The role of vitamin D in calcium homeostasis is well established. Recently, there has been growing interest in and evidence for previously unrecognized roles of vitamin D and its metabolites in the physiology of normal health and the pathophysiology of a wide range of clinical disorders. It is an appropriate time to reflect on this recent evidence and the implications for clinical chemists.

THE EVIDENCE FOR NEWLY DISCOVERED ROLES FOR VITAMIN D
An important clue to roles of vitamin D beyond calcium homeostasis came with the finding that the 1,25-dihydroxyvitamin D nuclear receptor is present in most tissues. A reevaluation of the physiological and pharmacological actions of vitamin D produced evidence that vitamin D can regulate the immune system and thereby is implicated in several immune-mediated diseases. There is also growing evidence that 1,25-dihydroxyvitamin D, the biologically active hormone, may regulate different cellular processes associated with carcinogenesis, including differentiation, proliferation, and apoptosis.

Epidemiological studies have suggested that vitamin D may play a role in protecting against cancer, heart disease, and type 1 diabetes—conditions that account for >60% of all deaths in the western world. In addition, the literature contains reports that vitamin D insufficiency may play a role in the development of multiple sclerosis, rheumatoid arthritis, and asthma, and increases the risk of tuberculosis, pneumonia, poor cognitive function, periodontal disease, and reduced muscle tone and lower-extremity function.

The strongest epidemiological evidence for non-calcium-related effects of vitamin D comes from investigations of its role in protection against cancer. For many years, common cancers have been known to occur more frequently in northerly populations across the world than in populations close to the equator. As long ago as 1980, Garland and his coworkers suggested that sunlight, and therefore vitamin D, may reduce the likelihood of colon cancer. Subsequent studies suggested that sunlight and vitamin D may have a protective effect on a range of common cancers including breast, prostate, ovary, and pancreas. These studies have become more convincing with the increasing sophistication of study design, the maturation of evidence based medicine, and the inclusion of 25-hydroxyvitamin D (25OHD) measurement. One meta-analysis demonstrated a 50% lower risk of colorectal cancer when the serum 25OHD was >83 nmol/L (>33 µg/L) compared to <30 nmol/L (<12 µg/L) (1). Another study using pooled analysis showed a 50% reduction in the risk of breast cancer for individuals with serum 25OHD of approximately 130 nmol/L (52 µg/L) compared to those with a serum 25OHD <33 nmol/L (<13 µg/L) (2). A further metaanalysis of second cancers after a diagnosis of nonmelanoma skin cancer concluded that solar ultraviolet-B (UVB) radiation reduces the risk of many internal cancers, including squamous cell carcinoma of the colon, stomach, and rectum (3).

Epidemiological studies provide only indirect evidence that vitamin D, resulting from exposure to UVB, is a protective agent. Therefore, vitamin D supplementation studies are important to establish whether vitamin D has a direct protective role.

Several vitamin D supplementation studies have been reported, especially related to individual cancer types, and most show a modest positive effect. However, the most striking recent study based on vitamin D supplementation is the metaanalysis of randomized control trials looking at total mortality (3). The authors identified 18 such trials of vitamin D intake that reported results for total mortality and found a 7% reduction in total mortality from any cause for patients, most taking relatively modest supplements of vitamin D (400–800 IU/day), compared to controls.

The importance of metaanalysis cannot be overstated because not all individual epidemiological and supplementation studies have showed clear-cut results. If vitamin D does have a protective effect against a range of cancers and immune-mediated diseases, then it is only one of a number of factors involved in this complex and disparate range of disorders. Patient populations will vary according to age, ethnic mix, body mass index, and social factors including smoking and...
alcohol intake. Single serum 25OHD measurements are of limited value because of the known seasonal variation and lack of consistency between methods (see below). Vitamin D replacement studies suggest even more variables, including the appropriate dose of the vitamin and the stage in life at which it should be administered to give a later protective effect.

More studies on the putative protective effect of vitamin D are required to clarify the etiology and mechanism of vitamin D action and therapeutic options. In the mean time it would seem sensible for individuals to consider their likely current vitamin D status. Studies suggest that a majority of Northern European and Canadian populations have serum 25OHD concentrations that are below 75 nmol/L (30 µg/L). A natural lack of UVB is being exacerbated by a sedentary and largely indoor lifestyle.

HOW MUCH VITAMIN D DO WE REQUIRE?
The doses of vitamin D that may be required for protection against cancer and immune-mediated diseases are higher than the minimum recommendations for the vitamin for the protection against bone disease. In the United States the current recommended daily allowance for vitamin D is 400 IU/day, and the tolerable upper intake is set at 2000 IU/day, although a recent study suggested that this upper limit could safely be increased to 10 000 IU/day. To achieve the serum concentrations of 25OHD that appeared to protect against colorectal and breast cancer (1, 2), the required intake of vitamin D would be 1000–2000 IU/day. A recent review has recommended a daily intake of >1000 IU for all adults to bring the 25OHD concentration to >75 nmol/L (>30 µg/L) in more than half of the population. This level of daily supplementation is justified by results of randomized control trials that evaluated thresholds for serum 25OHD concentrations in relation to bone mineral density, lower-extremity function, dental health, risks of fractures from falling, and colorectal cancer (4). The recent review by Holick gives a comprehensive analysis of vitamin D requirements and treatment strategies (5).

Human skin in white individuals exposed to UVB is very efficient at making vitamin D, and it has been estimated that 5–10 min exposure to the sun of hands, legs and arms, 2 or 3 times a week, is more than adequate to produce vitamin D in quantities sufficient for the protective effects. However, excessive exposure to UVB is recognized as being harmful, and has led to medical advice of restricted exposure to UV radiation and the need to use sunscreens. The sensible solution to acquiring vitamin D stores would seem to lie in a combination of a few minutes of limited and safe exposure to UV light when possible, coupled with appropriate vitamin D replacement therapy.

THE IMPLICATIONS OF NEW ROLES FOR VITAMIN D FOR CLINICAL CHEMISTRY
The measurement of 25OHD in blood and other biological fluids is challenging. Early methods that relied on competitive protein binding or immunoassay to measure 25OHD included a solvent extraction step to remove the sterol from vitamin D-binding protein. Manufacturers of 25OHD immunoassay methods have sought to replace this solvent-extraction step with blocking agents to facilitate the inclusion of 25OHD assays on automated platforms. Evidence in practice suggests that the success of this block-and-displace approach is limited. Clinical chemists are aware of spuriously high 25OHD results in individual patient samples and higher than expected imprecision. For example, the all-method mean CV for 20 recent specimens distributed through the Vitamin D External Quality Assessment Scheme was 18.7% (range 16.6%–20.2%), and the CV for some individual immunoassay methods was greater than this all-method mean.

A second challenge to the measurement of 25OHD arises from the application of the assay to assess the adequacy of vitamin D replacement therapy. Vitamin D that is used for replacement purposes occurs in 2 major forms. Cholecalciferol (vitamin D3) is the natural animal form, and ergocalciferol (vitamin D2) is derived from plants. Increasing globalization of health and medicinal products means that both vitamin D3 and D2 are used by the public and by patients in all countries. Although vitamins D3 and D2 are structurally similar, the differences are sufficient to cause a variable response in the immunoassays used to measure the hydroxylated form of these preparations. Some immunoassays are claimed to be equipotent for the measurement of 25OHD3 and 25OHD2, whereas others preferentially detect one form. Some immunoassays in use may lead to serious clinical misclassification of individual patients.

To address the problems arising from immunoassay methods of vitamin D measurement, clinical chemists are establishing 25OHD measurement techniques based on mass spectrometry. Such methods typically involve sample pretreatment to separate the 25OHD from its binding protein followed by liquid chromatography and quantification using tandem mass spectrometry. Although these methods allow simultaneous measurement of both 25OHD3 and 25OHD2, method harmonization is limited, and there is no reference preparation or agreed calibrant for 25OHD. Clinical chemists also must clarify whether they are reporting 25OHD3, 25OHD2 or total 25OHD.
As we seek to understand the importance of newly discovered roles for vitamin D in health and disease, the accurate and precise measurement of 25OHD in blood is essential. Better agreement among methods is needed both to allow more meaningful comparison among research studies and to facilitate agreement on appropriate minimum and optimum replacement targets for protective treatment with vitamins D3 and D2. Clinical chemists and manufacturers have a responsibility to agree on quality standards and performance targets for the measurement of 25OHD.

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