

Vitamin D across the lifecycle: physiology and biomarkers¹⁻³

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ABSTRACT

The field of vitamin D public health research has a pressing need to define sensitive and specific predictors of vitamin D status that can be used to determine whether an individual or population has a supply of vitamin D that is sufficient to meet requirements. The aim of this review is to highlight the considerations needed when evaluating evidence of the relations between vitamin D biomarkers and functional or health outcomes across the life cycle. It draws attention to the importance of distinguishing between biomarkers of supply, function, and outcome and of considering the many factors that could influence interpretation, such as life stage, ethnicity, body mass index, liver and kidney function, and dietary calcium and phosphorus intake. The vitamin D biomarkers that have shown the most utility to date are the plasma concentration of 25-hydroxyvitamin D (supply), the plasma concentration of parathyroid hormone (function), and the presence or absence of rickets (outcome). However, a single biomarker of vitamin D status or threshold value is unlikely to be valid in all situations. The field therefore needs research to refine existing biomarkers or establish new indicators that take the many factors into account and to identify useful functional biomarkers of vitamin D status for infants, children, women of reproductive age, and specific ethnic groups. However, evidence using the biomarkers currently available shows that frank vitamin D deficiency is a major public health problem in many parts of the world that requires urgent attention. *Am J Clin Nutr* 2008;88(suppl):500S–6S.

INTRODUCTION

Vitamin D plays a pivotal role in calcium homeostasis and skeletal metabolism throughout life. Classical vitamin D deficiency causes rickets in children and osteomalacia in children and adults (1). Vitamin D is also important for the functioning of many other systems, such as the immune, cardiovascular, and reproductive systems (2, 3).

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], acts in a wide range of tissues, and vitamin D receptors (VDRs) are present in many cells, including those of the liver, pancreas, brain, lung, breast, skin, muscle, and adipose tissue (4–6). Because of the wide-ranging involvement of vitamin D in many systems of the body, an inadequate vitamin D supply may have potential health impacts in addition to rickets and osteomalacia. For example, epidemiologic evidence has linked poor vitamin D status to osteoporosis, osteoarthritis, tuberculosis, diabetes mellitus, multiple sclerosis, preeclampsia, periodontal disease, and several cancers (3–5, 7–10).

Some of these potential health effects, such as susceptibility to infection and autoimmune diseases, may be relevant at all ages. Additionally, the adequacy of the vitamin D supply to tissues

might be particularly important at specific times of life such as in older persons for reducing susceptibility to chronic degenerative diseases, in the fetus for organogenesis, during puberty for breast development, and during childhood and adolescence for bone mineral accrual and somatic growth.

In this article, we discuss the considerations that those working in the field of vitamin D research and public health must take into account when reviewing evidence of the relations between vitamin D biomarkers and functional or health outcomes across the life cycle. We also discuss the need to distinguish between biomarkers of supply, function, and outcome and describe the many factors that could influence interpretation.

BIOMARKERS

Research scientists, health professionals, and policy makers need to gauge, either in populations or in individuals, whether the supply of vitamin D is sufficient to meet requirements at the tissue level at different times during the life cycle. Over the years, there has been a search for a biomarker (a biological factor that can be measured in living people) that provides information about the adequacy of vitamin D supply for maintaining good health and that thereby gives an indication of vitamin D status to inform public health policy and practice.

This parallels the search for biomarkers in other aspects of nutrition and health. It is important to recognize that there are several shades of meaning in the term *biomarker*, which is often used interchangeably to describe different attributes. This can lead to confusion and misunderstanding unless the term is strictly defined. The ideal nutritional biomarker would link quantitative information about nutrient supply directly to the impact on health or disease endpoints. However, this would require integrating information on all of the variables that could influence the nutrient's effectiveness once it is in the body, such as the efficiency of its absorption from the diet (and, in the case of vitamin D, skin synthesis), excretion, metabolic conversion, and the responsiveness of target systems. In practice, a single biomarker is only likely to provide information about one segment of the pathway

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between supply and outcome, and this limits the interpretations that can be made with it.

To introduce some clarity of thinking into this complex area, the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE) developed a system of definitions. The European Union funded this project to formulate a conceptual framework of scientific criteria for health claims regarding foods (11). This system (**Figure 1**) is now in common use by nutritional scientists in Europe to consider the role of biomarkers in nutritional surveillance. The schema divides biomarkers into the following interlinking categories:

- Markers of exposure that provide information about the supply of a nutrient to target tissues. An example is the plasma 25-hydroxyvitamin D [25(OH)D] concentration as a marker of vitamin D supply.
- Markers of function that provide information about the biological response to a nutrient. Examples are plasma des-carboxy-prothrombin and undercarboxylated osteocalcin as markers of vitamin K function.
- Markers of an intermediate health endpoint that provide information about disease risk. Examples include blood pressure as a marker of cardiovascular health and bone mineral density as a marker of bone health.
- Markers of disease. These include stigmata of disease such as bone deformity or fracture as markers of rickets and osteoporosis.

The National Institutes of Health and others have also developed conceptual frameworks for biomarkers (12, 13). However, regardless of the conceptual framework used, the general principle remains that the interpretation of a specific biomarker as an indicator of nutritional sufficiency depends on understanding the aspect of the pathway between supply and health that it represents. It also requires recognizing that the biomarker's utility and validity could differ at each life stage, depending on the biological requirement for that nutrient and the physiologic role of the selected biomarker.

SOURCES OF VITAMIN D

Persons acquire vitamin D from the diet and by cutaneous synthesis after exposure to sunlight. Investigators estimate that

the general Western European population derives most (80–90%) of its vitamin D from endogenous synthesis (14, 15). This proportion, however, varies considerably around the world, across population groups, and between individuals, mainly because of wide differences in skin exposure to ultraviolet B (UVB) radiation, the efficiency of cutaneous synthesis, and food fortification practices (16). Reliance on dietary sources of vitamin D is greatest during the winter at temperate latitudes and among people with restricted skin exposure to sunshine (3, 16).

The endogenous supply of vitamin D₃ (cholecalciferol) depends on exposure of the skin to UVB radiation at wavelengths of 290–315 nm, the efficiency of cutaneous vitamin D synthesis, and the extent to which vitamin D is degraded within the skin (2, 17, 18). The quantity of UVB radiation of the relevant wavelengths that reaches the skin depends on latitude, altitude, air quality, cloud cover, time of day, and, at northerly and southerly latitudes, month of the year. In addition, clothing habit and sunscreen use affect UVB skin exposure. The efficiency of vitamin D cutaneous synthesis depends on skin pigmentation and age. Intracutaneous degradation increases after prolonged continuous exposure to UV radiation and depends on the duration of each exposure rather than the frequency of repeated exposures (17).

Dietary sources contain vitamin D in the form of either vitamin D₃ or vitamin D₂ (ergocalciferol) (19, 20). The richest sources of vitamin D are oily fish, egg yolks, food supplements, and, in the countries where they are available, fortified products such as margarine, milk, and cereal (16, 21). Other animal products, such as muscle, liver, fat, and kidney, also contribute to dietary intake by delivering vitamin D as the metabolite 25(OH)D (14, 19).

After synthesis, cutaneous vitamin D enters the circulation and is transported to the liver bound to vitamin D binding protein (DBP). The liver produces DBP, which circulates at a much higher concentration than vitamin D and its metabolites (22). Dietary vitamin D is predominantly absorbed in the chylomicron fraction and is transported to the liver via the lymph (23). Dietary 25(OH)D is absorbed into lymph or directly into the portal vein and is transported to the liver bound to DBP (23, 24). The absorption of dietary vitamin D is generally high at all stages of life (25), although it is reduced when the intake of fat is low (19).

METABOLISM OF VITAMIN D

The liver hydroxylates vitamin D to 25(OH)D, which it secretes into blood plasma (26). 25(OH)D circulates mostly bound to DBP, where, because of its relatively long half-life of 2–3 wk (27), it serves as a reservoir for further hydroxylation to either the biologically active metabolite 1,25(OH)₂D or to 24,25-dihydroxyvitamin D [24,25(OH)₂D]. The hydroxylation of 25(OH)D takes place in the kidney and in extrarenal tissues via the 1 α -hydroxylase enzyme CYP27B1 to 1,25(OH)₂D and via the 24-hydroxylase enzyme CYP24 to 24,25(OH)₂D (28, 29). The 1,25(OH)₂D produced in the kidney acts as an endocrine modulator of calcium and phosphate homeostasis. The kidney secretes it into the circulation and it is transported, bound to DBP, to tissues involved in the regulation of calcium and phosphorus supply, namely intestine, bone, parathyroid glands, and the kidney itself. The half-life of 1,25(OH)₂D in the circulation is very short compared with that of 25(OH)D, \approx 4–6 h (15, 30). The 1,25(OH)₂D produced in extrarenal target tissues acts locally in an autocrine or paracrine fashion and generally does not reach the circulation. The function of 1,25(OH)₂D in cells of both the

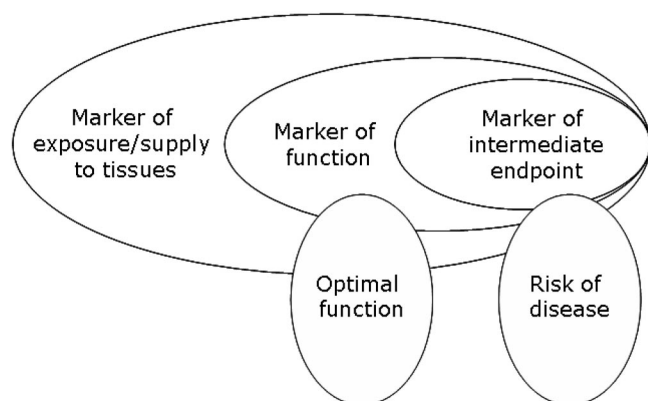


FIGURE 1. Conceptual framework for nutritional biomarkers. Adapted from the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE) schema for the scientific basis for health claims on foods. Reproduced with permission from the *British Journal of Nutrition* (11).

calcium-phosphate homeostatic system and other target tissues is to induce genomic and nongenomic responses mediated through its interaction with VDRs (2, 6). Vitamin D itself and 25(OH)D have little or no interaction with VDRs and therefore do not induce VDR-activated responses directly (26).

The alternative product of 25(OH)D hydroxylation in the kidney and extrarenal cells is 24,25(OH)₂D (2, 29). It circulates bound to DBP at a concentration in plasma that is directly related to that of 25(OH)D (31). Its production is generally the first step in the metabolic pathway to inactivate and degrade 25(OH)D and thereby prevent vitamin D intoxication (2, 26). However, significant concentrations of 24,25(OH)₂D are found in the circulation (22), even in persons with vitamin D deficiency. This suggests that this metabolite may also have a functional role (2). In support of this possibility, effects of 24,25(OH)₂D, acting in concert with other vitamin D metabolites and parathyroid hormone (PTH), have been described on osteoblast and osteoclast function, bone strength, and parathyroid gland function (26, 29, 32). However, 1,25(OH)₂D is regarded as the primary metabolite that elicits the biological actions of vitamin D.

VITAMIN D BIOMARKERS

Thus, there are multiple influences on the supply of vitamin D and its metabolites to the cells of the body. In addition, uncertainties are inherent in estimates of the amount of vitamin D entering the body from cutaneous synthesis and dietary sources. Gauging whether the supply of vitamin D to an individual or population is adequate to meet their needs by measuring skin exposure to sunlight and dietary intake is difficult.

The search for sensitive and specific biomarkers of vitamin D status has therefore been a priority, and many possible biomarkers have been researched that provide information about vitamin D supply, function, intermediate health endpoints, or disease (Figure 2). The search has mostly focused on the central role of vitamin D in calcium homeostasis and skeletal metabolism (7, 33–35). Recently, biomarkers have also been proposed based on other roles of vitamin D, such as in insulin secretion and the innate immune response (36).

However, all biomarkers of vitamin D have limitations because many physiologic factors and methodologic difficulties affect them. Of the possibilities researched to date, only the

plasma concentration of 25(OH)D, plasma concentration of PTH, and the absence or presence of rickets have found wide utility. Details of these biomarkers are below, followed by a short description of some of the other potential biomarkers.

Plasma 25-hydroxyvitamin D concentration

The plasma 25(OH)D concentration is a useful vitamin D biomarker because it has a long half-life in the circulation and its concentration is not under tight homeostatic regulation. This biomarker therefore reflects vitamin D supply and usage over a period of time (26).

The plasma concentration of 25(OH)D depends on the quantity of vitamin D delivered to the liver, the amount of 25(OH)D produced by the liver, and the half-life of 25(OH)D in the plasma. These are affected by many factors, including the amount of vitamin D entering the body from the skin and intestines, the amount of body fat and muscle, the 25-hydroxylase activity and DBP production in the liver, the volume of the extracellular compartment, the DBP plasma concentration and affinities that influence the delivery of vitamin D to the liver and of 25(OH)D to target tissues, and the efficiency of cellular uptake of 25(OH)D and its rate of conversion to 1,25(OH)₂D or 24,25(OH)₂D (22, 23, 31, 37–39). Interpretations of plasma 25(OH)D concentration, therefore, need to take into account such considerations as increased physiologic need, body adiposity, hemodilution, and the effects of aging, disease, and malnutrition on liver and kidney function. In addition, the methodologic difficulties with the accurate measurement of plasma 25(OH)D concentration are considerable (40, 41). The field of vitamin D public health needs research to determine how best to refine the interpretation of plasma 25(OH)D concentration to account for these various confounders. Despite these caveats, the plasma 25(OH)D concentration is a useful biomarker of vitamin D supply to target tissues in most situations.

This contrasts with the utility of the plasma 25(OH)D concentration as a biomarker of vitamin D function. 25(OH)D has little interaction with VDRs, and 1,25(OH)₂D usually elicits the biological actions of vitamin D. The extent to which the plasma concentration of 25(OH)D indicates the adequacy of the vitamin D supply to meet functional requirements, therefore, depends on many factors. These include the uptake of 25(OH)D by target

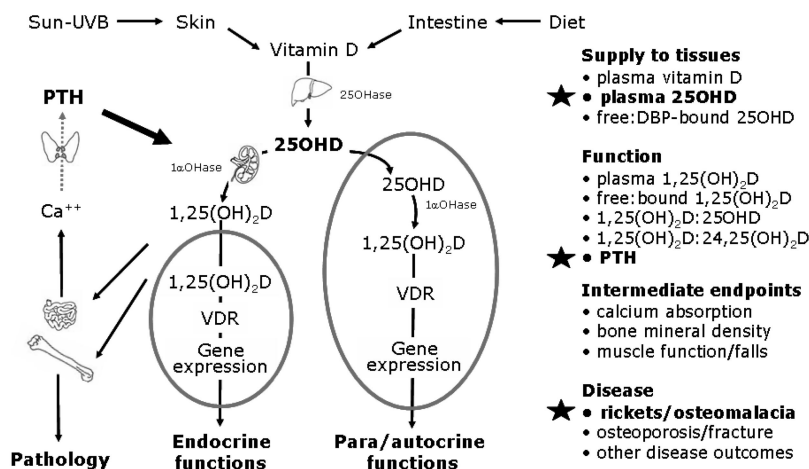


FIGURE 2. Potential biomarkers of vitamin D supply, function, and outcome. The stars highlight those markers that have found the widest utility to date and that are described in detail in the text.

cells; the rate of conversion to $1,25(\text{OH})_2\text{D}$ or $24,25(\text{OH})_2\text{D}$; the delivery of renal $1,25(\text{OH})_2\text{D}$ to, and its uptake by, target tissues; the expression and affinity of VDR in target tissues; the responsiveness of cells to activated VDR; and the efficiency of induced metabolic pathways. Because of this, there has been a search for a biomarker that reflects the body's response to vitamin D supply more directly. The plasma PTH concentration has received the most attention to date (5, 42).

Plasma parathyroid hormone concentration

PTH has been proposed as a functional marker of vitamin D status largely because an elevated plasma PTH concentration is a recognized risk factor for osteoporosis (43), and vitamin D supplementation can lower it (44). As illustrated in **Figure 3**, PTH is linked to $25(\text{OH})\text{D}$ through the calcium-phosphate homeostatic system (16, 45). PTH secretion is stimulated when plasma ionized calcium decreases as the result of low dietary calcium intake or poor calcium absorption as a result of an insufficient vitamin D supply or other factors, such as an increased physiologic calcium requirement. High dietary phosphorus intake and high plasma phosphate concentration can also induce PTH secretion.

An inverse relation between plasma concentrations of PTH and $25(\text{OH})\text{D}$ has been reported in many cross-sectional and intervention studies in the elderly (46–49), postmenopausal women (49, 50), and young persons (51). Some studies suggest that the plasma PTH concentration reaches a plateau as the $25(\text{OH})\text{D}$ concentration increases (47, 50), whereas others describe an exponential negative relation (linear when the data are expressed in logarithms) throughout the physiologic range of $25(\text{OH})\text{D}$ concentrations (46, 52). The reasons for these discrepancies are, as yet, unclear but they could reflect differences among the populations studied and methods used (16).

However, the plasma PTH concentration varies widely within and among individuals at any given concentration of $25(\text{OH})\text{D}$ (46, 47) because the plasma PTH concentration depends on many factors other than vitamin D status, such as stage of life, ethnic background, dietary calcium and phosphorus intakes, time of day, kidney function, physical inactivity, and drug use (16, 52–56). In addition, the choice of assay method is important because an assay could detect both PTH fragments and intact molecules (56). Currently, the most commonly used assays detect the 7–84 portion of the molecule as well as intact PTH (57). Circulating PTH fragments can accumulate in older persons with decreased

kidney function and could contribute to measurements of the plasma PTH concentration (38, 46).

Most of the studies supporting the use of PTH as a functional index of vitamin D status have been conducted among older white persons living in Europe and the United States. However, studies in other age groups and in people from different geographic and ethnic backgrounds caution against its universal applicability. Studies in Africa and China, for example, have shown that plasma PTH concentration is elevated in populations with a low calcium intake, even when vitamin D status is good, and that the inverse correlations between plasma PTH concentration and bone health indexes (bone mineral density and fracture) observed in Western countries are not found (58, 59). Furthermore, plasma PTH concentrations increase physiologically during puberty (60, 61), a period when more calcium is needed for skeletal growth, and decrease during pregnancy and lactation, when the PTH-related peptide plays a central role in calcium homeostasis (62, 63).

The field of vitamin D public health needs research to understand better the relations between plasma PTH concentrations and both vitamin D status and functional health endpoints at different stages of the life cycle and in different populations before this index can be used with confidence as a functional biomarker of vitamin D status in groups other than elderly white persons in countries with a Western lifestyle. Standardizing the plasma PTH concentration for kidney function, dietary calcium and phosphorus intake, assay method, and sampling time could improve its utility as a marker of vitamin D function in different populations.

Presence or absence of rickets

Traditionally, vitamin D deficiency has been defined as the presence of signs and symptoms that characterize rickets and osteomalacia. Diagnosis requires the use of X-rays, clinical evaluation of bone deformities, bone biopsies, and biochemical tests of metabolic bone disease, such as elevated plasma alkaline phosphatase activity. In general, only persons with severe deficiency disease are likely to come to the attention of health professionals. No simple screening tool for vitamin D deficiency disease is available for population surveillance, other than the identification of bone deformities consistent with rickets in children. Nevertheless, the presence or absence of rickets in children remains a useful index of the prevalence of frank vitamin D deficiency at the population level.

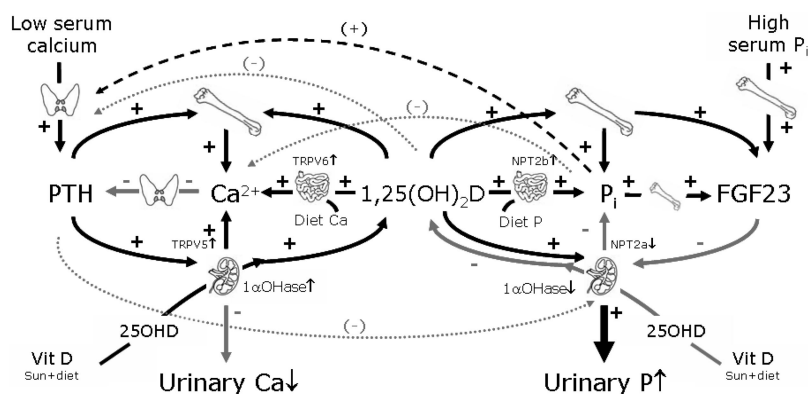


FIGURE 3. A schematic representation of the integrated calcium-phosphorus-vitamin D homeostatic system. Reproduced with permission from *Proceedings of the Nutrition Society* (45).

As with other markers of vitamin D status, there are caveats to the interpretation of rickets prevalence data. Recent studies of children in Africa and Bangladesh have identified cases of rickets that do not appear to be related to primary vitamin D deficiency (1, 16, 64, 65). These children had a low plasma phosphate concentration, normal or high plasma PTH and 1,25(OH)₂D concentrations, and a plasma 25(OH)D concentration above that generally seen in vitamin D–deficiency rickets. The children were from populations with very low calcium intake and the evidence to date suggests that they had a primary or secondary calcium deficiency. Caution therefore needs to be exercised in the use of the absence or presence of rickets in children as a marker of vitamin D status.

Other potential vitamin D biomarkers

The physiology of vitamin D suggests that other biomarkers may have the potential to give insights about vitamin D sufficiency (Figure 2). Potential markers of supply include the concentration of vitamin D (66, 67), the free (non-DBP bound) fraction, and the half-life of 25(OH)D in plasma (22). Potential markers of function include the total concentration and free fraction of 1,25(OH)₂D (4, 68) and its ratio to the concentration of 25(OH)D or 24,25(OH)₂D in plasma. Potential markers of intermediate health outcomes and disease include fractional calcium absorption (69), bone mineral density (7), muscle function (7), fracture incidence (7), insulin sensitivity (36), and innate immune response (36). However, to date, none of these have found widespread utility either because they lack specificity for vitamin D or because applying them to population surveys is associated with logistic or methodologic difficulties.

Potential biomarkers of supply

The plasma concentration of vitamin D is generally very low, and the appearance of significant quantities of this parent compound in the circulation is considered to indicate the saturation of muscle and fat depots and of the 25-hydroxylase enzyme system in the liver (66, 67). Plasma vitamin D concentrations may be useful in population groups with a moderate to high vitamin D supply but not in those with a marginal or deficient supply. However, interpretation will depend not only on the supply of vitamin D from the skin and intestines but also on body composition and liver function.

The free fraction of 25(OH)D in plasma may be an index of the amount available for cellular uptake, although the free hormone theory does not fully explain the experimental data in this regard (22). The half-life of 25(OH)D in plasma provides information about the balance between supply and metabolism at the tissue level. Exploration of the utility of these potential biomarkers of vitamin D supply has been hampered, among other factors, by methodologic problems and assay difficulties (4).

Potential biomarkers of function

PTH and other factors regulate the total concentration of 1,25(OH)₂D and its free fraction in plasma (26) and, except in cases of severe vitamin D deficiency, 1,25(OH)₂D and 25(OH)D concentrations correlate with each other very little, if at all (38, 52, 70). Thus, the 1,25(OH)₂D concentration is likely to have limited value as a biomarker of vitamin D function, except when the 25(OH)D supply is low. The free fraction may reflect the 1,25(OH)₂D available for VDR binding, although, as with

25(OH)D, the free hormone theory does not fully explain the experimental data (22).

Total and free 1,25(OH)₂D plasma concentrations vary depending on life stage, liver and kidney function, and dietary calcium and phosphorus intake. Expressing the plasma concentration of 1,25(OH)₂D relative to that of 25(OH)D or 24,25(OH)₂D may give a more informative indication of the extent to which the supply of 25(OH)D is sufficient to meet the cellular requirements for 1,25(OH)₂D. However, methodologic difficulties and the narrow range of plasma 1,25(OH)₂D concentrations observed in people without severe vitamin D deficiency have, thus far, limited the use of any of these potential biomarkers.

Potential biomarkers of outcome

Fractional calcium absorption has been proposed as an index of vitamin D adequacy because 1,25(OH)₂D regulates the active transport of calcium across the intestine. However, this and other measures of health and disease endpoints, such as bone mineral density, insulin sensitivity, and fracture incidence, are not specific for vitamin D, and many factors influence them, including stage of life, ethnicity, dietary intake, and intestinal calcium and phosphorus availability. In addition, the methods of assessment are difficult and expensive, which is likely to restrict their application to research studies rather than to population surveillance.

ASSESSMENT OF VITAMIN D STATUS

Because of the uncertainties in the use of functional or outcome biomarkers, attempts have been made to define thresholds of vitamin D supply, as measured by plasma 25(OH)D concentration, that denote adequacy in terms of functional and health endpoints. For many years, the threshold has been set at a 25(OH)D concentration that marks the top end of the range observed in patients with vitamin D–deficiency rickets or osteomalacia: 25 nmol/L (10 ng/mL) in the United Kingdom (3, 71) and 27.5 nmol/L (11 ng/mL) in the United States (72). More recently, there have been proposals to raise this threshold to take into account the possible relations between a low vitamin D supply and health or disease endpoints, such as low bone mineral density, fracture risk, and poor muscle function (7, 14, 38, 67). Other articles in this supplement discuss these considerations, the evidence behind these proposals, and their applicability at different stages of life.

GLOBAL PREVALENCE OF VITAMIN D DEFICIENCY

Based on either the presence or absence of rickets or a plasma 25(OH)D concentration <25 nmol/L, the current evidence shows that the prevalence of vitamin D deficiency is high in many parts of the world and is a cause of considerable public health concern (3, 16). High prevalence rates have been reported, for example, among elderly people in the United Kingdom, especially those living in residential care (73); infants and pregnant women from ethnic minorities at northerly and southerly latitudes (74–78); persons living in or near the tropics who wear concealing clothing or who spend little time out of doors (79, 80); and children from Asia and the Middle East (16, 81). Public health researchers and policy makers must find solutions to address this burden of deficiency and not delay action because of



the current scientific debate about what thresholds of 25(OH)D should be used to denote optimal function and health (16).

SUMMARY

The aim of this overview was to set the stage for reviewing the evidence presented elsewhere in this volume of the relations between biomarkers of vitamin D and functional or health outcomes. The ultimate objective of these discussions is to define sensitive and specific predictors of whether an individual or population has a supply of vitamin D that is sufficient to meet their needs and that can therefore be used to assess vitamin D status. As has been discussed, it is important to distinguish between biomarkers of supply, function, and outcome, and to consider the many factors that may influence interpretation of such biomarkers, such as life stage, ethnicity, body adiposity, liver and kidney function, and dietary intake of calcium and phosphorus. It is unlikely, therefore, that a single biomarker of vitamin D status or a single threshold value will be identified that can be used in all situations. The field of vitamin D public health needs research to refine existing biomarkers or establish new indicators to take these many factors into account and to identify useful functional biomarkers of vitamin D status for infants, children, women of reproductive age, and different ethnic groups.

The contributions of the authors were as follows—AP, GRG, and IS: conceived the presentation and article, discussed the contents, and debated the conclusions; AP: wrote the first draft of the article; GRG and IS: contributed to the content and agreed on the final version. The authors had no conflicts of interest.

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