 20, identified by data source. Data sources include values from the 1980 Provisional Table on Vitamin D, US Standards of Identity, calculated values provided by the food industry based on declared label claims, analytical values from contract analyses and from the food industry, and values calculated by the USDA Nutrient Data Laboratory (NDL).

their distribution by food type in the SR20 (released in September 2007) is shown in **Figure 1**. The SR20 contains a limited amount of analytic data for vitamin D. Values for ready-to-eat breakfast cereals account for >50% of the vitamin D data in the SR, with vitamin D data provided by manufacturers on the basis of calculations from label declarations of percent Daily Values. Dairy product data were imputed by NDL staff from federal fortification standards. Some data were migrated into the SR from the USDA's "Provisional Table on the Vitamin D Content of Foods," which was released in 1980 (4).

Some second-generation databases, such as the Nutrition Data System for Research (5), a popular database for nutrition studies that was developed and licensed by the University of Minnesota, and the Genesis R & D SQL and the Food Processor SQL (6), which were developed by ESHA company (Portland, OR), use vitamin D data from the USDA's SR and supplement those data with other data obtained from individual food companies or the literature.

THE NATIONAL FOOD AND NUTRIENT ANALYSIS PROGRAM

In 1997, the Nutrient Data Laboratory developed the National Food and Nutrient Analysis Program (NFNAP) to generate new analytic data for highly consumed foods (7). The NDL has used many of the standard protocols developed under this program in selecting and analyzing foods for vitamin D content. The NFNAP was initiated through an interagency agreement between the US National Institutes of Health and the USDA to support the analysis of up to 140 nutritional components in foods and to update the data to be disseminated to the research community through the NDL Web site.

The attention of the scientific community on the status of vitamin D adequacy in the population, coupled with the lack of complete and reliable datasets for vitamin D in foods, has led the NDL to identify this vitamin as a critical nutrient for NFNAP analysis. In general, the first step in prioritizing foods for sampling would be the determination of the Key Foods for a particular nutrient. Key Foods are defined as those major sources of a nutrient that together contribute 75% of the intake of that nutrient for the population (8). Because it has not yet been possible to assess the dietary intake of vitamin D, the NDL used alternate methods to develop a priority list of foods for vitamin D analysis. Resources reviewed included the USDA 1980 Vitamin D Provisional Table, federal regulations listing foods to which vitamin D can be added, and the market availability of foods with added vitamin D. Lawful additions of vitamin D under the Generally Recognized as Safe regulations or by the Food Additive petition to the Food and Drug Administration have cleared the way for fortification of some foods historically not fortified with vitamin D: calcium-fortified fruit juice, some grain products and macaroni products, yogurt, some cheese and cheese products, and certain meal replacement bars or beverages used for weight control (9). Many of these foods are now fortified with vitamin D, but

values are not yet available in the SR. Although fortification of macaroni and noodle products is permitted, vitamin D–fortified versions have not been seen in local markets as of the date of the preparation of this article and were not selected for sampling. After review of the above-mentioned resources, the NDL identified the following foods to be analyzed for vitamin D_2 and D_3 contents: representative finfish and shellfish, which are known to be natural vitamin D contributors (10–14), and the following vitamin D–fortified foods: orange juice, ready-to-eat breakfast cereals, fluid milk, margarines, sliced American cheese, and yogurt.

SAMPLING PLANS FOR FOODS SELECTED FOR VITAMIN D ANALYSES

NDL staff collaborated with statisticians at the USDA's National Agricultural Statistics Service to develop the general approach for food sampling (15), eg, the sampling frame, which includes a stratified sampling approach. A three-stage sample selection process using Chromy's probability minimum replacement PPS (probability-proportional-to-size, where size equals population density) sample-selection procedure (16) was developed. It was based on the 2000 US Bureau of the Census population data and food product market share data from AC Nielsen Inc or as identified by a specific industry, trade association, or government agency.

The sampling method was used to identify a self-weighting, nationally representative sample including 12–24 locations across the country and nationally representative of the 48 contiguous states and consolidated metropolitan statistical areas of the nation and regions. Census regions, states, and counties were selected at the first stage; retail stores, restaurants, homes, or manufacturing plants within the selected counties were selected at the second stage. At the third stage, units (eg, packages or bottles) of a specific food product were purchased for nutrient analyses. This selection process allowed for inclusion of urban and rural sites.

Twenty-four samples were analyzed for fluid milks to assess the variability across the nation. According to the US Code of Federal Regulations, the addition of vitamin D to fluid milks is optional. If added, however, the level of vitamin D should be 400 IU per quart (17). In 1993 the National Dairy Council reported that ≈98% of all homogenized milk sold in the United States was fortified with vitamin D (18). As recently as 2003, the US Public Health Service/Food and Drug Administration Grade A Pasteurized Milk Ordinance stated that if vitamin D is added to fluid milk, the acceptable range is 100–150% of label claims, or 400–600 IU per quart (19).

However, a survey by the Food and Drug Administration published in 1988 reported that only 26% of 669 milk samples obtained in 3 states across the country were within the fortification range (20). In 1992 Holick et al (21) reported that the vitamin D levels were outside the fortification range in 71% of a sample of 42 containers of milk from 5 Eastern states. In 1993 Chen et al (22) reported that milk samples obtained from 10 states (including the earlier 5 Eastern states) showed that 80% of the samples contained either 20% less or 20% more than the label claim. Murphy et al (23) reported the values for milk samples collected in New York between 1997 and 2000. The frequency distribution of values from these samples ranged from <160 IU to >680 IU, with significant numbers of observations falling outside of the required range.

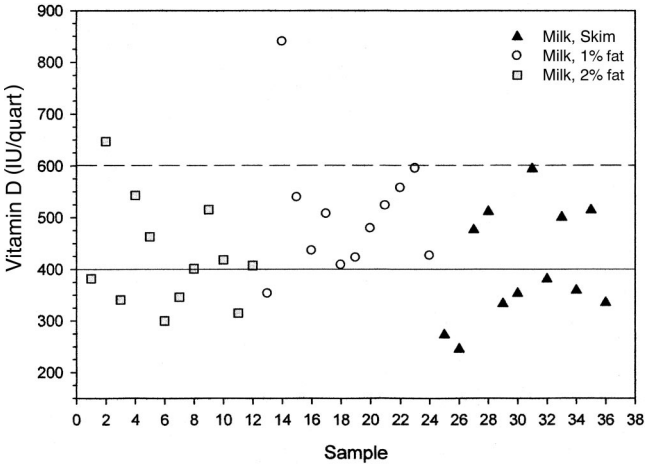

FIGURE 2. Vitamin D content of 3 types of milk from 12 locations nationwide, sampled in 2001 as part of the US National Food and Nutrient Analysis Program (1 quart = 946 mL). Values were determined by a commercial laboratory using AOAC method 999.05 (liquid chromatography). The laboratory's uncertainty on their in-house quality control material is ±25%. The solid line indicates the target fortification level; the dashed line indicates the maximum fortification recommended.

The USDA data on 3 types of fluid milk (skim, 1% fat, and 2% fat) obtained from 12 locations sampled across the United States in 2001 according to the NFNAP sampling plan are shown in **Figure 2**. A large commercial laboratory, using AOAC (Association of Official Analytical Chemists) method 995.05, a liquid chromatographic technique, measured vitamin D values. Although a few of the values for the 36 samples were above the 600 IU fortification maximum, one-third of the samples of all 3 types fell below the minimum required level of 400 IU. Means and SDs of milk samples, in IU per quart (946 mL), were as follows: skim, 423 ± 103; 1% fat, 507 ± 126; and 2% fat, 406 ± 109. This variability in the amount of vitamin D found in fluid milk samples could be due to the variability in fortification of milk across the United States or it could be due to laboratory variability or accuracy issues. Insight into the sources of vitamin D variability in milk should be gained under the present sampling and analysis program, which incorporates both increased sampling and the use of methods validated by laboratories with demonstrated proficiency in vitamin D analysis.

ANALYTIC ASSESSMENT OF VITAMIN D FOOD CONTENT AND QUALITY CONTROL

Samples of the seafood and vitamin D–fortified foods to be analyzed during the current project were purchased from locations designated by the sampling protocol and were shipped to the Food Analysis Laboratory Control Center at Virginia Polytechnic Institute and State University (VPI&SU). On arrival, the samples were verified, logged into the data system, and processed for analysis. Scientists at that institution had developed standard protocols, including homogenization procedures, for handling the samples. Also, quality-control materials similar in food type (eg, dairy, cereal, and fish) and concentration to the nationwide samples were developed by scientists at VPI&SU to monitor precision and support accuracy determinations. Sample

aliquots and these quality-control food materials were stored at VPI&SU until they were distributed to the analytic laboratories.

The uncertainty of estimates for food components is determined by both the variability attributable to the food itself, ie, difference in brands, forms, and fortification practices, as well as variability due to the measurement process, ie, different laboratories, different methods, and day-to-day laboratory performance. At the initiation of the vitamin D project, a discussion among vitamin D analysts indicated that various methods were available, including the specific AOAC international methods (24), and other methods used in various expert laboratories (ie, those laboratories that have been reporting vitamin D values in recent years).

At the same time that the food sampling phase was being planned and executed, analysts from 6 laboratories (2 commercial laboratories and 4 expert laboratories) began a comparison of analytic methods by using the 5 quality-control food materials and the US Pharmacopeia vitamin D standard. Scientists at the USDA's Food Composition and Methods Development Laboratory, one of the expert laboratories, also developed an improved method for vitamin D analysis that combines the best aspects of the 2 existing AOAC methods. This method, which will be applied to various food types, is more efficient in the use of extracting solvents and uses an internal standard for the best quality of quantification.

In addition to the 5 quality-control food materials, selected Standard and Certified Reference Materials were obtained from 2 official organizations, the National Institute of Standards and Technology, Department of Commerce (25), and the Institute for Reference Materials and Measurements, which maintains the reference materials formerly prepared and certified by the BCR (Bureau Communautaire de Référence), Belgium (26). Samples of all the quality-control food materials were distributed by VPI&SU to the various laboratories to provide standardized homogeneous test material to support the characterization of the materials. This phase of the project resulted in consensus values for the quality-control materials and the qualification of 4 laboratories to analyze vitamin D. Those data will be presented in a separate publication.

Presently, 3 expert laboratories have completed the harmonization of their procedures with other laboratories involved in the study. Samples of specific foods are being analyzed by selected laboratories for the various food types. A separate project on the composition of dietary supplements is being conducted by various collaborators. The resulting data will be released in the Dietary Supplement Ingredient Database (27).

FUTURE PLANS

After the analysis of food samples and the review and evaluation of results, acceptable values for vitamin D_2 and D_3 will be migrated into the USDA National Nutrient Databank System to be compiled and disseminated for use in the assessment of the intake of vitamin D. Existing vitamin D values, when not replaced by analytic values, will be reviewed for currency and accuracy. Vitamin D in the SR will be populated first with analytic values (from USDA contract analyses, the scientific literature, and food industry), then augmented with values calculated by food industry (based on fortification levels) or calculated by the NDL (from percent Daily Value food label declarations.) Food industry representatives will be consulted as to the particular form of vitamin D added to fortified foods. When vitamin D data are expanded to include all SR foods in the subset for the USDA Food and Nutrient Database for Dietary Studies, the NDL will calculate remaining vitamin D values by using standard imputation procedures (28), including calculating values for similar foods with appropriate concentration adjustments (eg, solids, fat), calculating values by recipe or estimated formulation, or assignment of an assumed zero (eg, most vegetables).

After vitamin D intake has been estimated by What We Eat in America, NHANES, the formal process of developing a Key Foods list can be undertaken to provide more complete information for future updates. The USDA will monitor formulation changes in foods and dietary supplements as well as possible updates in federal nutrition policies for vitamin D. Additional foods may be identified for analysis on the basis of their ranking in the Key Foods list for vitamin D. Market share data will be considered in selecting specific brands or product types for analysis.

The expansion and quality improvement of vitamin D values in the USDA SR will provide accurate and current data to epidemiologists and other investigators who use the vitamin D database to better assess the adequacy of dietary vitamin D intake in the US population. It is likely that results of these analytic studies will resolve some of the outstanding questions about the apparent variability in vitamin D values in food and dietary supplement products.

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