Calcium and bone metabolism

Calcium metabolism or calcium homeostasis is the mechanism by which the body maintains the blood levels of circulating calcium within narrow limits.[1] In this process, bone acts as a reservoir for calcium enabling the increase/decrease of blood plasma calcium levels by absorption/deposition from/to the bone tissue as part of the continuous process of bone turnover. Disruption to this process leads to hypo- or hypercalcemia with associated health effects.

Calcium levels within the blood are regulated by parathyroid hormone (PTH) and calcitonin. PTH is produced in the parathyroid glands, which are located on the thyroid gland. It is secreted in response to low calcium levels and is responsible for increasing calcium levels, which it does in different ways; initially by reducing excretion by the kidneys and stimulating intestinal absorption. If this process is not sufficient to restore normal calcium levels, PTH will also stimulate the maturation of bone osteocytes to increase bone resorption and the release of calcium. Conversely, calcitonin is released by the thyroid gland in response to elevated plasma levels in order to decrease the levels of circulating calcium.

The active absorption of calcium from the intestine is regulated by 1,25-dihydroxy vitamin D (also referred to as calcitriol) which is derived from cholesterol (via 7-dehydrocholesterol, the vitamin D_3 precursor[2]). When the skin is exposed to ultraviolet light, cholesterol is converted to pre-vitamin D_3 which is then isomerised to Vitamin D_3 and converted to 25-OH vitamin D_3 in the liver. PTH regulates the renal conversion of 25-OH vitamin D to the active hormone 1,25-dihydroxy vitamin D, which acts on the epithelial cells of the small intestine to alter the rate of calcium absorption. When calcium levels are high, PTH levels are reduced which effectively prevents the conversion of 25-OH vitamin D to 1,25-dihydroxy vitamin D, thereby inhibiting calcium absorption from the intestine. When calcium levels are low, on the other hand, PTH production is upregulated, which in turn increases conversion of 25-OH vitamin D to 1,25-dihydroxy vitamin D in the kidneys, stimulating intestinal absorption of calcium.

What is vitamin D?

- Vitamin D is a secosteroid with two forms[3] (D_2 and D_3), which are differentiated by the presence of a single methyl group and double bond on the vitamin D_2 molecule.
- Vitamin D_2 is derived from ergosterol (plant sterol), while vitamin D_3 is derived from cholesterol.
- Vitamin D is readily hydroxylated at carbon 25 and subsequently on carbon 1 to produce the active form of vitamin D.

Sources of vitamin D?

The major source of vitamin D is exposure to sunlight.[2] Variation is observed in many individuals as a result of seasonal changes and the 'strength' of the available UV light due to the angle of the earth in relation to the sun. It is therefore very common for individuals in northern locations to have very low vitamin D levels, particularly during winter. Furthermore, the use of sun screens with a high protection factor can reduce the skin's ability to produce vitamin D by up to 95%[4]. In addition to the limitation caused by a lack of exposure to the sun, there are only very few foods that contain naturally occurring vitamin D (e.g. some fish, mushrooms and fish oils).

What to measure?

Because both vitamin D_3 and vitamin D_2 may contribute to an individual's vitamin D level, the assay method should respond equally to metabolites of both forms of vitamin D, even if concentrations of 25-OH vitamin D_2 and D_3 are not reported separately. Furthermore, a structural isomer of 25-OH vitamin D known as 3-epi-25-OH vitamin D has been reported in certain serum samples, particularly those from infants. Although its biological activity in humans has not yet been determined, it can be a potential source of measurement bias.[5]

The determination of an individual's vitamin D status based on the total circulating levels of both forms of vitamin D should be used to inform the clinical decision-making process.

Levels of vitamin D?

There is no universal agreement on the optimal concentration of 25-OH vitamin D. Ranges should be based on clinical decision values that apply to both sexes of all ages rather than population-based reference ranges. There are also many other factors that may influence 25-OH vitamin D values: diet, time of day, sun exposure, season of year, geographic location, age, use of sunscreen and/or protective clothing and skin pigmentation.[4, 6–9]

Recommendations for 25-OH vitamin D levels can be summarised as:

Deficient	**< 20 ng/mL**	**< 50 nmol/L**
Insufficient	**20 – 30 ng/mL**	**50 – 75 nmol/L**
Sufficient	**> 30 – 100 ng/mL**	**> 75 – 250 nmol/L**

Function of vitamin D

Non-skeletal

Apart from its role in maintaining calcium homeostasis, vitamin D has additional functions. A variety of studies have investigated these extra-skeletal functions which include (but are not limited to) vitamin D's role in obesity and diabetes, the prevention of falls, cancers and cardiovascular disease.

A comprehensive investigation by the Endocrine Society[10] suggested that while there was considerable observational data to support the association between vitamin D and the above-mentioned conditions, further studies and long-term randomised controlled trials were required to definitively conclude the positive association between Vitamin D level and non-skeletal health benefits.

Effect of elevated PTH levels

Elevated PTH levels will stimulate bone resorption due to the maturation of osteoclasts digesting the bone matrix, which can lead to an increased risk of fracture, osteopenia and osteoporosis. Increased levels of PTH can also have an impact on bone mineralisation and cause e.g. osteomalacia, as a result of the associated increased renal loss of phosphate. It is therefore necessary for individuals to maintain sufficient levels of 25-OH vitamin D to avoid elevations in PTH.

Standardisation

Vitamin D testing is a widely discussed and intensely scrutinised topic in recent history. The increase in interest is largely due to the startling prevalence of vitamin D deficiency worldwide and the growing number of studies linking deficiency to multiple clinical conditions unrelated to bone health.

Experts noticed a disparity between laboratories and assay results with the dramatic increase in vitamin D tests performed. Numerous reports demonstrate that the variability in 25-OH vitamin D measurements are hindering international efforts to develop evidence-based clinical guidelines. Many organisations and scientists have expressed the need for standardisation of 25-OH vitamin D measurements. Thus, the Vitamin D Standardisation Program (VDSP) was established in 2010 with the goal of promoting 25-OH vitamin D concentration measurements that are accurate and comparable over time and across locations and laboratory procedures to improve the practice of clinical and public health world-wide.[11] VDSP is an international effort conducted by the US Office of Dietary Supplements, National Institutes of Health (NIH) in collaboration with the US Centres for Disease Control and Prevention (CDC), the National Centre for Environmental Health (NCEH), the US National Institute of Standards and Technology (NIST) and the Belgian Laboratory for Analytical Chemistry, Faculty of Pharmaceutical Sciences, at Ghent University.

- There has been considerable discussion regarding the concentrations associated with vitamin D deficiency.
- The conflicting 25-OH vitamin D cut-off values in different guidelines can be explained by their different intentions.[12] For example, the purpose of the Endocrine Society's guideline[13] was to establish cut-off levels for vitamin D deficiency aimed at clinicians for the treatment of patients at risk of deficiency, rather than to make recommendations for a minimal level for normal healthy individuals, which is covered by the Institute of Medicine (IOM) report.[14]
- Although there is no consensus on the appropriate 25-OH vitamin D cut-off values, many leading authorities believe that health-based reference values are preferable.
- The development of evidence-based guidelines for the diagnosis and management of diseases related to vitamin D deficiency requires the combination and interpretation of data generated in different research and epidemiological studies using different analytical methods.
- Many organisations and scientists agree that there is a need for the standardisation of 25-OH vitamin D measurements in order to overcome these problems. However, this requires 25-OH vitamin D assays to be sufficiently accurate and aligned over time, location and methodology.

The VDSP's objectives are to:

- Standardise the measured 25-OH vitamin D concentrations in national health surveys in accordance with the reference measurement procedures (RMP) ID-LCMS/MS.
- Evaluate the differences in measured 25-OH vitamin D concentrations among standardised national health surveys.
- Expand standardisation efforts from national surveys to include assay manufacturers, clinical and research laboratories.
- Enable the use of standardised data in patient care and public health activities.

Immunoassay product portfolio

ELISA portfolio		ChLIA portfolio	
Product name	**Code**	**Product name**	**Code***
25-Hydroxy Vitamin D[S]	AC-57SF1	25 VitD[S]	IS-2500N/IS-2530N
25-OH Vitamin D	EQ 6411-9601[a]	1,25 VitD[XP]	IS-2000[c]/IS-2030
1,25 Dihydroxy Vitamin D	AC-62F1	1,25-Dihydroxy Vitamin D	IS-2400[d]/IS-2430
Intact-PTH	EQ 6421-9601[b]	Intact PTH	IS-3200/IS-3230

a Validated in combination with EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P and Sprinter XL (EUROIMMUN devices).
b Validated in combination with EUROIMMUN Analyzer I and EUROIMMUN Analyzer I-2P.
c Sample purification fully on-board
d Unique and proprietary immunocapsules for a simplified sample purification procedure without the need for organic solvents

*** IS-##30 are the corresponding control sets.**

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