Skeletal

Vitamin D

Analyte Information

Vitamin D

Introduction

Vitamin D is a lipid soluble vitamin that acts as a hormone. It stimulates intestinal calcium absorption and is important in maintaining of adequate phosphate levels for bone mineralization, bone growth, and remodelling.
No longer is vitamin D regarded only as a steroid hormone that prevents rickets in children. It's also believed to be involved in the regulation of cell growth proliferation and apoptosis (programmed cell death), as well as modulation of the immune system and other functions. Its roles in cancer prevention, diabetes, motor function, and immunologic processes have opened the door for large population-based studies. The role of vitamin D in these disease states and how the alterations of its levels may affect the risk of diseases development has been verified.

Metabolism

There are two main forms of vitamin D: vitamin D_2 (ergocalciferol - Fig.1) and vitamin D_3 (cholecalciferol – Fig.2), the main form obtained from animal sources. Synthesis of vitamin D_3 occurs also naturally in the skin through exposure to ultraviolet B (UVB) radiation from sunlight (Fig.3). The amount of cholecalciferol produced in the skin is dependent on the surface area exposed, skin pigmentation, age, season, latitude and use of sun blockers. The sufficient amount of vitamin D can be synthesized during careful exposure of the arms and legs to sunlight for approximately 10 - 15 minutes per day in the summer months.

Fig.1: Ergocalciferol

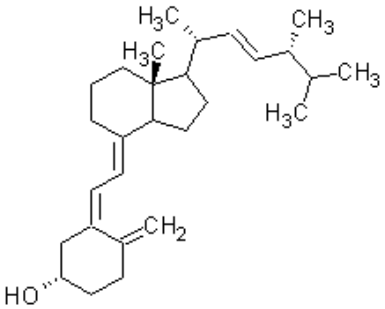


Fig.2: Cholecalciferol

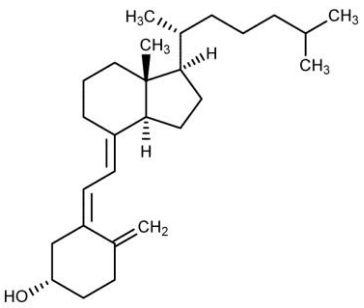
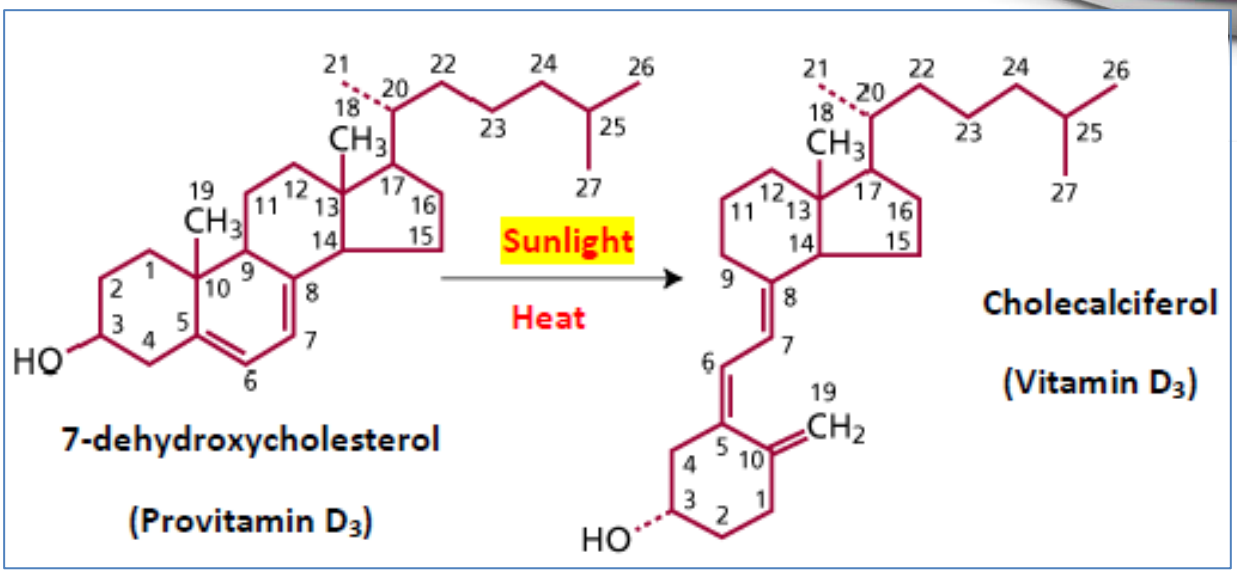


Fig.3: Synthesis of cholecalciferol in the skin



Vitamin D_2 (ergocalciferol) does not occur naturally but is manufactured by the UV irradiation of provitamin D_2 (ergosterol), which occurs in different kind of plants, molds, and yeasts (Fig.4).

Fig.4: Production of ergocalciferol

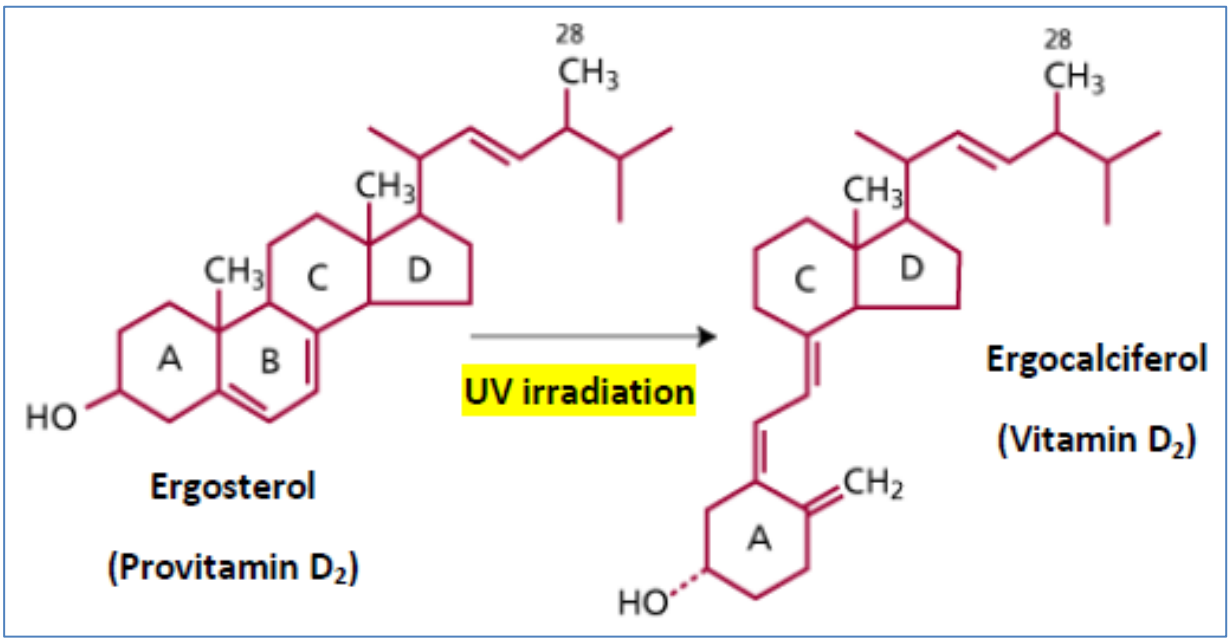
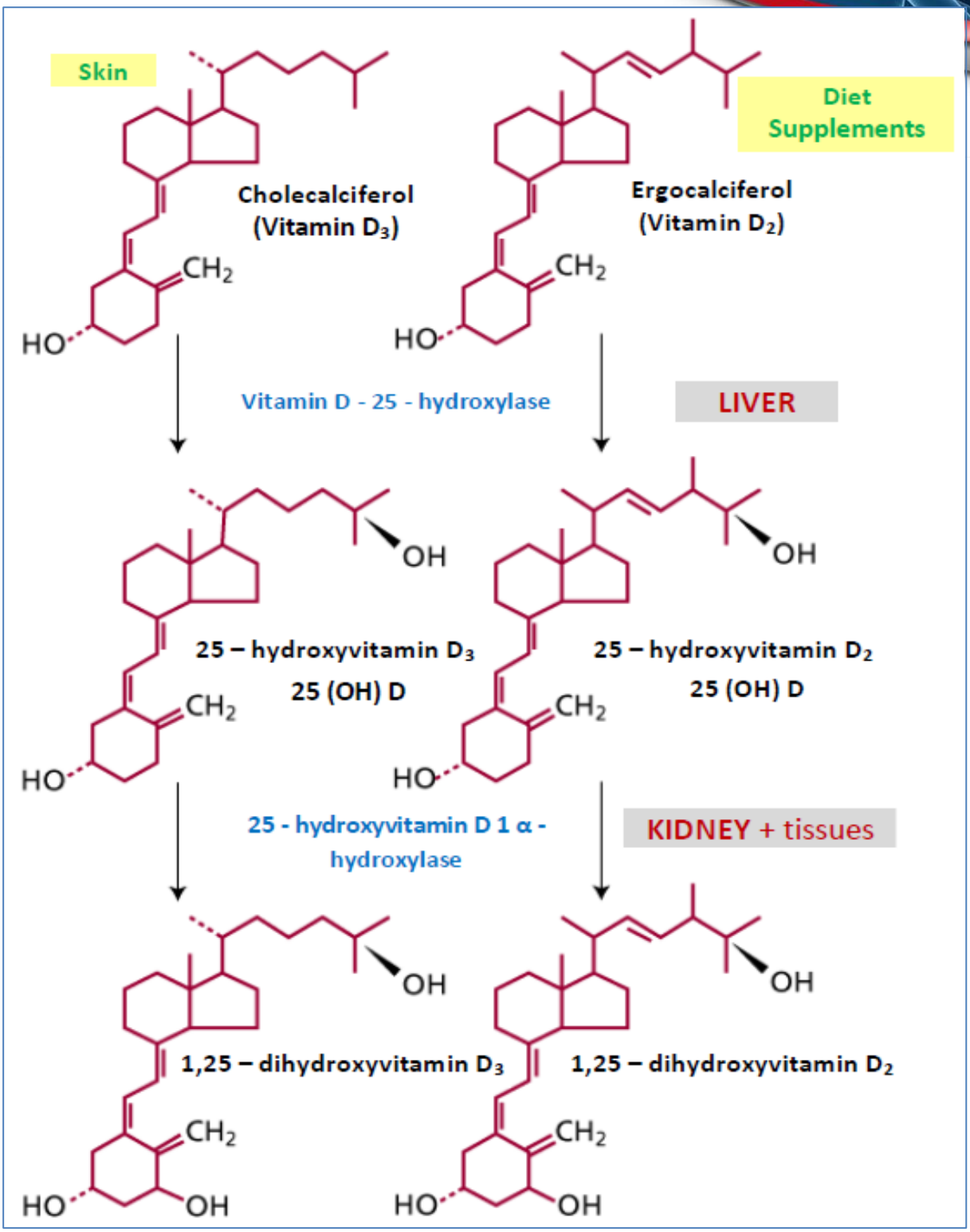


Fig.5: The pathway of vitamin D biological activation



Vitamin D from diet, supplements, or sunlight exposure undergoes hydroxylation in the liver first, producing 25-hydroxyvitamin D, abbreviated as 25(OH)D. 25(OH)D is released into the plasma, where it is bound to vitamin D binding protein (VDBP). The halflife of 25(OH)D is 3 weeks and its daily production is approximately 10mg/kg. A second hydroxylation occurs primarily in the kidneys but also in other tissues, and produces active form of vitamin D: 1,25-dihydroxyvitamin D or calcitriol (Fig.5). Calcitriol is released into the circulation and, after its binding to VDBP calcitriol is transported to various target organs. The biological effect is mediated by binding to the vitamin D receptor (VDR), which is located in the nuclei of target cells. The halflife of 1,25 dihydroxyvitamin D is only about 4 hours. Synthesis of 1,25 dihydroxyvitamin D is regulated by plasma parathyroid hormone (PTH), calcium and phosphorus levels. The overview of vitamin D synthesis and degradation is shown in Fig.6.
The overview of vitamin D forms is given in Tab.1.

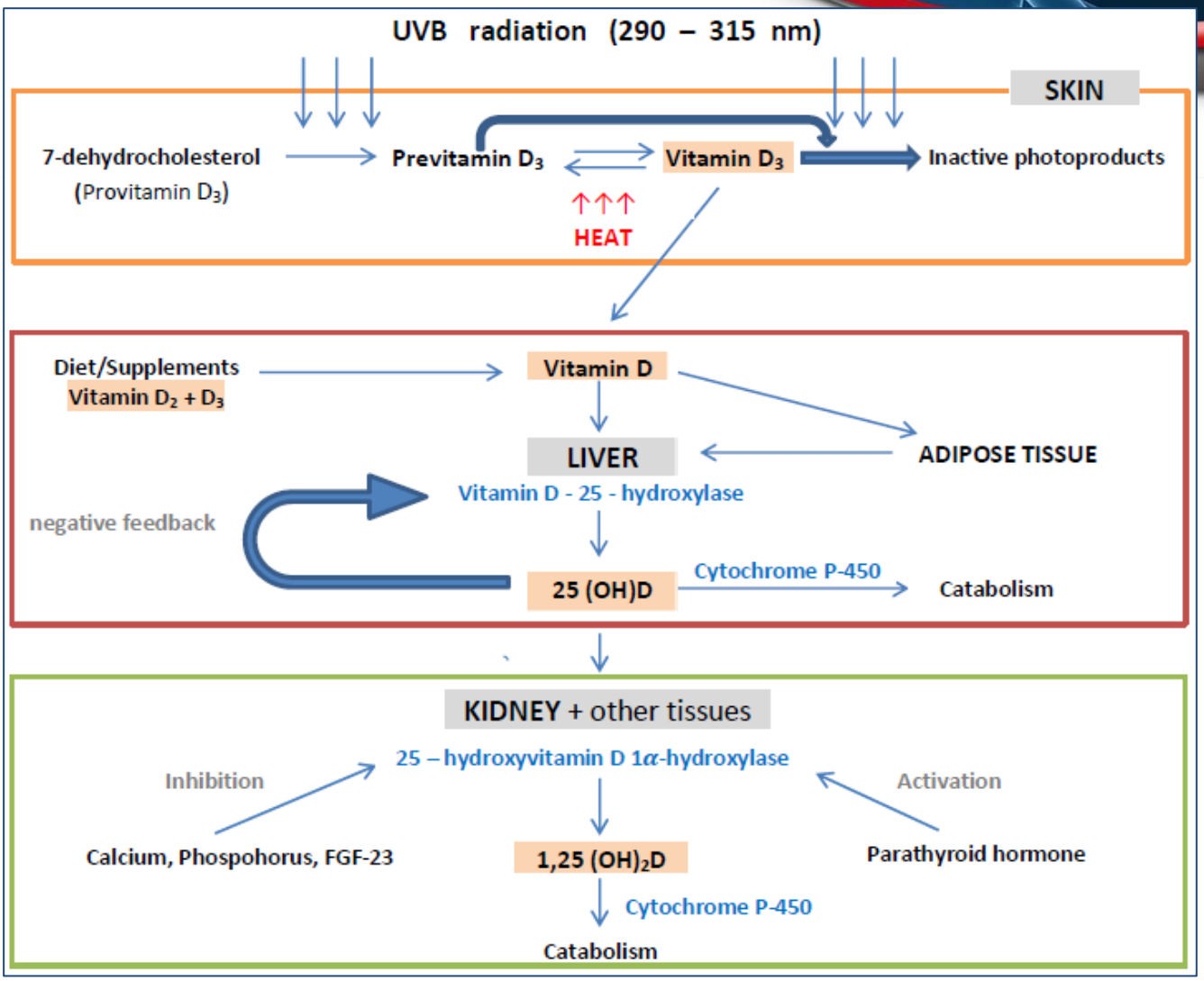
Tab.1: Types of Vitamin D

Vitamin D	Name and description
Vitamin D_2	**Ergocalciferol** Present in plants.
Vitamin D_3	**Cholecalciferol** Animal origin (fish) or produced in the skin.
25-hydroxyvitamin D or 25(OH)D	**Calcidiol** Produced by hydroxylation of vitamin D_2 or D_3 in the liver.
1,25- hydroxyvitamin D	**Calcitriol** Active metabolite produced by hydroxylation of calcidiol in the kidneys or other tissues.

Physiological function

Vitamin D promotes calcium absorption in the gut and maintains adequate plasma calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts. Without sufficient vitamin D levels, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D also helps to protect older adults from osteoporosis. Vitamin D has other

Fig.6. Metabolism of vitamin D



Metabolism of vitamin D: 7-dehydrocholesterol (provitamin D3) in the skin absorbs ultraviolet B (UVB) radiation with wavelengths of 290–315 nm and is converted to previtamin D3. Previtamin D3 undergoes thermal isomerization to vitamin D3. Continued exposure to UVB radiation can result in the breakdown of previtamin D3 and vitamin D3 to inactive products. Dietary vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are absorbed in the gastrointestinal tract. Vitamin D (vitamin D2 + D3) from the diet and skin enters the circulation bound to the vitamin D binding protein. As a fat-soluble molecule, it can be stored in adipose tissue. Circulating vitamin D is metabolized in the liver to 25-hydroxyvitamin D by the enzyme vitamin D-25-hydroxylase. Vitamin D-25-hydroxylase activity is inhibited by the negative feedback of 25(OH)D. 25(OH)D is then metabolized in the kidney and other tissues to the active metabolite - 1,25-dihydroxyvitamin D - by 25-hydroxyvitamin D-1α-hydroxylase. Renal production of $1,25(OH)_2D$ is inhibited by elevated serum levels of phosphorus, calcium and fibroblast growth factor 23 (FGF-23). Parathyroid hormone enhances renal production of 1,25(OH)2D. Catabolism of 25(OH)D and $1,25(OH)_2D$ into biologically inactive molecules is primarily mediated by the cytochrome P-450.

roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. This may be a possible mechanism for the effect of vitamin D in cancer.

Levels

The reference ranges are usually determined by measurement of the values in the healthy population and calculation of the 95% confidence interval for the Gaussian distribution. However, this methodology can't be used for vitamin D reference ranges. Due to our lifestyles, the levels of vitamin D in general population are inadequate for the physiological requirements of target tissues. Moreover, the levels of vitamin D depend on the season, especially in the countries that are distanced from equator, where the winter values are much lower than summer values. The vitamin D levels were measured in the employees of the bay-watch and compared to the general population in the recent studies. The levels in bay-watch employees were 2.5 times higher than those found in the "healthy" population. The conclusion is that the general population suffers from a lack of vitamin D. That is why the "healthy" population cannot be considered as a reference one.

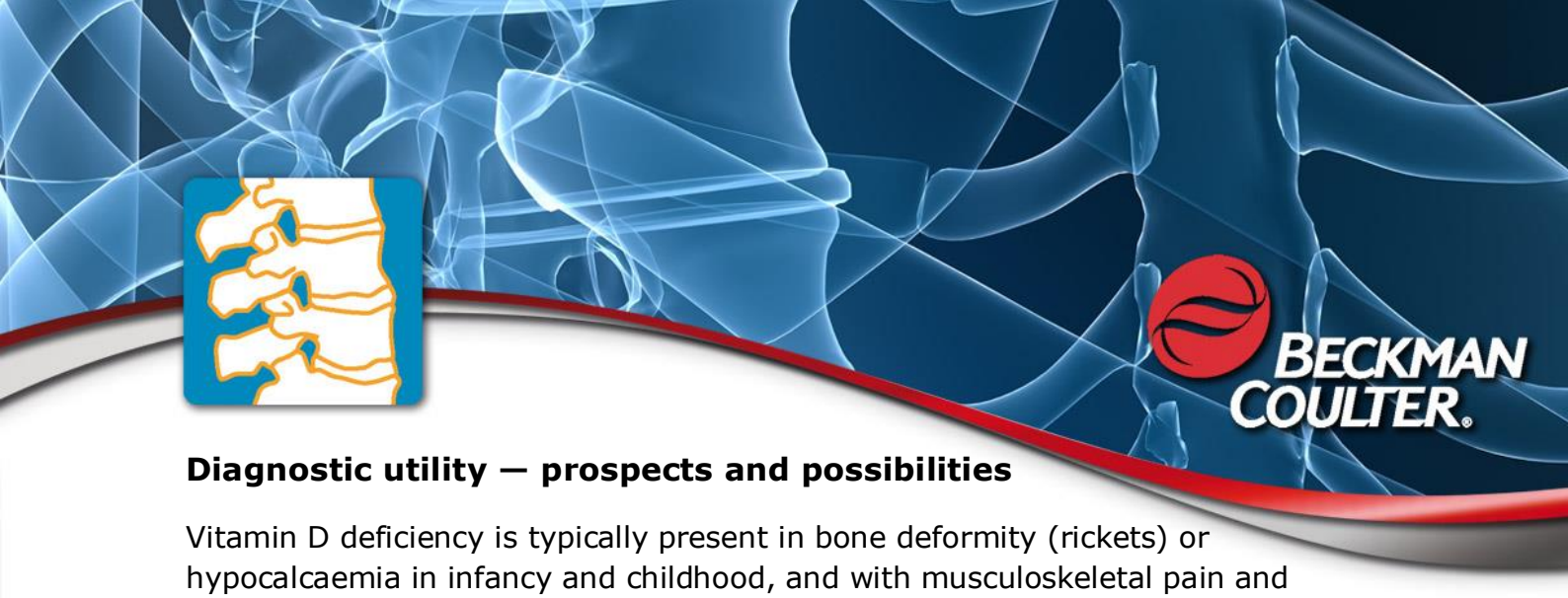
Similarly to cholesterol, the reference values were determined arbitrarily on the basis of the optimal level evidence.

Reference vitamin D levels are given in Tab.2. For each assay, the relevant reference values are shown in the appropriate Instructions for Use (IFU).

Tab.2: Reference vitamin D levels in serum in adults

Specimen (serum)	Reference interval (ng/mL)
Deficiency:	**<10**
Insufficiency:	**10-29**
Sufficiency:	**30 - 100**
Potential toxicity:	**>100**

Equation for the conversion of units for vitamin D: 1 ng/mL x 2.5 = nmol/L



Diagnostic utility — prospects and possibilities

Vitamin D deficiency is typically present in bone deformity (rickets) or hypocalcaemia in infancy and childhood, and with musculoskeletal pain and weakness in adults. In recent years, however, non-musculoskeletal conditions including cancer, metabolic syndrome, infectious and autoimmune disorders have also been found associated with low vitamin D levels. The observational studies have demonstrated that vitamin D insufficiency is widespread in many regions of the world, including industrial countries. In contrary, increased levels of vitamin D are observed in an intoxication which can arise only from inappropriate massive supplementation.

Increased vitamin D levels are associated with:

- Vitamin D intoxication

Decreased vitamin D levels are associated with:

- Inadequate dietary vitamin D
- Inadequate exposure to sunlight
- Increased catabolism
- Nephrotic syndrome
- Severe hepatocellular disease
- Vitamin D malabsorption

Vitamin D measurement

Although the active form of vitamin D - 1,25 dihydroxyvitamin D - can be measured in the circulation, more reliable information is provided by measurement of 25(OH)D. This is due to its long serum halflife (approximately 3 weeks) and because of the 25-hydroxylation step is unregulated, thus reflecting substrate availability. 25(OH)D concentration is directly related to the body's storage of vitamin D, whilst the concentration of 1,25(OH) Vitamin D correlates more with kidney function than with vitamin D deficiency. It has been concluded that determination of both forms of 25(OH)D (25-dihydroxyvitamin D_2 and 25-dihydroxyvitamin D_3) is necessary. For a good assessment of the vitamin D status, the amount of cholecalciferol,

which is a marker of the vitamin D self-production,
and also the amount of ergocalciferol which originates from the external resources (diet or supplementation) are needed.
In other words, if we are thinking about 25(OH)D determination we are talking about the determination of both forms of vitamin D - cholecalciferol (D_3) and ergocalciferol (D_2).

Diagnostic schema in children

Severe vitamin D deficiency may cause hypocalcaemic seizures or tetany, particularly in the neonatal period and also during the phase of rapid growth in adolescence. From the age of 6 months, children with vitamin D deficiency commonly suffer from bone deformity (rickets). Bowing of the legs is typical, but knock knees can also occur. Children with vitamin D deficiency can manifest impaired growth. An increased susceptibility to infections and respiratory symptoms in children with vitamin D deficiency may be a manifestation of "rachitic lung". Severe vitamin D deficiency can result in cardiomyopathy and potentially fatal heart failure.

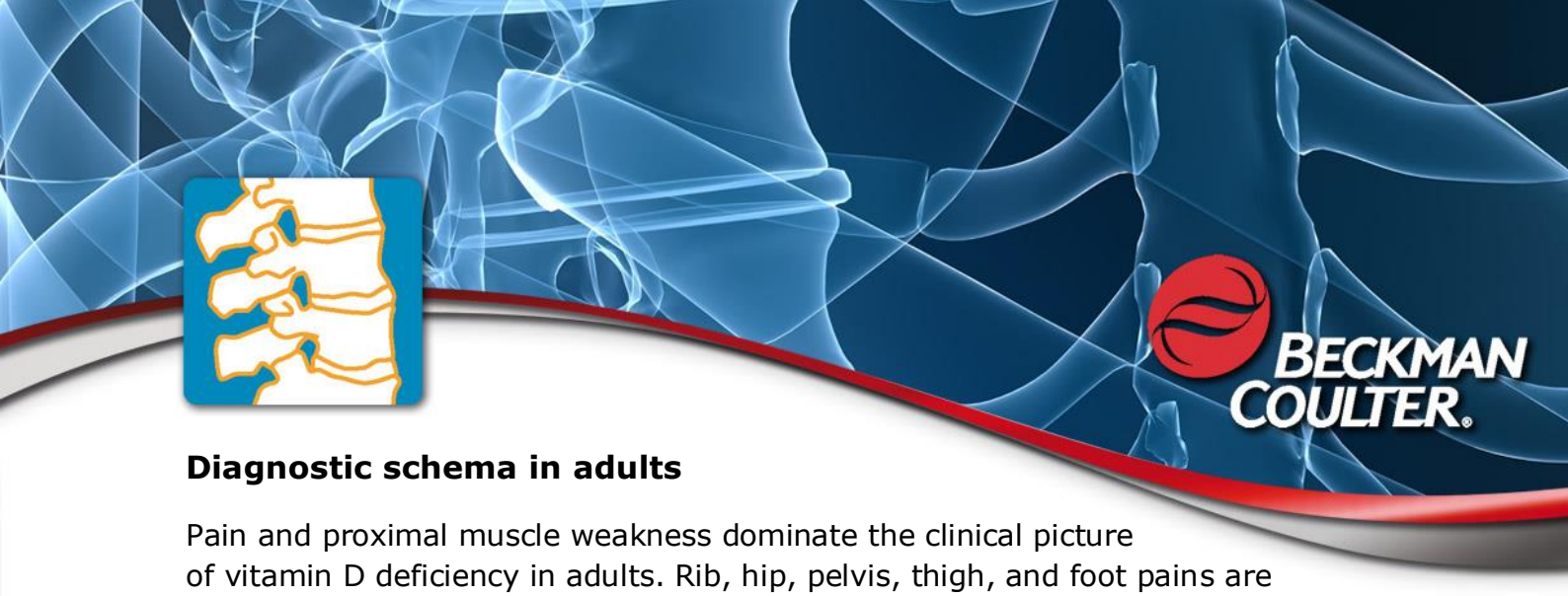
Investigations

Vitamin D deficiency should be suspected if any of the above mentioned symptoms appear. Measurement of calcium, phosphate, alkaline phosphatase, and serum 25(OH)D levels are done primarily. Haemoglobin levels should also be measured because iron deficiency anaemia frequently coexists with rickets. PTH concentrations are typically elevated in neonates and young infants with vitamin D deficiency.

If there is diagnostic uncertainty because of atypical clinical manifestations, a lack of risk factors, atypical biochemistry, the radiographs should be arranged to confirm rickets. In addition, a small number of children may have hereditary or renal rickets. The clinician must be vigilant for a secondary cause of vitamin D deficiency in both children and adults, such as covert coeliac disease or cystic fibrosis causing malabsorption.

Treatment

Oral calciferol in the bioequivalent forms of either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) is the treatment of choice for children with rickets. The principal aim of therapy is to replenish vitamin D stores.



Diagnostic schema in adults

Pain and proximal muscle weakness dominate the clinical picture of vitamin D deficiency in adults. Rib, hip, pelvis, thigh, and foot pains are typical. Diffuse muscular aches and muscle weakness, including in the limbs and back, are also common and may be labelled as "fibromyalgia" or as a somatisation of depression. Low bone density on dual energy X-ray absorptiometry scanning, or osteopenia on plain radiography, may also reflect osteomalacia, and these findings warrant assessment of vitamin D status.

Investigations

More than 80% of adults with osteomalacia have a high concentration of plasma alkaline phosphatase. Hypocalcaemia and hypophosphataemia are less consistently present, depending on the severity and chronicity of the disease and the patient's dietary calcium intake. Elevation of plasma PTH is typical for osteomalacia but is not found in about 20% of adults with vitamin D insufficiency.

Treatment

The principal aim of therapy is the same as in children therapy – replenishment of vitamin D stores. Calciferol has a high therapeutic index. It has been estimated that a regular daily dose of 1000 IU raises plasma 25(OH)D by 10 ng/mL; however, vitamin D toxicity occurs at 25(OH)D values above 100 ng/mL., Calciferol treatment, in a daily dose of 10 000 IU or a weekly dose of 60 000 IU, will lead to restoration of body stores of vitamin D during 8 – 12 weeks in adults.

Diagnostic utility – practical applications

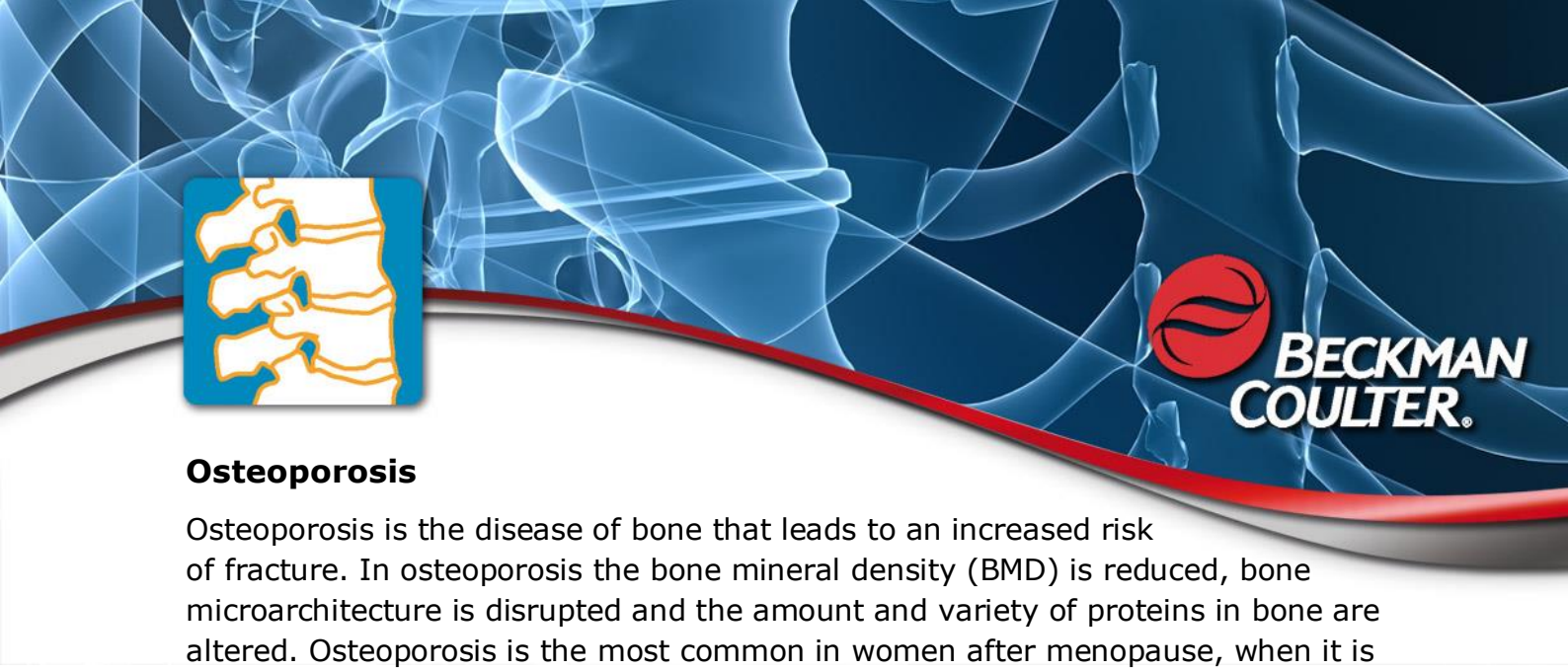
Rickets
Osteomalacia
Osteoporosis
Vitamin D and cancer
Vitamin D and melanoma
Supplementation of vitamin D

Rickets

Rickets is defective mineralization of bones before epiphyseal closure, in childhood. The primary cause of rickets is a vitamin D deficiency. Vitamin D is required for proper calcium absorption from the gut. In the absence of vitamin D, dietary calcium is not properly absorbed, resulting in hypocalcaemia. The children bones are still growing and low levels of calcium lead to skeletal and dental deformities and neuromuscular symptoms, e.g. hyperexcitability. The majority of cases occur in children suffering from severe malnutrition. Treatment involves increasing intake of calcium, phosphates and vitamin D. As mentioned above, vitamin D is essential for calcium uptake and proper bone calcification and maintenance. Children who do not get adequate amounts of vitamin D are at increased risk of rickets. Preventive recommendations regarding vitamin D supplementation for infants and children are established in many countries.

Osteomalacia

Osteomalacia is a similar condition as rickets occurring in adults, after epiphyseal closure. It is the pathological condition in bone in which the osteoid matrix remains uncalcified. Symptoms include bone pain and muscle weakness. The condition is usually caused by vitamin D deficiency. In the absence of vitamin D, dietary calcium is not properly absorbed. Osteomalacia can be treated by increasing intake of calcium, phosphates and vitamin D. Some people are more at risk of osteomalacia, particularly pregnant or breast-feeding women, black or Asian people and the elderly. People with an increased risk of osteomalacia can take vitamin D supplements to prevent it developing.



Osteoporosis

Osteoporosis is the disease of bone that leads to an increased risk of fracture. In osteoporosis the bone mineral density (BMD) is reduced, bone microarchitecture is disrupted and the amount and variety of proteins in bone are altered. Osteoporosis is the most common in women after menopause, when it is called postmenopausal osteoporosis as the result of estrogen deficiency. Rapid bone loss accompanies the decline of estrogen levels at the onset of menopause or after ovariectomy, as a result of the combined effects of an imbalance in bone remodelling and an increase in bone turnover. Hormone replacement therapy (HRT) is often used for the prevention of osteoporotic fractures in postmenopausal women. However many women cannot avail themselves of HRT, because of the increased risk of cancer and the resumption of menstrual bleeding. Therefore, other compounds such as bisphosphonates and vitamin D supplementation have been used to treat osteoporosis. Osteoporosis may also develop in men. Particularly, it may occur in the presence of certain hormonal disorders and other chronic diseases or as a result of medications, specifically glucocorticoids (so called steroid- or glucocorticoid-induced osteoporosis). Measurements of vitamin D levels are not useful for the diagnosis of osteoporosis; diagnosis of osteoporosis should be made on the basis of bone density. The measurement of 25(OH)D is used for monitoring of sufficient supplementation of vitamin D.

Vitamin D and cancer

Vitamin D has a variety of physiological effects which can be applied to neoplastic process. It is mainly the effect of anti-angiogenesis, vitamin D supports the cell apoptosis and cell differentiation. Therefore, it is not surprising that a significant difference in vitamin D levels were found in population studies comparing group of healthy people and group patients with cancer. The high incidence of reduced vitamin D levels in patients with tumours was found. The first cancer with significant correlation was colorectal cancer, the breast cancer was the second one. The most frequently occurring low levels of vitamin D have been demonstrated in lung cancer. The research of the relationship between vitamin D levels and the development and progression of the tumour takes more than fifteen years. It has been shown that substitution of vitamin D reduces the risk of cancer.

Vitamin D has also positive effect during chemotherapy. According to the last observation, the decreased levels of vitamin D may have the negative effect of the chemotherapy efficiency. This effect was found, for example, in lung cancer therapy. It was shown that the normalisation of the vitamin D level increases the effect of chemotherapy up to three times.

It seems that the prostate tumours are the only exception. Similarly as with other types of tumours, low levels of vitamin D significantly increase the frequency of cancer, but values above 120 nmol /L paradoxically increase incidence of cancer too. We are talking about "U effect". This situation depends on the specific situation when the vitamin D receptors are presented.

Vitamin D and melanoma

Very frequent question in professional discussions regards vitamin D and melanoma. On the one hand, the protection against the sunray is strongly recommended in general. On the other hand, the effect of sunray is important for desired improvement of vitamin D levels in the body. As always, finding the optimal middle way is necessary. As it was mentioned above, 10 - 15 minutes per day spend on the sun in the summer months is sufficient for maintaining appropriate vitamin D levels.

Supplementation of vitamin D

In the past, the supplementation was mainly recommended in patients with osteoporosis and malabsorption. Nowadays, the supplementation with vitamin D is recommended in autoimmune diseases, repeated inflammatory diseases, diabetes, in patients with cardiovascular and neurological disorders. Vitamin D supplementation is also recommended as part of adjuvant cancer therapy.

The risk groups that need to be monitored and preferably supplemented with vitamin D are: babies, children, pregnant women, elderly people, obese people, and people working in night shifts.

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