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## Review article

**Osteoporosis in rheumatoid arthritis: role of the vitamin D/parathyroid hormone system**Mattia Bellan<sup>a,\*</sup>, Mario Pirisi<sup>a,b</sup>, Pier Paolo Sainaghi<sup>a,b</sup><sup>a</sup> Internal Medicine and Rheumatology Unit, AOU Maggiore della Carità, Novara, Italy<sup>b</sup> Interdisciplinary Research Center on Autoimmune Diseases (IRCAD), Department of Translational Medicine, Università del Piemonte Orientale A. Avogadro, Novara, Italy

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## ABSTRACT

Osteoporosis is a well-established extra-articular feature of rheumatoid arthritis (RA). Systemic inflammation seems to play a crucial role in causing an alteration of multiple homeostatic systems implied in bone health, such as the RANK/RANKL/Osteoprotegerin and Wnt/ $\beta$  catenin pathways; several other causal factors have been called into question, including the chronic use of corticosteroids. Since vitamin D exerts important immune-regulatory roles, it has been claimed that derangement of the vitamin D/parathyroid hormone (PTH) system, a well-known determinant of bone health, may play a pathogenic role in autoimmunity; animal models and clinical data support this hypothesis. Furthermore, RA patients seem to be relatively refractory to vitamin D-induced PTH suppression. Therefore, the link between RA and osteoporosis might in part be due to alterations in the vitamin D/PTH system. A better understanding of the pathophysiology of this system may be crucial to prevent and cure osteoporosis in patients with inflammatory/autoimmune diseases. A major clinical correlate of the strict cooperation and interdependence between vitamin D and PTH is that correction of the vitamin D deficiency, at least in autoimmune diseases, should be targeted to PTH suppression.

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**Osteoporose na artrite reumatoide: papel do sistema vitamina D/hormônio paratireóideo**

## RESUMO

A osteoporose é uma característica extra-articular bem estabelecida da artrite reumatoide (AR). A inflamação sistêmica parece ser essencial para causar uma alteração em múltiplos sistemas homeostáticos implicados na saúde óssea, como as vias RANK/RANKL/osteoprotegerina e Wnt/ $\beta$  catenina; vários outros fatores causais têm sido implicados, como o uso crônico de corticosteroides. Como a vitamina D exerce funções

## Palavras chave:

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Hormônio paratireóideo

Osteoporose

Artrite reumatoide

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imunorreguladoras importantes, tem-se afirmado que o desarranjo do sistema vitamina D/hormônio paratireóideo (HPT), um determinante bem conhecido da saúde óssea, pode desempenhar um papel patogênico na autoimunidade; estudos com animais e dados clínicos apoiam essa hipótese. Além disso, os pacientes com AR parecem ser relativamente refratários à supressão de HPT induzida pela vitamina D. Portanto, a ligação entre a AR e a osteoporose pode ser em parte causada por alterações no sistema vitamina D/HPT. Uma melhor compreensão da fisiopatologia desse sistema pode ser crucial para prevenir e curar a osteoporose em pacientes com doenças inflamatórias/autoimunes. A maior evidência da correlação clínica de cooperação e interdependência entre a vitamina D e o HPT é que a correção da deficiência de vitamina D, pelo menos nas doenças autoimunes, deve ser orientada para a supressão do HPT.

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## Introduction

Osteoporosis is a frequent complication of autoimmune inflammatory diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), systemic lupus erythematosus (SLE) and multiple sclerosis (MS).<sup>1</sup> The reasons why osteoporosis occurs in these diseases are multiple and not completely understood. The failure of several bone regulation systems has been claimed to be responsible for this complication of systemic inflammatory diseases even though this issue remain partially unresolved.

The vitamin D/PTH system is a well known determinant of bone health in the general population. Recently, a failure in Vitamin D metabolism has been described in patients affected by inflammatory rheumatic diseases,<sup>2</sup> even though its relationship with the pathogenesis of autoimmune diseases and the consequences on bone health remain not completely understood. In this paper we will review the role of the vitamin D/PTH system in the pathogenesis of osteoporosis in autoimmune diseases and, in particular, in RA.

To perform the present review, we interrogated PubMed (accessed on-line on July 1st, 2013), limiting our search to papers published in English until June 30th, 2013, and using the following string: "(vitamin D PTH system) OR (vitamin D rheumatoid arthritis) OR (rheumatoid arthritis secondary hyperparathyroidism) OR (rheumatoid arthritis hypovitaminosis D)" yielded 856 articles. Eight-hundred papers were excluded for the following reasons: (a) dealt with topics non-relevant for the present review, (b) letters, case reports, (c) small sample size, (d) unobtainable full text articles, or a combination of the above reasons. We reviewed all the remaining papers, plus additional relevant articles identified from the references of selected articles or through personal knowledge of the authors.

### Osteoporosis and autoimmune inflammatory diseases

Osteoporosis is a clinical condition characterized by a high risk of vertebral and non-vertebral fractures, due to the reduction of Bone Mineral Density (BMD). It also represents a well established extra-articular feature of RA.<sup>3</sup>

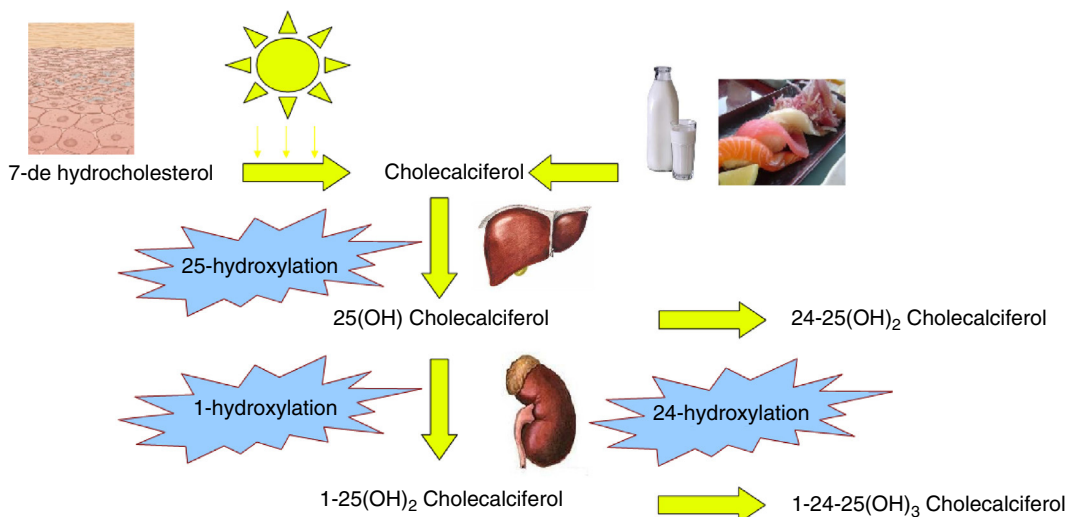
Patients affected by RA have been reported to be at higher risk of vertebral and non vertebral fractures.<sup>4,5</sup> With respect

to the reference population values, female RA patients display lower bone mineral density (BMD) values at the hip and the spine; the risk of osteoporosis seems to be higher among patients who are older, postmenopausal, positive for Rheumatoid Factor, treated with corticosteroids, have longer disease duration and higher burden of disability.<sup>6</sup> Recently,<sup>7</sup> in a perspective cohort of 102 RA patients who completed a 5-years follow-up, an annual incidence of vertebral fractures of 3.7/100 patients/year has been reported, higher than in the general population according to other prospective studies.<sup>8,9</sup> The annual incidence of non vertebral fractures was also increased. These data have been confirmed by other studies<sup>10</sup> in which the risk of all fractures in RA patients was 1.5-fold higher than healthy controls.

However, the increased risk of osteoporosis is not limited to RA patients but has been also reported for other autoimmune diseases such as MS, AS, and SLE.<sup>1</sup> The reasons why patients affected by autoimmune inflammatory diseases are prone to develop osteoporosis are complex. A central role seems to be played by systemic inflammation. Receptor Activator of Nuclear Factor Kappa B (RANK) is a nuclear receptor expressed by osteoclast precursors and mature osteoclasts, which mediates osteoclastogenesis after binding to its ligand, RANKL.<sup>11</sup> In patients with RA the overexpression of several inflammatory cytokines (TNF- $\alpha$ , interleukin (IL)-1, IL-6 and IL-17) favors the activation, differentiation and proliferation of osteoclasts induced by RANKL.<sup>12,13</sup> This system is further regulated by osteoprotegerin (OPG), a decoy receptor expressed by osteoblasts which competes with RANK for the binding to RANKL; so, OPG is an inhibitor of osteoclast activation *in vitro* and *in vivo*.<sup>14</sup>

Furthermore TNF $\alpha$  can also induce osteocytes to synthesize sclerostin and Dkk-1,<sup>15</sup> two inhibitors of the Wnt/ $\beta$  catenin pathway, a crucial system for osteoblastic differentiation.<sup>16,17</sup>

In a recent study on RA patients, a osteoprotegerin/RANKL ratio 5 times lower than that observed in healthy controls has been reported, with an inverse correlation between circulating osteoprotegerin and the disease activity score DAS28, and a positive correlation between RANKL and C-reactive protein. Furthermore, Dkk-1 and sclerostin levels were higher in RA patients than in healthy controls. After two months of treatment with tocilizumab (a humanized anti-interleukin-6 receptor antibody), the osteoprotegerin/RANKL ratio increased proportionally to clinical improvement and suppression of



**Fig. 1 – Vitamin D metabolism. Cholecalciferol, both deriving from dietary intake and from endogenous synthesis, circulates in the blood bound to a vitamin D binding protein. Then, it is hydroxylated to 25(OH) cholecalciferol by a liver enzyme and further hydroxylated to 1,25(OH)<sub>2</sub>D by CYP27B1, expressed by kidney, but also by several other tissues. The inactivation is mediated by 24-hydroxylation.**

inflammation; furthermore, sclerostin increased while Dkk-1 decreased with respect to baseline.<sup>18</sup>

Similar data have also been obtained with other biologics; in fact, the improvement of inflammation control with infliximab has been associated with a reduction in bone loss.<sup>19</sup>

One other major factor in the pathogenesis of osteoporosis in rheumatic diseases is the long term use of corticosteroids. It is known that glucocorticoids can induce osteoporosis through different mechanisms<sup>20</sup>: in fact, the use of glucocorticoids reduces the number and the function of osteoblasts<sup>21</sup> and impairs their differentiation and maturation<sup>22</sup> through interference with Wnt/ $\beta$ -catenin signaling.<sup>23</sup> In this context, the apoptosis of osteoblasts and osteocytes is enhanced,<sup>24</sup> the expression of RANK-L increased and that of OPG decreased<sup>25</sup> favoring the activation of osteoclasts. In the clinical setting glucocorticoid treatment is an independent risk factor for bone loss<sup>26</sup>; in a meta-analysis on 2891 steroid users glucocorticoid treatment has been linked dose dependently to bone loss and risk fractures, in particular in the first months of treatment. The risk decreases after stopping therapy. However, doses as low as 5 mg/day have been reported to increase the risk of fractures of approximately 20%; interestingly, higher initial doses are stronger related to the risk of bone loss than higher cumulative doses.<sup>27</sup> According to ACR guidelines, any patient starting a long term (>3 months) steroid regimen should receive calcium and vitamin D; furthermore, bisphosphonates should be started according to the assessed osteoporosis risk.<sup>28</sup>

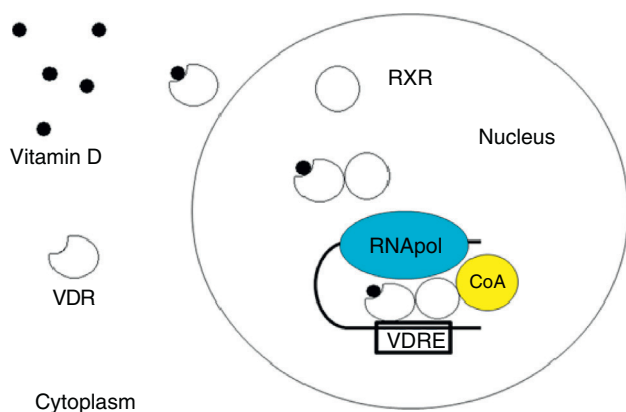
#### Vitamin D metabolism

Vitamin D is a secosteroid hormone that in the body is derived both from dietary intake and endogenous synthesis. Several foods are dietary sources of Vitamin D, either as Vitamin D<sub>2</sub>

(ergocalciferol) or Vitamin D<sub>3</sub> (cholecalciferol), including cod oil, fish and fortified milk. However, the greater part of Vitamin D required for metabolism originates from endogenous synthesis. The metabolic pathways start from the activation of a precursor (7-dehydrocholesterol) in the skin which is photolysed into cholecalciferol following sun exposure; cholecalciferol is then hydroxylated to 25-hydroxyvitamin D (25(OH)D) by different isoforms of a 25-hydroxylase (CYP2C11, CYP2J3, CYP2R1, CYP3A2, CYP27A1) in the liver. 25(OH)D is the circulating form of the vitamin, bound to Vitamin D binding protein (DBP). 25(OH)D can also be stored in fat tissue, and is the intermediate metabolite usually measured to define the vitamin D status, because it is more stable and has a longer life than the active form.<sup>29</sup>

1,25-Dihydroxyvitamin D (1,25(OH)<sub>2</sub>D, also called calcitriol) represents the active form, and it is the result of a further hydroxylation step mediated by 1- $\alpha$ -hydroxylase (CYP27B1) expressed in kidney (Fig. 1). This is the most important check-point of Vitamin D metabolism and CYP27B1 activity is strictly controlled: in particular PTH induces its activity,<sup>30</sup> while high serum calcium concentration and 1,25(OH)<sub>2</sub>D down regulate its expression.<sup>31</sup> While the expression of 25-hydroxylase seems to be restricted to the liver, CYP27B1 is expressed by many other tissues, including placenta, endothelium, prostate, monocytes and macrophages, skin, colon and brain.<sup>32</sup>

1,25(OH)<sub>2</sub>D acts on its target cells through a nuclear receptor (vitamin D receptor – VDR). The binding between 1,25(OH)<sub>2</sub>D and VDR induces the heterodimerization with RXR (retinoid X receptor); the VDR/RXR complex modulates both positively and negatively the expression of many downstream genes, linking specific DNA regions (VDRE – vitamin D responsive elements). Corepressors and coactivators can contribute to VDR/RXR complex activity (Fig. 2).<sup>33</sup>



**Fig. 2 – Mechanism of action of vitamin D.** The active form of vitamin D reaches the cytoplasm, where it binds to VDR (vitamin D receptor), inducing nuclear translocation and further association to RXR (retinoid X receptor), thereby creating a transcriptional complex together to RNA polymerase (RNAPol) and a co-activator (CoA) able to induce or repress genes transcription. This complex recognizes specific DNA-sequences, also known as VDRE (Vitamin D responsive elements).

### Vitamin D/PTH system and bone health

The classic and best defined function of vitamin D is the regulation of calcium/phosphorus metabolism, which is essential to grant bone health. In particular 1,25(OH)<sub>2</sub>D induces gut absorption of calcium and phosphorus<sup>34</sup>; furthermore it acts on kidney tubules determining an increase in calcium reabsorption.<sup>35</sup> The global result is an increase in plasma calcium and phosphorus concentration.

1,25(OH)<sub>2</sub>D has various effects on bone cells: in particular, it increases the expression of osteopontin<sup>36</sup> and osteocalcin<sup>37</sup> in osteoblasts, increases the RANKL expression on the plasma membrane of osteoblasts and inhibits the synthesis of OPG. Thereby, vitamin D increases the number of RANKL molecules able to bind to RANK, allowing a physiological bone turnover.<sup>38</sup>

It is well known that vitamin D actions on bone are strictly linked to PTH activity, because vitamin D needs PTH to play its role on bone, but also because vitamin D down regulates PTH synthesis both indirectly (increasing calcium concentration) and directly (activating a VDRE in the promoter of the PTH gene). Vitamin D also inhibits parathyroid cells proliferation<sup>39</sup> and modulates the sensitivity to calcium, increasing the transcription of CasR (Calcium Sensing Receptor).<sup>40</sup>

These molecular pathways have important clinical implications. First, even though in the last decade other bone health determinants have been used to define vitamin D status, the definition of a normal plasma vitamin D concentration is mainly based on the identification of a 25(OH)D plasma concentration able to suppress PTH. This threshold of normality has been set by some at 30 ng/mL (75 nmol/L).<sup>41</sup>

Therefore, patients affected by severe vitamin D deficiency can suffer a secondary hyperparathyroidism responsible of an inadequate bone mineralization leading to rickets or osteomalacia; in the modern era these conditions have become rare, although hypovitaminosis D is extremely common in the general population. The impact of hypovitaminosis D on bone outcomes is still debated, in particular with regard to the relationship between lower vitamin D levels and the risk of falls and fractures. In a systematic review vitamin D concentration correlated positively with BMD and inversely with the risk of falls,<sup>42</sup> while a correlation with an increased risk of fractures lacked consistence. These data confirmed the direct relationship between vitamin D concentrations and BMD observed in a large population of postmenopausal women,<sup>43</sup> as well as in the community-dwelling men and women aged at least 20 years who participated to the US NHANES III survey.<sup>44</sup> However, recently, hypovitaminosis D has been also associated to an increased risk of vertebral fractures.<sup>45</sup>

Even though the definition of normal 25(OH)D plasma concentration is still debated, a classification of vitamin D status on which many would agree is: deficiency 25(OH)D <20 ng/mL (<50 nmol/L), insufficiency 20–30 ng/mL (50–75 nmol/L), normality >30 ng/mL (>75 nmol/L).<sup>46</sup>

Vitamin D supplementation seems to be linked to favorable clinical outcomes. In particular the resolution of vitamin D deficiency is associated with a recovery in bone mineral density<sup>47</sup>; furthermore vitamin D supplementation reduces the risk of falls and fractures according to the results of a meta-analysis.<sup>48,49</sup>

It was suggested that a 25(OH)D plasma concentration of at least 60 nmol/L is necessary for prevention of falls and fractures.<sup>50</sup> However the preferable regimen of vitamin D supplementation has not been established yet and several different schemes have been suggested.<sup>51–53</sup> Recently<sup>46</sup> published guidelines suggested the use of high doses (2000 IU/day or 50,000 IU once weekly for 6 weeks) ergocalciferol or cholecalciferol in the correction of hypovitaminosis D, followed by a maintenance daily dose of 400–1000 IU/day.

However, a randomized clinical trial in 2010 surprisingly reported an increased risk of falls and fractures in patients treated with one single annual dose of 500,000 IU cholecalciferol.<sup>54</sup> Although these data await confirmation in other studies, this observation question the safety of administering single high dose cholecalciferol.

### Vitamin D in autoimmune diseases

In the past few years, several reports pointed out a putative role for the active metabolite 1-25(OH)<sub>2</sub>D in immune system regulation. This hypothesis is based on the observations that 1-25(OH)<sub>2</sub>D has immuno-modulatory effects on cells of the innate immunity. In fact, in vitro, it induces the differentiation of monocytes with inhibition of inflammatory cytokines production (TNF- $\alpha$ , IL-6, IL-1). Moreover, 1-25(OH)<sub>2</sub>D decreases dendritic cells (DC) maturation down-regulating the expression of molecules of the class II major histocompatibility complex, and of CD40, CD80, CD83 and CD86, and favoring the activation of CD4 T lymphocytes with a Th2 phenotype.<sup>55–57</sup>

The importance of vitamin D metabolites in immune regulation is confirmed by recent data showing that macrophages and monocyte-derived DCs express the enzyme CYP27B1. In this way, 1-25(OH)<sub>2</sub>D is generated locally and binds to VDR in immune cells, thereby exerting concentration-dependent anti-inflammatory autocrine and paracrine effects in lymphoid microenvironments.<sup>32</sup> In addition, some epidemiological studies have correlated hypovitaminosis D to the development of autoimmune diseases, such as multiple sclerosis, type I diabetes, systemic sclerosis, SLE and RA.<sup>58</sup> In fact, a lower dietary intake of vitamin D has been linked to a higher risk of RA development in a meta-analysis of several studies<sup>59</sup>; furthermore, plasma 25(OH)D concentration has been reported to be lower in RA patients when compared to healthy controls,<sup>60</sup> although these results were not confirmed in other studies.<sup>61</sup> Plasma 25(OH)D concentration has also been inversely correlated with disease activity.<sup>59,62</sup>

Further evidence for an involvement of vitamin D metabolism in the pathogenesis of RA is based on the following observations, suggesting a local action for vitamin D metabolites in the modulation of joint inflammation:

1,25(OH)<sub>2</sub>D and 25(OH)D are detectable in synovial fluid<sup>63</sup>; VDR is expressed in the synovial membrane of RA patients<sup>64</sup>; VDR-knock-out mice develop a more aggressive form of TNF-induced arthritis with respect to mice with a normal vitamin D function<sup>65</sup>; TNF-blockade requires the presence of 1,25(OH)<sub>2</sub>D to suppress Th17 activity and, consequently, synovial inflammation in joint tissues cultures.<sup>66</sup>

The importance of understanding the role of vitamin D in autoimmune diseases is enforced by the very high prevalence of hypovitaminosis D in this particular setting.<sup>61,67</sup>

#### **Relative hypovitaminosis D and secondary hyperparathyroidism in autoimmune rheumatic diseases**

An impairment of Vitamin D system has already been postulated as a concausal factor in the pathogenesis of osteoporosis in inflammatory arthritis. In fact, a VDR polymorphism has been linked to bone loss in RA; in particular<sup>68</sup> Rass and colleagues found a lower BMD in RA patients carrying the BB and Bb genotypes of the VDR BsmI polymorphism with respect to carriers of the bb genotype. These results suggest that the B allele may be a marker for increased bone reabsorption and bone loss in RA. In addition, we recently described a significantly higher PTH concentration in 105 patients affected by autoimmune rheumatic diseases with respect to 1020 controls despite similar plasma vitamin D concentration. These findings held true also categorizing patients in different groups according to different vitamin D thresholds. We also observed that patients showed a higher prevalence of hyperparathyroidism than controls. Finally, at stepwise logistic regression analysis, plasma 25(OH)D <75 nmol/L, age ≥65 years and the presence of an autoimmune rheumatic disease were independent predictors for hyperparathyroidism.<sup>2</sup>

This result has been confirmed in a further paper, in which we observed that suppression of secondary

hyperparathyroidism in autoimmune rheumatic patients was impaired with respect to patients affected by osteoarthritis after a cholecalciferol regimen used to correct hypovitaminosis D.<sup>69</sup>

Similarly, in a multicentric study, RA patients with erosive evolution presented with higher PTH and lower BMD in comparison to patients with less aggressive arthritis, despite possessing similar vitamin D concentrations.<sup>70</sup>

These observations raise the attention on the possible presence of a “relative hypovitaminosis D” in RA and in other inflammatory rheumatic diseases, which could explain the reduction in the physiological actions of this molecule; in these patients, indeed, the mechanisms that regulate PTH synthesis seem to be more refractory to vitamin D suppression.

A possible explanation for these findings is that chronic inflammation may reduce parathyroid cells sensitivity to 1,25(OH)<sub>2</sub>D; the reduced sensitivity of parathyroid cells could reflect a more complex refractoriness to 1,25(OH)<sub>2</sub>D actions, involving immune cells too. Alternatively, since the active vitamin D metabolite has immunosuppressive and immunoregulatory effects, during chronic inflammation, immune cells may consume higher quantities of 1-25(OH)<sub>2</sub>D, lowering its availability to parathyroid cells with consequent PTH hyperproduction.

So in this setting, the vitamin D/PTH system seems to be somehow altered and this dysfunction could participate significantly in the pathogenesis of osteoporosis in rheumatic patients, and may represent a promising therapeutic target.

At the best of our knowledge, very few studies evaluated the best supplementation regimen of cholecalciferol in rheumatic patients. We have demonstrated that only a high dose supplementation regimen (300,000 IU orally once) followed by a maintenance dose was superior, also if still suboptimal, in the correction of hypovitaminosis D and of secondary hyperparathyroidism in rheumatic patients.<sup>70</sup>

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## **Conclusions**

In the last few years the same definition of “vitamin D” has been debated and remains the subject of intense controversy. In fact, vitamin D is not only obtained by dietary intake, like the other vitamins, since its greater amount derives from endogenous synthesis. Importantly, the concept of “vitamin” is static, while vitamin D and PTH participate in dynamic system acting on several biological targets. For these reasons vitamin D is currently, more correctly, defined as “hormone D”; in fact the relationship between vitamin D and PTH shares many similarities with the other endocrine networks.

Obtaining a normal vitamin D status is paramount in preventing RA related osteoporosis and the correction of a deficient vitamin D status should be suggested to each rheumatic patient. However, since the correlation between vitamin D and PTH is less linear in RA, this system is not correctly defined by the single evaluation of plasma 25(OH)D concentration. Many patients with a normal vitamin D concentration are affected by secondary hyperparathyroidism and present an inadequate vitamin D status. Therefore, we propose that in rheumatic diseases both Vitamin D and PTH

are measured to establish the need for cholecalciferol supplementation, and that Vitamin D supplementation is targeted to the correction of hyperparathyroidism rather than to the normalization of plasma 25(OH)D concentration alone.

### Conflicts of interest

The authors declare no conflicts of interest.

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