Vitamin D assessment in population-based studies: a review of the issues1–4

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ABSTRACT
In the past decade, research on the relation between vitamin D exposure and disease in population-based studies has increased exponentially. These studies have involved measurement of vitamin D exposure by means of several methods: blood assays, self-reported dietary and supplemental intakes, and sunlight exposure questionnaires or diaries. As with all exposure measurements, researchers must consider the validity of their assessment tools for capturing vitamin D exposure. The purpose of this article is to summarize our current understanding of the various approaches to measuring vitamin D status within populations as reviewed at the 2007 Experimental Biology symposium, “Assessment of Vitamin D in Population-Based Studies.” In summary, serum 25-hydroxyvitamin D is the accepted biomarker for short-term vitamin D status, but estimates of long-term dietary and supplemental intakes of vitamin D and long-term sunlight exposure may be the most logically feasible indicators of lifetime vitamin D exposure in population-based studies. Also discussed are issues investigators should consider when analyzing relations between vitamin D exposure and disease outcomes in population-based studies as well as research avenues that need further exploration. The best method for assessing vitamin D status in population-based studies will depend primarily on the research question asked and the critical window of vitamin D exposure hypothesized to be most important. Am J Clin Nutr 2008;87(suppl):1102S–5S.

INTRODUCTION
Vitamin D deficiency is prevalent throughout the world. In the past 5 y, numerous epidemiologic analyses have associated vitamin D insufficiency with adverse health conditions. The accuracy with which vitamin D status is assessed in epidemiologic studies is one of the primary factors determining the quality of study findings and the public health or clinical recommendations that can be drawn from them. Nutritional epidemiologists have long studied the validity of dietary assessment and its effect on our ability to relate dietary exposures to disease. In the specific case of vitamin D, for which sunlight exposure can account for most (≈90%) of the vitamin D in circulation (2), we must be concerned with the validity of vitamin D quantified not only from diet and supplements, but also from sunlight exposure. We do not fully understand how well the assessment of vitamin D from questionnaires about diet, supplements, or sunlight exposure truly captures individual or population vitamin D status.

It is widely accepted that serum 25-hydroxyvitamin D [25(OH)D] is the best indicator of a person’s short-term vitamin D status, but not all investigators have the resources to assay 25(OH)D. Moreover, as discussed below, a one-time measurement of 25(OH)D may not be ideal for all research questions. The objective of this article is to discuss the strengths and limitations of serum 25(OH)D assessment and whether other measurements of vitamin D status, ie, estimates of vitamin D intake and sunlight exposure, can be used in population-based studies to accurately estimate vitamin D status. We highlight issues that should be considered when conducting research with these measurements and address areas of research that need further exploration.

SERUM 25(OH)D CONCENTRATIONS TO ESTIMATE VITAMIN D STATUS
As reviewed by Zerwekh (3), serum 25(OH)D is the most valid estimate for determining vitamin D status in humans, but this measurement is not without limitations when used in population-based research. First, a baseline measurement of serum 25(OH)D may not accurately reflect a person’s vitamin D status over the course of a year. It is well known that serum 25(OH)D varies by season: concentrations are highest in the summer and the fall and are lowest in the spring (4). We do not fully understand whether a person’s peak concentration during the year, their average concentrations throughout the year, or the difference between their zenith and nadir concentrations is most relevant for disease pathophysiology. It would be ideal to obtain measurements of serum vitamin D in each season, but logistically this may not be affordable or possible, especially in large population studies. For this reason, analyses of serum 25(OH)D should at least be adjusted for season of blood draw. Second, because the cutoffs for

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defining vitamin D deficiency and sufficiency are being debated (5), investigators would be well served to consider exploring multiple cutoffs for serum 25(OH)D with respect to disease and relating these measurements to functional indicators (eg, blood parathyroid hormone concentrations).

Third, 25(OH)D concentrations may need to be considered in the context of genotype. We do not fully understand whether genetic variation in proteins involved in vitamin D transport, function, and metabolism (6), such as vitamin D binding protein (DBP), vitamin D receptor (VDR), 25-hydroxylase (CYP27A1), 1α-hydroxylase (CYP27B1), and 24-hydroxylase (CYP24), partially determine a person’s 25(OH)D status.

Fourth, for many outcomes of interest in epidemiologic studies, long-term nutritional exposure is most relevant. Yet, for vitamin D, we do not fully understand the intra-individual variation in 25(OH)D over many years. Measurements of serum 25(OH)D at entry into a study may not reflect a person’s long-term vitamin D status. This may be the case especially for persons who spent significant portions of their life in regions with differing annual ultraviolet B (UVB) radiation, or if persons have made significant alterations in lifestyle practices over time that affect vitamin D exposure (eg, use of sunscreen, dietary supplements, etc). For example, Knight et al (7), using retrospectively reported sunlight exposure, found a reduced risk of breast cancer related to increased sun exposure among women in adolescence (10–19 y), during the critical period of breast development. Measures of sunlight exposure at other periods of time were not related to cancer risk. Similarly, in another study, men born in regions of the United States with high versus low solar radiation had a reduced risk of prostate cancer (8). These findings illustrate that exposure to vitamin D at different times during the course of a person’s life may influence disease. The only methods available to assess vitamin D status over the lifetime, in the absence of repeated, longitudinal serum 25(OH)D measurements, are repeated, longitudinal or retrospective assessment of vitamin D intake and sunlight exposure.

Dietary and Supplemental Intake of Vitamin D to Estimate Vitamin D Status

Estimating the dietary intake of vitamin D from foods requires a nutrient database to analyze the vitamin D content of foods. The US Department of Agriculture’s (USDA’s) Nutrient Database for Standard Reference (version 20) (9) is one of the most widely cited nutrient databases. However, this database is not complete for vitamin D. Currently, the USDA is in the process of revising the vitamin D nutrient database [reviewed by Holden and Lemar (10)].

After the USDA’s vitamin D nutrient composition data are updated, it will be important for researchers to investigate how this database performs compared with the earlier provisional version, as was done previously for carotenoids (11). Specifically, investigators will need to compare the old and new databases’ estimated average intakes of vitamin D in population groups. It also will be useful to determine whether use of the old and updated databases result in similar or different rankings of individuals with respect to their usual vitamin D intake. It likewise should be determined whether correlations between dietary vitamin D estimates and serum 25(OH)D concentrations improve with the updated nutrient database. Such research will help us verify whether previous studies using the older USDA nutrient composition database were valid.

Despite the known inaccuracies in assessing vitamin D from existing nutrient databases, and the fact that vitamin D status is greatly influenced by sunlight exposure (12), several studies have been published showing positive relations between intake of vitamin D and the prevalence or incidence of several different diseases (13–18). There is significant potential for vitamin D exposure misclassification in such studies. Therefore, it is debatable how well the results of these studies effectively establish the influence of vitamin D on such outcomes.

Indeed, diet is not the only, and sometimes not the most important, determinant of a person’s serum 25(OH)D status. Determinants often identified include age, race, time spent outdoors or sunlight exposure, geographic region of residence, season of blood draw, vitamin D intake from the diet or supplements, body mass index or body fatness, and physical activity (19, 20). Giovannucci et al (19) found that, among men in the Health Professionals Follow-Up Study, physical activity and darker skin were stronger predictors of serum 25(OH)D status than was dietary vitamin D intake. In another study, Brot et al (21) showed that active sunbathing was the greatest determinant of serum 25(OH)D concentrations in Danish women (ages 45–58 y). For this reason, in the absence of serum 25(OH)D, investigators should consider using information on predictors of this biomarker in addition to estimates of vitamin D intake to predict a person’s vitamin D status, as shown in the study by Giovannucci et al (19).

By contrast, some investigators (20, 22–24) have reported that oral intake of vitamin D ranks individuals well with respect to serum 25(OH)D measurements. However, the strength of this relation may vary depending on the population being studied or the time period during which serum 25(OH)D was assessed. For example, serum 25(OH)D and vitamin D intake from oral supplements may be stronger when the influence of solar radiation is weaker, such as among African Americans (23) or in winter months, especially in areas of the world with limited UVB exposure during significant portions of the year (25).

At the same time, assessment of oral vitamin D intake in population-based studies can be used to estimate a person’s lifelong exposure to vitamin D when obtaining longitudinal serum 25(OH)D measures is not feasible. However, dietary assessment must cover intake over a long enough period of time (eg, at least a 3-mo window) to capture less commonly consumed foods (ie, fatty fish) to best classify persons with respect to their usual vitamin D intake. Additionally, investigators must also query for dose, frequency, and duration of vitamin D supplement use. Supplemental sources of vitamin D contain significantly more of the vitamin (400 to 2000 IU) than do most vitamin D–containing foods (most < 400 IU) (1) and are likely consumed more frequently among consistent supplement users than are vitamin D–rich foods.

Latitude and Sunlight Exposure to Estimate Vitamin D Status

Some of the earliest studies that proposed a relation between vitamin D and nonskeletal outcomes were ecologic studies. These studies showed associations between population mortality or incidence rates of certain diseases (eg, cancer and multiple sclerosis) by geographic latitude of residence or season (26, 27).
Latitude or season was seen as a proxy measure of vitamin D exposure, because most vitamin D comes from the skin’s synthesis of the vitamin after exposure to solar radiation (2). Although instrumental in generating hypotheses, these ecologic studies alone should not be used to support a role for vitamin D in disease pathophysiology. Latitude of residence does not capture individual-specific sun exposure or dietary practices, and individuals are also not equally distributed by latitude with respect personal factors that affect vitamin D status, such as race and age (28, 29). Indeed, vitamin D deficiency is prevalent even in areas experiencing high UVB exposure (30–32).

For this reason, population-based studies interested in vitamin D exposure should assess individual-specific sun exposure when possible instead of relying on region of residence for this estimate. Two fields of research, skin cancer (33) and age-related eye disease (34), frequently use estimates of self-reported individual sunlight exposure. However, despite decades of assessing sunlight exposure with respect to these diseases, no validated sunlight questionnaires are widely used (35).

Of the studies validating different sunlight exposure questionnaires or diaries, few have investigated associations between these measurements and serum 25(OH)D as a primary focus. Some of these studies have presented correlations between serum 25(OH)D and self-reported sun exposure in the range of 0.16 to 0.39 (21, 22, 36, 37). Although these correlation coefficients are significant, they are not particularly strong, which suggests that sun exposure assessed with these methods does not explain most of the variation in serum 25(OH)D concentrations. In a study by Brustad et al (38), serum 25(OH)D values were directly related to UV hours per day, but this relation leveled off after 1.1 to 2.0 UV hours. This suggests that serum concentrations and sunlight exposure measurements may not be linearly related but may plateau after a certain amount of sunlight exposure is achieved. More research is needed in large population studies to gauge how well self-reported sunlight exposure assessed with questionnaires and diaries ranks individuals with respect to their vitamin D status.

As with assessment of vitamin D intake, a major strength of solar radiation questionnaires is that they provide a means of estimating retrospective sunlight exposure throughout a person’s lifetime, which is especially relevant for chronic diseases that develop over many decades. If researchers choose to use sunlight exposure questionnaires as proxy measures for vitamin D status, they should ask additional questions with respect to anatomical distribution of sunlight (ie, use of sunscreen and clothing for coverage of skin) and other factors that influence the skin’s ability to synthesize vitamin D, such as skin color. Other methods that are not questionnaire-based, such as personal UV dosimetry (39) or measures of histologic solar skin damage (40) should also be explored to assess sunlight exposure as it relates to vitamin D status.

Development of a standard sun exposure questionnaire validated specifically in relation to serum 25(OH)D is needed to better understand how individuals are ranked by sunlight exposure questions and to identify the types of questions that are most useful for capturing vitamin D status. Such questionnaires should be validated in different age and racial/ethnic groups and by latitude of residence and season. Researchers should determine whether additional questions regarding clothing or sunscreen use, which offer protection from UVB rays, help to improve the validation.

CONCLUSION

A combination of baseline blood 25(OH)D concentrations and questions about lifetime dietary, supplemental, and sunlight exposure is likely the best way to estimate vitamin D status throughout a person’s lifetime. Deciding to use any one of these measurements or a combination of all 3 will primarily depend on the question being asked and the time period during which one hypothesizes that vitamin D exposure is most important, whether this is short-term or long-term exposure.

It is clear that we need to better understand intra-individual variation in 25(OH)D concentrations among people and how this variability relates to disease status. Validation of dietary assessment tools for intake of vitamin D by use of the updated USDA nutrient database will be needed. We will also need to develop and validate sunlight exposure questionnaires to accurately capture vitamin D status. Currently, the limitations of assessing vitamin D status with serum biomarkers or self-reported dietary intake or sunlight questionnaires should be considered when reviewing the body of literature with respect to vitamin D status and disease risk. This will allow us to make the most informed recommendations for the public concerning requirements for vitamin D intake and sunlight exposure to optimize health outcomes.

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