

# VITAMIN D

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Dear Colleagues,

The tissues that make up bones and skeletal muscles are intimately interconnected from a bio-mechanical standpoint, and it has been hypothesised that vitamin D may be considered a "director" molecule of the inter-tissue cross-talk that governs the structural and functional efficiency of the musculoskeletal system. In this issue you will find an update on the relationship between vitamin D deficiency and osteosarcopenia, and specifically on the mechanisms through which it seems that vitamin D influences muscle strength.

Muscles also have receptors for vitamin D. In animal studies it has been observed that their deletion leads to sarcopenia and muscle function deficits<sup>1</sup>. Furthermore, for some time now, it has been known that a condition of severe vitamin D deficiency can manifest in severe muscle weakness, especially among the proximal muscles, and thus in an increased risk of falls. Nevertheless, it is still being vigorously debated whether vitamin D supplementation in community-dwelling elderly adults will lead to an increase in muscle strength and reduce the risk of falls, or whether over-supplementation may exacerbate these outcomes.

Though a 2014 meta-analysis concluded that vitamin D supplementation improved muscle strength<sup>2</sup>, this was not confirmed by two more recent meta-analyses<sup>3,4</sup>. Two recent meta-analysis studies, included in this issue's bibliography, found no significant reduction in the risk of falls and fractures with vitamin D supplementation<sup>5,6</sup>. Instead, two earlier meta-analysis studies showed that the risk of falls in deficient subjects was significantly reduced, but not, understandably, in non-deficient subjects<sup>7,8</sup>. Still, two recent RCTs<sup>9,10</sup>, including the VITAL Study, observed no effect on the risk of falls, even though the participants were largely vitamin D deficient! On the other hand, as you may recall, the administration of a 500,000 IU vitamin D bolus, more-over largely among non-deficient subjects, was seen to be associated with an increased risk of falls after three months<sup>11</sup>. In addition, two other studies showed an increased risk of falls in those participants who achieved high serum 25(OH)D levels<sup>12,13</sup>, whilst in the STOP-IT trial, a "U-curve" in the relationship between serum 25(OH)D levels and the risk of falls was observed, which indicated that a concentration of between 20 and 40 ng/mL could be viewed as optimal<sup>14</sup>. It is likely that the differences in the vitamin D status and the clinical profiles of the subjects receiving treatment (e.g. muscle performance, body mass index and comorbidities), the treatment schedule variability and the lack of well-defined primary endpoints could justify this discordance in results and thus generate an unfortunate level of uncertainty and confusion. The other article in this issue has been dedicated to the possible anti-inflammatory role of vitamin D. The regulation of inflammation and cytokine expression is of crucial importance not only for many inflammatory diseases but also in view of the recent "inflammaging" hypothesis, which states that with increasing age there seems to in fact be a shift towards a pro-inflammatory state that would create and maintain a chronic basic state of inflammation, which would result in organ damage and progression towards various chronic diseases typical of ageing (e.g. rheumatological, metabolic, cardiovascular and cancerous conditions). That be-

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ing the case, it has recently been observed that elderly people with vitamin D deficiency have higher levels of C-reactive protein in their blood <sup>15</sup>. Just a few and sometimes discordant studies have evaluated the effect of cholecalciferol administration on inflammatory status, particularly among deficient subjects, whilst bias has often limited the interpretation of these studies, especially when considering pathological conditions. In a group of young, healthy subjects who were vitamin D-deficient, we have recently observed that cholecalciferol supplementation brought about a progressive reduction in the levels of IL-6 and IL-17 <sup>16</sup>, two key cytokines in the pathogenesis of rheumatoid arthritis and spondyloarthritis, respectively. Therefore, vitamin D deficiency could accelerate inflammaging whilst increasing the risk or progression of inflammatory diseases or reduce response to their treatment. What are your thoughts?  
Happy reading!

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# Vitamin D deficiency and osteosarcopenia

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## INTRODUCTION

The musculoskeletal system can be considered one of the most advantageous anatomic-functional outcomes that have appeared throughout the evolutionary history of the animal world. This complex system sees the interaction of several organs and tissues, mostly of the same embryogenetic derivation, which integrate different vital functions, and which go beyond the primary purpose of locomotion, into a single "organ" that develops during the period of growth, and is modelled and remodelled throughout a person's life <sup>1,2</sup>. At least three fundamental tissues are involved in this anatomical-functional interaction: bone tissue, striated muscle tissue and adipose tissue.

These three tissues, which have the same embryological derivation, develop from the mesodermal germ layer, which can be divided into three basic regions: paraxial, intermediate and lateral mesoderm. Somitogenesis is a fundamental step that occurs in the paraxial mesoderm where cells divide into somites. Each somite contains specific precursors for the development of the axial skeleton (sclerotome), tendons (syndetome), skeletal muscles (myotome) and the dermis (dermatome) <sup>3</sup>. The sclerotome develops into pre-cartilage, then into cartilage, which finally undergoes ossification. The precursors derived from the paraxial mesoderm that turn towards myogenesis are under the control of Pax3/7 (Paired Box 3/7), followed by the activation of differentiation and fusion into multinucleated syncytium, i.e. myotubes, driven by the expression of myogenic factors, such as Myf5 (Myogenic Factor 5) and MyoD (Myogenic Differentiation).

The fusion of myotubes gives rise to muscle fibres, which then group into bundles and the bundles join together to form muscle tissue. Some of these cells, so-called "satellite cells", are Pax7+ muscle precursors that localise under the basal lamina of muscle

fibres in a latent state and act as a source of myonuclei during postnatal growth and after muscle injury <sup>4</sup>.

Bone and skeletal muscle tissue are intimately connected to each other from a biomechanical standpoint. Whilst bone plays a supportive role, muscle enables motor activity through the interaction of contractile proteins within sarcomeres and through their insertion through tendons onto skeletal structures. Both tissues also regulate energy metabolism through the production and release of several molecules, especially cytokines. Molecules produced by bone tissue and released into the circulation to carry out local or remote biological activity are called "osteokines". These include Wnt, sclerostin, RANK-L (Receptor Activator of Nuclear Kappa B Ligand), osteocalcin, FGF-23 (Fibroblast Growth Factor-23), BMP (Bone Morphogenetic Protein), PGE-2 (Prostaglandin E2), and IGF-1 (Insulin like Growth Factor-1). These molecules all have one or more roles modulating muscle's biological and functional activity. At the same time, muscle tissue produces other cytokines, known as myokines, including irisin, myostatin, various interleukins, and neurotrophic factors, which act in an autocrine, paracrine and endocrine manner. The cross-talk among the component tissues that make up the locomotor system is due precisely to the production and circulation of these different substances <sup>5</sup>.

Deep knowledge of the function of the molecules involved in these complex interconnected tissue systems is necessary to identify useful therapeutic strategies for the management of musculoskeletal disorders, particularly osteosarcopenia.

There is speculation that vitamin D may be considered a "director" molecule of the inter-tissue cross-talk that governs the structural and functional efficiency of the musculoskeletal system <sup>6</sup> (Fig. 1).

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## Conflict of interest

The Authors declare that they have received funding or have contracts or other forms of funding in place with Abiogen, Amgen and UCB.

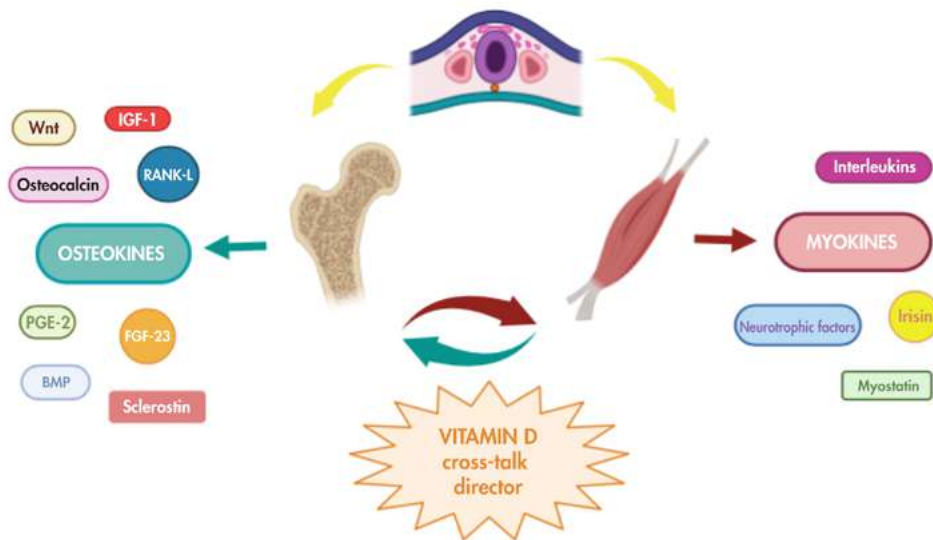
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IGF-1: Insuline like Growth Factor-1; RANK-L: Receptor Activator of Nuclear Kappa B Ligand; PGE-2: Prostaglandin E2; FGF-23: Fibroblast Growth Factor-23, BMP: Bone Morphogenetic Protein.

**FIGURE 1.**

Role of vitamin D in embryonic skeletal muscle development.

### VITAMIN D AND OSTEOSARCOPENIA

The relationship between the low concentration of vitamin D [ $25(\text{OH})\text{D}_3$ ] in the blood and age-related pathological conditions, such as osteoporosis and sarcopenia, has long been known. Equally well known is the close relationship between vitamin D deficiency and increased risk of falls, linked to consistent decrease in muscle strength, primarily due to the depletion of type 2 muscle fibres, which are principally engaged in postural changes<sup>7-9</sup>. For example, a decrease of type 2 muscle fibres will necessarily cause a significant increase in the risk of falling when one stands up from a seated position. After all, the replenishment of the serum level of  $25(\text{OH})\text{D}_3$  of patients with established vitamin D deficiency through supplementation can induce a significant recovery of muscle strength, which can lead to an important reduction in the risk of falling among elderly patients<sup>10</sup>.

As is well known, vitamin D primarily acts through a genomic pathway, which is mediated by binding to vitamin D nuclear receptors (VDRs). In the presence of a significant decrease in the serum level of vitamin D, which is frequent if not constant among many elderly patients, muscles are effected negatively, with histological signs of age-related muscle atrophy, char-

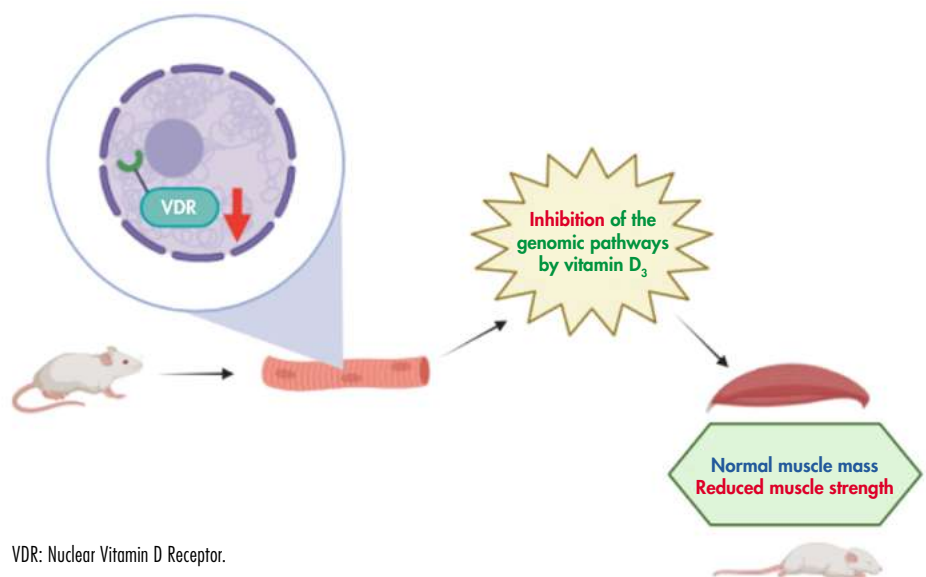
acterised mainly by the depletion of type 2 fast-twitch muscle fibres. Experimental evidence has shown that inhibition of the vitamin D genomic pathway leads to muscle weakness in mature mice with muscle fibre-specific VDR deficiency, with no affect on muscle mass<sup>11</sup> (Fig. 2).

This finding appears to have been con-

firmed by a longitudinal epidemiological study of community residents, in whom serum  $25(\text{OH})\text{D}_3$  levels had no significant effect on muscle mass, but were significantly correlated with muscle strength<sup>11</sup>. The aforementioned experimental and epidemiological data could well lead to the conclusion that vitamin D deficiency on mature muscle fibres exerts its negative effects primarily on muscle strength. As a consequence, it has therefore been hypothesised that since low serum vitamin D levels are closely related to age-related muscle weakness,  $25(\text{OH})\text{D}_3$  dosage could be considered a good predictor of muscle weakness and therefore a biomarker of sarcopenia.

Although it could also be supposed that vitamin D deficiency does not act primarily through a depletion of muscle mass, it is probable, instead, that it acts to a greater extent through a reduced contractile function of individual muscle fibres, an impairment in motor unit activity due to decreased motor neuron discharge frequency, reduced nerve conduction velocity, and even excitation-contraction uncoupling. A non-secondary role in the genesis of the strength deficit could also be played by an increase in fat and fibrous tissue inside the muscle.

A recent animal study has revealed that the genomic pathway regulates muscle strength by modulating the expression of



VDR: Nuclear Vitamin D Receptor.

**FIGURE 2.**

Role of vitamin D on muscle mass and strength.

the calcium-dependent ATPase<sup>12</sup>. SERCA, which is a calcium pump in the sarcoplasmic reticulum membrane, concentrates calcium in the lumen of the sarcoplasmic reticulum (SR). Three distinct genes encode SERCA 1, 2 and 3, which are known to produce more than 10 isoforms. Typical isoforms are the following: SERCA1 is the fast twitch muscle isoform, SERCA2a is the slow twitch muscle isoform. VDR deficiency reduces SR Ca<sup>2+</sup> ATPase activity in mature myofibres, which is hypothesised to be induced by reduced SERCA gene expression. Therefore, it seems that Vitamin D alters muscle contraction dynamics by decreasing Ca<sup>2+</sup> reuptake in the SR, thus prolonging the relaxation phase of muscle contraction. In conclusion, reduced serum vitamin D levels lead to diminished VDR signalling in myofibres and causes an excitation-contraction uncoupling.

The non-genomic pathway by which vitamin D enters directly through the caveolae on cell membranes is modulated by a molecule interaction with a separate VDR (mVDR) pool or with a different membrane-bound or intracellular receptor. One candidate that has been proposed for such a membrane-bound protein that mediates the rapid non-genomic effects of vitamin D is PDIA3 (protein disulphide isomerase) also called 1 $\alpha$ ,25D<sub>3</sub>-MARRS. This protein, which is associated with several cell membranes, including the plasma membrane and endoplasmic reticulum, is also known for its important role in protein folding. It has been reported that some of the new non-classical vitamin D hydroxy-metabolites, formed by CYP11A1, interact with both the nuclear VDR and membrane-bound 1 $\alpha$ ,25D<sub>3</sub>-MARRS<sup>13</sup>.

The interactions between vitamin D and the aforementioned membrane receptors realise the activation of a plethora of intracellular signal transduction pathways. It has been hypothesised that the non-genomic action of vitamin D activates a cascade of mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1 and 2 through several intermediate effectors, which are activated when vitamin D binds to the VDR. Activated VDR stimulates calcium influx, which, in turn, activates calcium-driven intracellular pathways such as protein kinase C (PKC). Furthermore, vitamin D could activate G-protein-coupled receptors (GPCRs), which, in turn, would stimulate several downstream pathways,

including phosphoinositide 3-kinase (PI3K), adenylate cyclase (AC), Ras and phospholipase C gamma (PLC $\gamma$ ). Through different signals, each of these pathways could converge on the activation of ERK-MAPK 1/2, which would then interact with the classical VDR-driven genomic pathway to modulate gene expression.

### NEW VITAMIN D TARGETS IN MUSCLE FIBRE

The contractile function of skeletal muscle is regulated by cytosolic calcium, which is provided by transport from the sarcoplasmic reticulum and supplied by ATP hydrolysis produced by SERCA. Vitamin D causes an upregulation of SERCA expression by supplying ionised calcium in the cytosol and thus contributing to the maintenance of muscle strength. Clearly, therefore, vitamin D acts in muscle cells by promoting ATP consumption. It has further been hypothesised that vitamin D upregulates LIN-43 expression in a dose-dependent manner, promoting the release of inorganic phosphates, such as pyrophosphate, into cell surface niches where they play an important role in ATP metabolism<sup>14</sup>. Indeed, extra-skeletal pyrophosphates suppress ectopic calcification in muscle tissue. Ectopic calcification in skeletal muscle has been observed in mouse models showing impaired muscle function, such as Duchenne muscular dystrophy or focal skeletal muscle injury<sup>15</sup>. Vitamin D should have a controlling activity on skeletal muscle calcification, which is essential for maintaining proper locomotor activity.

### CONCLUSION

Among vitamin D's extra-skeletal actions, what happens on striated muscle certainly impacts people's health considerably. Abundant scientific evidence has been found that confirms the activity of vitamin D in promoting muscle structure development during embryonic and foetal life as well as skeletal muscle regeneration and repair during adult life.

Furthermore, vitamin D plays a key role in the functional capacity of muscle fibre by promoting maximal efficiency of excitation/contraction coupling and in counteracting age-related structural and functional impairment of muscle and other sarcopenia conditions.

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# Effects of vitamin D deficiency on inflammatory cytokines

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Besides playing a key role in maintaining bone health, vitamin D has also been recognised for its antibacterial, antiproliferative, immunomodulatory and anti-inflammatory actions <sup>1,2</sup>, whilst its immunomodulatory functions have increasingly become of scientific interest. Indeed, in recent years, both clinical and epidemiological data supporting the link between vitamin D status and the incidence and severity of immunocorrelated conditions, such as multiple sclerosis, psoriasis, diabetes, rheumatoid arthritis, inflammatory bowel disease and infectious diseases, have been published <sup>1,2</sup>. Whereas the association between these pathological events and vitamin D deficiency has been widely demonstrated, the effect of cholecalciferol supplementation on the same phenomena has not. To complicate the picture, published studies are extremely heterogeneous in terms of the populations considered, the basal 25(OH)D levels, the extent of supplementation and the modality applied to administration (daily rather than boluses).

Attention to the effect of cholecalciferol supplementation on immune cells and inflammatory cytokines was certainly rekindled by publication of the VITAL study last year. In this study, 25,571 subjects were enrolled and randomised to take 2,000 IU of cholecalciferol per day (with or without omega-3 supplementation) versus placebo for five years, demonstrating a 22% reduction in the incidence of autoimmune diseases, including rheumatoid arthritis, polymyalgia rheumatica and psoriasis <sup>3</sup>. The regulation of inflammation and cytokine expression is also of crucial importance for the recent “inflammaging” hypothesis, which proposes that with increasing age there appears to be a shift towards a proinflammatory state that tends to create and maintain a chronic low-grade inflammation (only partially detectable by serum biomarkers such as C-reactive protein [CRP]) with a subsequent slow accumulation of damage. This acceleration toward ageing, driven by chronic inflammation, is believed to be the basis for progression to

several chronic diseases <sup>4</sup>. This has also been confirmed by a recent Anglo-Saxon biobank study of 397,737 subjects, aged between 37 and 73 years. Vitamin D deficiency was found to be associated with increased mortality from several causes, although not with classical serum inflammatory markers. If this is valid in the general population, however, it may be different in patient populations with high levels of inflammation, such as those with cancer, diabetes mellitus or acute cardiovascular disease, where supplementation in deficient subjects showed a reduction in high-sensitivity PCR <sup>5</sup>.

## VITAMIN D MECHANISM OF ACTION

Though vitamin D can act through an endocrine mechanism (the typical regulatory action of bone metabolism), it can also act through autocrine-paracrine signalling because of the presence of the enzyme 1  $\alpha$ -hydroxylase capable of producing the active metabolite 1,25(OH)<sub>2</sub>D within individual cells. Actually, it is the autocrine-paracrine action, which is responsible for the effect on immune system cells and consequently on proinflammatory cytokine production. The action of the active metabolite produced this way is modulated by binding to its receptor (VDR). The VDR found in the nucleus of multiple cell types mediates two types of actions, namely <sup>1-6</sup>:

- The non-genomic pathway: binding of the ligand to VDRs present in the cytosol triggers multiple pathways in intracellular signalling cascades, which lead to immediate responses independent of gene transcription in the cells;
- The genomic pathway: the retinoic acid receptor forms a heterodimer with the VDR bound to 1,25(OH)<sub>2</sub>D. The heterodimer translocates into the cell nucleus and binds to specific vitamin D response elements (VDREs) on target genes, thereby regulating nuclear transcription.

Both VDR and 1  $\alpha$ -hydroxylase are expressed by different types of immune cells, including

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## Conflict of interest

The Author declares no conflict of interest.

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macrophages, T-cells, dendritic cells, monocytes and B-cells. Evidence from preclinical studies has shown that vitamin D exerts biological effects on both the innate and adaptive immune systems (Tab. I). Extra-renal 1- $\alpha$ -hydroxylase is not up-regulated by PTH (parathyroid hormone). Therefore, the production of 1,25(OH)<sub>2</sub>D<sub>3</sub> depends on the levels of the 25(OH)D<sub>3</sub> substrate and can be regulated by inflammatory signals, such as polysaccharide (LPS) and the same cytokines. Vitamin D is believed to have a direct effect on cytokine production, the main mechanisms of which are summarised in Table II <sup>6</sup>.

### VITAMIN D DEFICIENCY AND PRO-INFLAMMATORY CYTOKINES

Vitamin D deficiency, often associated with increased serum levels of pro-inflammatory mediators, including IL-6 and tumour necrosis factor-alpha (TNF- $\alpha$ ), are related to both the development and progression of rheumatic and vascular inflammatory diseases <sup>1,2</sup>. Apart from what has by now been considered outdated evidence that observed an association between vitamin D deficiency and pro-inflammatory cytokines in classic inflammatory rheumatological diseases, such as rheumatoid arthritis or connective tissue disorders, a study was recently published that documented a linear correlation between the extent of vitamin D deficiency and increased levels of IL-6 and IL-8 in fibromyalgia. Specifically, reduced vitamin D levels were found to be associated with higher scores for both widespread pain as well as for disease activity <sup>7</sup>.

In a similar manner, in another study by the same authors on patients with osteoarthritis of the knee, a correlation was observed between vitamin D deficiency and higher IL-6 levels, whilst the IL-6 levels in turn were found to be associated with the radiographic stage of the disease and with the patient's functionality scale <sup>8</sup>.

Finally, in a study on obese patients, it was shown that reduced serum 25(OH)D concentrations were usually correlated with increased levels of other biomarkers of vascular inflammation, such as high-sensitivity PCR and fibrinogen. Similar conclusions were also reached for severely obese children <sup>1</sup>. All these studies support the hypothesis that among vitamin D-deficient subjects there is a concomitant rise in pro-inflammatory cytokines regardless of whether the subjects were healthy or were suffering from various

**TABLE I.**

Main effects of vitamin D on the activity of cells involved in innate and adaptive immunity.

| Innate immunity                          | Adaptive immunity                   |
|------------------------------------------|-------------------------------------|
| Increases differentiation of macrophages | Decreases Th1 cytokines             |
| Bactericidal action                      | Increases Th2 cytokines             |
| Inhibits dendritic cell maturation       | Reduces differentiation to Th17     |
| Inhibits antigen presentation            | Increases differentiation of T-regs |
|                                          | Reduces B-cell proliferation        |
|                                          | Induces B-cell apoptosis            |
|                                          | Inhibits plasma cell production     |
|                                          | Inhibits immunoglobulin secretion   |

Th1: T Helper 1; Th2: T Helper 2; Th17: T Helper 17, T-regs: Regulatory T cells.

**TABLE II.**

Main mechanisms by which vitamin D exerts its anti-inflammatory effect.

| Molecular target         | Mechanism                                               | Effect                                                                          |
|--------------------------|---------------------------------------------------------|---------------------------------------------------------------------------------|
| MAP kinase phosphatase 5 | Activates the enzyme which in turn inhibits p38         | Blocks amplification of the inflammatory cascade mediated by p38                |
| NF-kB                    | Inhibits NF-kB transcription factor through VDR binding | Reduces transcription/production of TNF- $\alpha$ , IL-1b and consequently IL-6 |
| Cyclooxygenase 2         | Directly inhibits prostaglandin production              | Reduces cell proliferation and angiogenesis                                     |

TNF- $\alpha$ : tumour necrosis factor-alpha; NF-kB: nuclear factor kappa B; IL-1 $\beta$ : interleukin-1 beta; IL-6: interleukin-6.

rheumatological and non-rheumatological diseases.

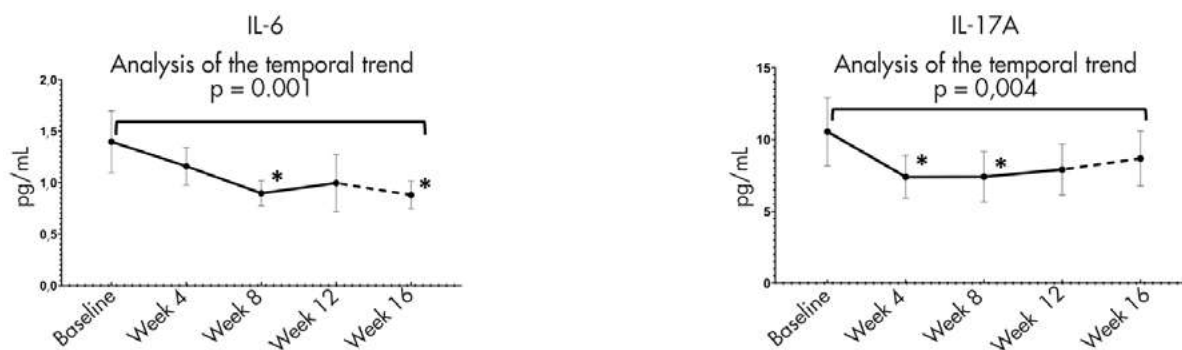
### EFFECT OF CHOLECALCIFEROL ADMINISTRATION IN CHOLECALCIFEROL-DEFICIENT SUBJECTS

Although a great deal of evidence has been found that supports the association between vitamin D deficiency and increased inflammatory cytokines, few studies have evaluated the effect of cholecalciferol administration on inflammatory status, whereas, often there has been bias that has limited interpretation. In a group of young, healthy subjects, who were however vitamin D-deficient, the effect of cholecalciferol administered over 12 weeks on the production of IL-17A, IL-6, IL-8, IL-10, IL-23 and TNF- $\alpha$  was recently evaluated. Though we observed a progressive reduction in IL-6 and IL-17A levels, no significant differences were found in the serum concentrations of the other cytokines (Fig. 1) <sup>9</sup>. IL-6 and IL-17 are two key cytokines in

rheumatoid arthritis and spondyloarthritis, respectively. The reduction in serum levels observed in this study could support a possible role of vitamin D supplementation for patients with rheumatological diseases to optimise their therapeutic response to specific drugs. In support of this option, it was also observed that, depending on serum 25(OH)D levels, vitamin D supplementation would have different (positive) effects on pain and disease activity among patients with rheumatoid arthritis <sup>10</sup>.

### CONCLUSIONI

Gli studi che hanno valutato l'effetto della supplementazione con vitamina D sulle citochine infiammatorie sono ancora pochi, talvolta con risultati discordanti e spesso non confrontabili tra loro in quanto condotti su popolazioni a volte carenti, a volte no e con comorbidità differenti. Tuttavia negli studi condotti su soggetti giovani, sani e carenti di vitamina D, dove i fattori confondenti sono ridotti, ed è possibile così



IL-6: interleukin-6; IL-17A: interleukin-17°.

**FIGURE 1.**

Effects of cholecalciferol supplementation on serum levels of IL-6 and IL-17A in young, healthy, vitamin D-deficient subjects °.

valutare l'effetto "puro" del colecalciferolo, si evidenzia un effetto della supplementazione nel ridurre le citochine pro-infiammatorie. Se questi dati si confermassero, la vitamina D potrebbe diventare un trattamento complementare nella prevenzione e nel trattamento di numerose patologie reumatiche e infiammatorie.

Instead, in another study on healthy but elderly subjects (average age over 70 years), cholecalciferol administration did not change gene expression or serum levels of IL-6, IL-8, IL-10, TNF- $\alpha$  or IFN- $\gamma$ . Regardless, it should be noted that basal serum 25(OH)D levels were higher than in the previous study and the cholecalciferol dosage varied among treatment groups <sup>11</sup>. The effect on cytokine reduction was also studied in a small group of healthy men undergoing intense endurance exercise. Compared to placebo, the supplemented subjects showed positive effects in terms

of increased blood levels of 25(OH)D, of CD4+/CD8+ ratio (immune response) and of aerobic capacity, through the inhibition of inflammatory cytokines (IL-6 and to a lesser extent TNF) and CK(creatine kinase) and LDH (lactate dehydrogenase) (indicators of muscle damage) <sup>12</sup>.

Table III summarises the main features of studies that have assessed the effects of cholecalciferol supplementation on serum levels of inflammatory cytokines. The effect has been found to be more controversial in disease conditions. Some years ago, a meta-analysis of over 80 studies on different disease conditions, showed no significant effects of vitamin D supplementation on inflammatory biomarkers, including C-reactive protein, IL-6 and TNF- $\alpha$ . It should be noted that in addition to the heterogeneity of the pathological conditions and their pathogenesis, IL-6 was assayed in only 22 of these studies, whereas TNF- $\alpha$  was measured in only 25 <sup>13</sup>.

Assessing some specific clinical conditions, Corrado et al. recently showed that in vitro exposure to increasing doses of 1-25(OH)<sub>2</sub>D in deficient subjects was associated with a significant reduction in IL-17A and profibrotic cytokines (FGF2, TGF- $\beta$ , CTGF) whether the patients had systemic sclerosis or were healthy, both with a dose-dependent effect <sup>14</sup>.

Instead, in 44 vitamin D deficient multiple sclerosis patients, an increase in serum levels of anti-inflammatory cytokines (IL-10, TGF- $\beta$ ) and the regulatory IFN- $\gamma$  was observed after 12 months of supplementation with 500-1000 IU/day [depending on basal 25(OH)D levels] of cholecalciferol, while IL-17 (proinflammatory) remained unchanged <sup>15</sup>.

Among patients with cardiovascular disease, in deficient subjects, vitamin D supplementation was able to reduce the expression of pro-inflammatory and pro-atherogenic cy-

**TABLE III.**

Studies that evaluated the effect of cholecalciferol administration on pro-inflammatory cytokines.

| Author          | No. of patients | Median age (years) | 25(OH)D (ng/mL) | Dose administered                                                                                                                                                                                      | Duration | Effect                  |
|-----------------|-----------------|--------------------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|-------------------------|
| Fassio et al.   | 75              | 34                 | 13.7            | <ul style="list-style-type: none"> <li>• 10,000 IU/day for 8 weeks then 1,000 IU/day for 4 weeks</li> <li>• 50,000 IU/week for 12 weeks</li> <li>• 100,000 IU every other week for 12 weeks</li> </ul> | 12 weeks | Reduces IL-6 and IL-17a |
| Berlanga et al. | 305             | 72                 | 20              | <ul style="list-style-type: none"> <li>• 4,000 IU/day</li> <li>• 2,000 IU/day</li> <li>• Placebo</li> </ul>                                                                                            | 1 year   | No significant effect   |
| Liu et al.      | 18              | 22                 | 22              | <ul style="list-style-type: none"> <li>• 5,000 IU/day</li> <li>• Placebo</li> </ul>                                                                                                                    | 4 weeks  | Reduces IL-6            |

IL-6: interleuchina-6; IL-17A: interleuchina-17A.

tokines such as IL-2 and interferon- $\gamma$  (IFN- $\gamma$ ), which are responsible for T-helper-1 cell activation and vascular inflammation<sup>1</sup>. The condition of obesity deserves a separate discussion. Chronic low-grade inflammation appears to play a crucial role in the development of obesity-associated comorbidities such as insulin resistance, cardiovascular disease and cancer. The systemic inflammatory response brought about by obesity appears to mainly originate from adipose tissue, promoting the infiltration of inflammatory cells (macrophages) and the release of pro-inflammatory mediators, leading to low-grade systemic inflammation. In support of this, previous studies showed some positive correlations between adipose tissue volume and the secretion of pro-inflammatory cytokines<sup>4</sup>. One recent study evaluated the effect of supplementation with probiotics (strains of lactobacilli and bifidobacteria), omega-3 and omega-6 and vitamin D on low-grade inflammation among overweight and obese individuals. The study showed no differences on the primary outcome, which was hs-CRP (high-sensitivity C-reactive protein) levels. However, among the subjects treated, serum levels of IL-6 decreased after administration indicating an albeit modest effect on inflammation<sup>16</sup>. The main limitations of this study were, apart from the limited case series, the simultaneous administration of omega-3-6 probiotics and cholecalciferol, which do not allow the effect of the individual elements to be distinguished, and the low dose of vitamin D administered (200 IU/day, well below the doses that have so far demonstrated extra-skeletal effects). Although the rationale is very strong, only one study documented a reduction in serum IL-6 concentration after cholecalciferol administration alone among obese subjects<sup>17</sup>.

## CONCLUSIONS

Though there are few studies that have evaluated the effect of vitamin D supplementation on inflammatory cytokines, they sometimes had discordant results, were often not comparable with one another because they were conducted on populations that were sometimes deficient, sometimes not and often the subjects had different comorbidities. However, in studies conducted on young, healthy, vitamin D-deficient subjects, where confounding factors were reduced, and the "pure" effect of cholecalciferol could thus be assessed, it was shown that one effect

was that supplementation reduced pro-inflammatory cytokines. If these data were to be confirmed, vitamin D could then become a complementary therapy in the prevention and treatment of numerous rheumatic and inflammatory diseases.

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