Vitamin D Deficiency in HIV-Positive Patients

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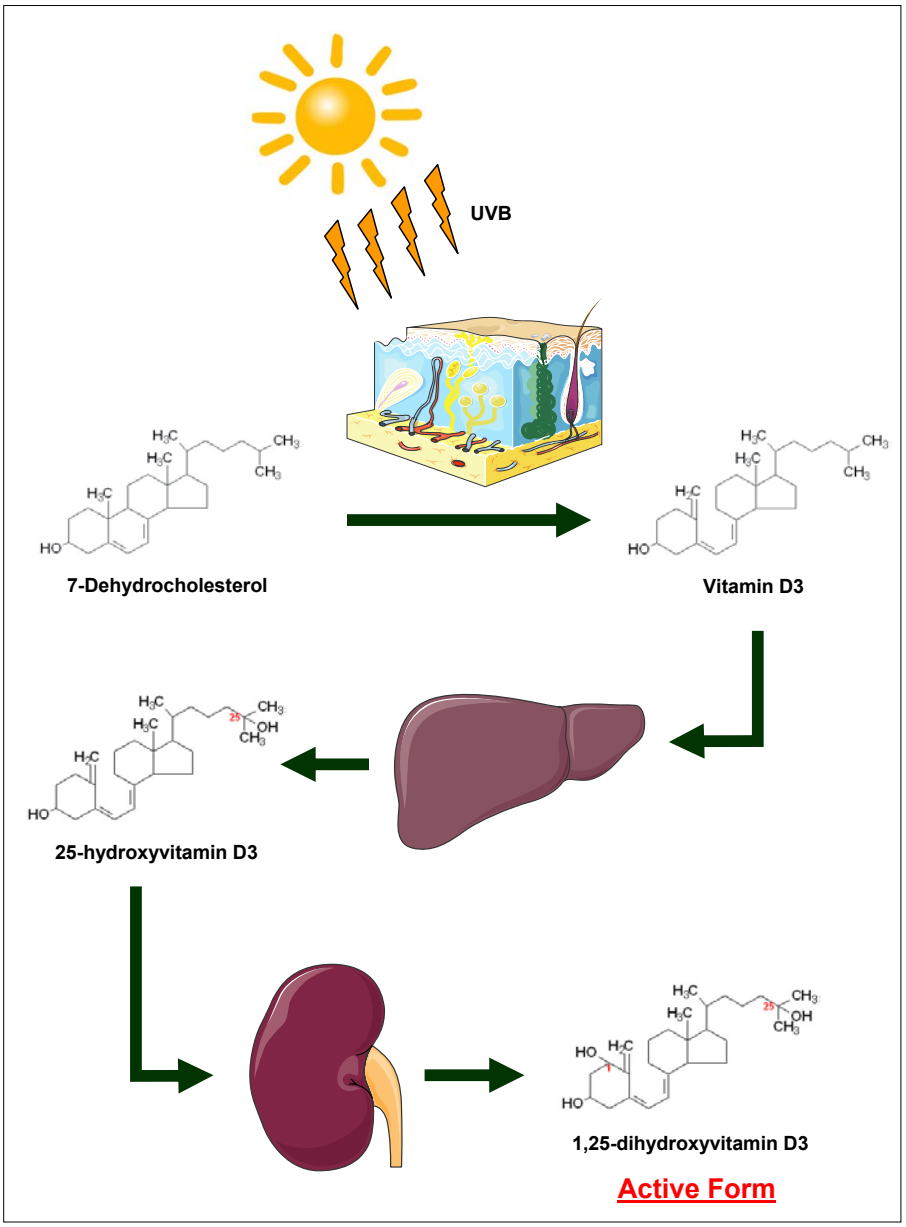
Increasing evidence have shown that vitamin D is fundamental in several processes inside human body. It is well known that a lack of vitamin D during childhood causes rickets, a severe disease characterized by bone softening and several other signs (bone deformities, fractures, rachitic rosary, Harrison's groove, muscular weakness, hypocalcaemia and sometimes also tetany). This condition is very frequent in developing countries and, particularly, among children with malnutrition (starvation) or malabsorption (chronic diarrhoea) (Favus 1999).
When vitamin D deficiency occurs in adults, bones have been already built up and therefore skeletal deformities cannot be appreciated. In this very case the disease takes the name of osteomalacia that is characterized by reduced bone mineralization and a consequent higher risk of fractures (Favus 1999). Beyond its skeletal actions, vitamin D is involved in many other physiological processes and its deficiency have been associated with several diseases and pathological states. An important paper published in 2006 showed that vitamin D values lower than 75-80 nmol/L are significantly associated with both increased risk of colon cancer and muscular weakness, together with a higher rate of dental loss (Bischoff-Ferrari et al. 2006). Vitamin D not only regulates calcium and phosphorus metabolism but can stimulate the pancreas to produce insulin and it downregulates the renal production of renin (Holick 2006). Vitamin D also interacts with many tissues and cells and helps maintain normal cell proliferation and differentiation. In fact the enzymes converting vitamin D into its active form are contained in a wide variety of cells, including colon, prostate, and breast, for the autocrine production of calcitriol (see below for vitamin D metabolism). It is believed that the autocrine production of calcitriol is important for regulating cell growth and maturation, thus decreasing the risk of their malignant transformation (Holick 2006). Also macrophages contain the 1-alpha-hydroxylase to produce calcitriol and following infective stimuli macrophages increase their nuclear expression of cathelicidin, which is a cationic peptide that causes the destruction of infective agents including mycobacterium tuberculosis (Holick 2006).

Vitamin D is synthesized in the skin where ultraviolet B radiation (UVB) converts 7-dehydrocholesterol (7-DHC) into cholecalciferol. This molecule faces two other transformations before becoming active. The first one takes place in the liver where cholecalciferol is converted into 25-hydroxyvitamin D (25-D), that can be stored in adipose tissue from where it can be mobilized during winter (Holick et al. 1989). The blood half-life of 25-D is around 2-3 weeks and this is the reason why it is considered the best indicator of vitamin D status. The last step toward the production of the active form takes place in the kidneys where 25-D becomes 1,25-dihydroxyvitamin D (1,25-D) under a strict control of parathyroid hormone (Fig. 1) (Holick 2004).
Normal human diet cannot provide all the vitamin D men need as very few foods contain it. Thus sunlight exposure is fundamental in order to achieve a sufficient amount of circulating vitamin D. Some categories are at higher risk for hypovitaminosis D. For example the adipose tissue of obese subjects sequesters vitamin D, thus reducing the quantity of cholecalciferol that can reach the liver to be transformed into 25-D (Bell et al. 1985; Wortsman et al. 2000). Also aging reduces vitamin D production by both a lowering of available 7-DHC in the skin and a thickening of epidermis, that lessens UVB penetration. As a matter of facts a high prevalence of hypovitaminosis D have been reported in several elderly populations, particularly in those living in institutions (1998; Chapuy et al. 1997; Finch et al. 1998). A study conducted in Boston at the end of August 2002 showed that 30% of old Caucasians had low plasma vitamin D concentration and that this rate grew up to 42% in Hispanics and up to 84% in coloured people (Holick 2002). Some studies revealed that also young people can have high rates of hypovitaminosis D, particularly if they are inside workers (Nesby-O'Dell et al. 2002; Tangpricha et al. 2002).
A definitive consensus on the optimal serum 25-D concentration does not exist, but many studies allow to identify a cut off beneath which hypovitaminosis D can be diagnosed. A value of 75 nmol/L is considered the minimal cut-off for vitamin D sufficiency. Insufficiency is identified by values between 30 nmol/L

Figure 1: Biosynthesis of vitamin D



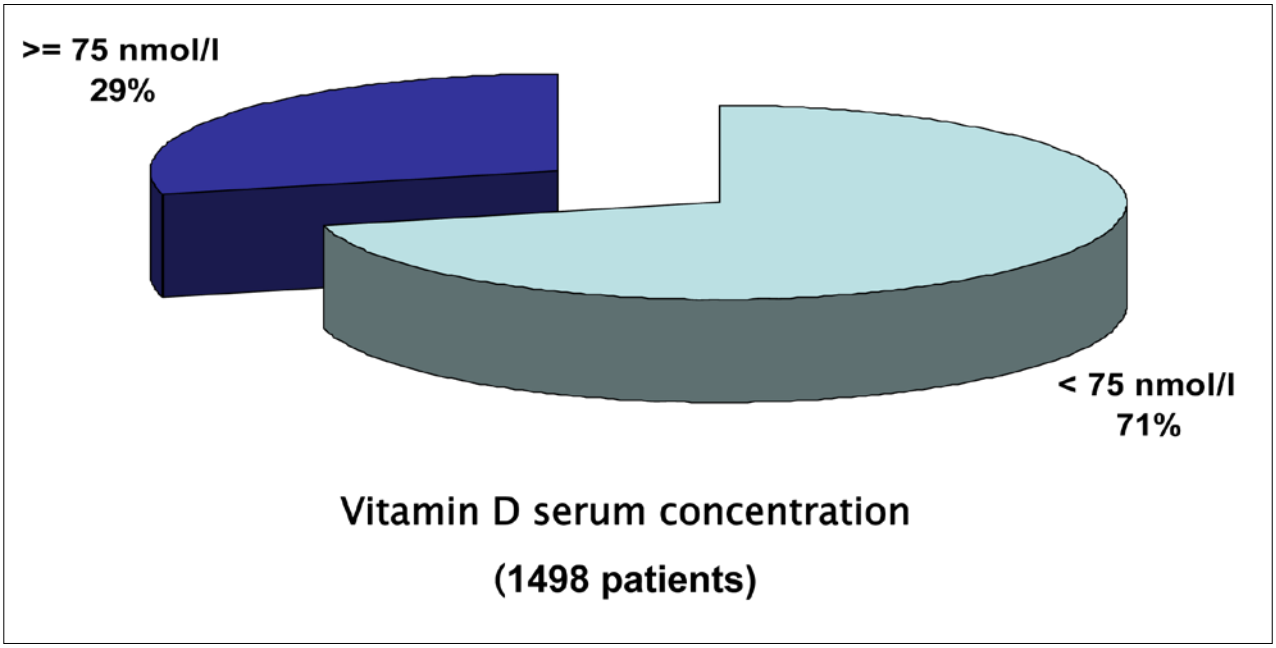
and 75 nmol/L and vitamin D deficiency occur under 30 nmol/L (Dawson-Hughes et al. 2005).

Since the end of 90s a growing body of evidence has shown an increased prevalence of osteopenia and osteoporosis in HIV-infected patients (Brown and Qaqish 2006). Many explanations have been adduced in order to explain these findings. A reduction of osteoblastic bone deposition together with an increased osteoclastic bone resorption are the pathogenetic mechanism at the basis of bone loss in HIV infected patients (Borderi et al. 2009). By linking to osteoblasts precursors the envelop viral protein gp120 can force the mesenchymal stem cell commitment toward adipocytes instead of osteoblasts thus producing a fat-rich bone which has a lower ability to deposit bone matrix and to mineralize it (Cotter et al. 2007; Cotter et al. 2009; Wang et al. 2002). *In vitro* studies demonstrated that cultured HIV-infected osteoblasts can produce a lower amount of alkaline phosphatase, a well known marker of their activity (Cotter et al. 2007). Finally also osteoblasts survival is reduced as HIV stimulates these cells to produce TNF-alpha that, by autocrin/paracrin way, enhances osteoblasts apoptosis (Gibellini et al. 2008). Together with this reduction of osteoblastic bone formation, also osteoclasts activity is increased. In fact HIV promotes both a higher production of RANK ligand (RANKL), a cytokine that enhances osteoclastogenesis, and a reduction of osteoprotegerin (OPG), a decoy receptor that counteracts RANKL action on RANK receptor (Fakruddin and Laurence 2003; Fakruddin and Laurence 2005; Gruber et al. 1995). Therefore RANKL/OPG ratio is augmented and bone resorption increased.

Another important determinant of bone loss in HIV-infected patients is combined antiretroviral therapy (cART). Despite controversial results regarding the negative effect on bone of each antiretroviral molecule, several groups found out a possible role for specific antiretroviral classes. A metanalysis by Brown a Qaqish revealed a 2.38 folds increase in the risk of osteoporosis in cART treated patients compared with naïve ones (Brown and Qaqish 2006). Also being on protease inhibitors (PI) therapy increased the risk of osteoporosis by 1.57 folds (Brown and Qaqish 2006). Finally nucleoside analogues (NRTIs) can reduce bone mass, depending on the grade of their mitochondrial toxicity (Borderi et al. 2009).

Besides the direct effects of HIV and cART on bone loss, a possible aetiopathogenetic role can be ascribed also to vitamin D deficiency. As mentioned above vitamin D deficiency is an important determinant for low bone mass and fragility fractures. Recently a high prevalence of 25-D deficiency has been reported in some HIV-infected cohorts (Arpadi et al. 2004; Bang et al.; Curtis et al. 2006; Garcia Aparicio et al. 2006; Haug et al. 1994; Haug et al. 1998; Madeddu et al. 2004; Mueller et al. 2010; Paul et al. 2010; Seminari et al. 2005; Stephensen et al. 2006; Teichmann et al. 2003; Villamor 2006; Wasserman and Rubin 2010) and therefore osteomalacia must be taken into account. Very recently we were able to demonstrate a very high prevalence of hypovitaminosis D (Fig. 2) in 1498 HIV-infected patients, that, to our knowledge, represents the largest cohort ever published (Borderi et al. 2010). Up to now none of the published papers can discriminate whether bone loss should be ascribed to osteoporosis, osteomalacia, or, more probably, to the combination of these two diseases. Nevertheless,

Figure 2: Prevalence of hypovitaminosis D among HIV-positive patients enrolled in a large Italian cohort. (derived from: Borderi et al. for the Icona Foundation Study Group. CROI, San Francisco 2010)



as vitamin D deficiency has been demonstrated to be more frequent in HIV-positive patients than in healthy age and sex matched controls, this particular aspect must be also considered.

Many hypotheses have been formulated in order to explain why HIV-positive patients display such a high prevalence of hypovitaminosis D. Undoubtedly these patients share common risk factors for vitamin D deficiency with general population (i.e. ageing, low sunlight exposure, highly represented fat tissue, coloured skin, low consumption of fortified foods, etc.), but some specific clues have been investigated. Some studies found that, besides forcing osteoblasts apoptosis, HIV-induced cytokines, such as TNF-alpha, can interfere with the enzymatic activity of renal 1-alpha hydroxylase, causing a reduced conversion of 25-D into 1,25-D (Villamor 2006). Also PI can inhibit this important enzyme as well as all the other enzymes belonging to cytochrome P450 family (Haug et al. 1994). In fact both 1-alpha-hydroxylase and 25-hydroxylase enzymes are cytochrome P450 monoxygenases (Whitfield et al. 1995) and PI are potent *in vivo* inhibitors of human hepatic cytochrome P450s namely CYP3A4 (Dusso et al. 2004; Eagling et al. 1997; von Moltke et al. 1998). Ritonavir, but also indinavir and nelfinavir, negatively affect 1-alpha-hydroxylase and, to a lesser extent, 25-hydroxylase activity reducing 1,25-D production (Cozzolino et al. 2003; Dusso et al. 2004). A case report described severe 25-D deficiency after efavirenz treatment, but others did not confirm this finding (Gyllensten et al. 2006). In contrast some Authors found higher 25-D levels in patients receiving HAART compared to untreated patients (Ramayo et al. 2005).

Moreover some studies, focusing on extra-skeletal actions of vitamin D, showed interesting data. As an example a good paper by Mehta, S et Al. is cited (Mehta et al. 2009). Vitamin D is known to be a strong immunomodulator that may protect against adverse pregnancy outcomes, mother-to-child transmission (MTCT) of HIV and child mortality. The Authors demonstrated that low maternal vitamin D level are associated with a 50% higher risk of MTCT of HIV; moreover they found a 2-fold higher risk of MTCT of HIV through breast-feeding among children who were HIV uninfected at 6 weeks of age and a 46% higher overall risk of HIV infection. Finally children born to women with a low vitamin D level had a 61% higher risk of dying during follow-up (Mehta et al. 2009).

Even though the mechanism by which HIV or cART induce hypovitaminosis D is not completely understood, 25-D deficiency remains a clear feature of HIV-infected patients. As reduced BMD is well described in these patients it is noteworthy to consider that hypovitaminosis D can be a part of the problem. Vitamin D deficiency causes osteomalacia, increasing the risk of fracture as well. Unlike osteoporosis, which is a painless disease, osteomalacia causes aching bone pain that is often misdiagnosed as fibromyalgia or chronic pain syndrome or is simply dismissed as depression (Holick 2007). The histological feature of osteomalacia is the deposition of large amounts of non-mineralized bone matrix (Favus 1999). Therefore both osteoporosis and osteomalacia induce a reduction of bone mineralization and it appears clear that a differential diagnosis cannot be achieved simply by means of a dual X-rays absorptiometry. Nonetheless a correct diagnosis must be done in order to prescribe a correct therapy. In fact osteoporosis is commonly treated with anti-resorptive or anabolic agents (Kanis et al. 2008) while osteomalacia requires high doses of vitamin D (Holick 2007).

Further studies, particularly intervention trials, are required in order to demonstrate whether cholecalciferol supplements can increase bone mineral density, either alone or in combination with anti-resorptive drugs.

Finally due to their safety, low cost and easy schedule of administration, cholecalciferol supplements should be considered for the treatment of HIV-infected patients. Even though data from longitudinal studies are lacking the important skeletal and extra-skeletal action of this vitamin should not be disregarded.

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