



## A Review on the Potential Role of Vitamin D and Mineral Metabolism on Chronic Fatigue Illnesses

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### Abstract

The aim of this report is to review the effects of vitamin D-deficiency on chronic mineral deregulation and its clinical consequences. Recent research data are presented including the effects of vitamin D3-induced calcium sensing receptor (CaSR), fibroblast growth factor 23 (FGF23), the cofactor of FGF1-receptor  $\alpha$ -klotho ( $\alpha$ KI) and the interplay with each other and with vitamin D3-repressed parathormone (PTH). The importance of persistent calcium- and phosphate deregulation following long-standing vitamin D3-deficiency for cellular functions and resistance to vitamin D3 treatment is discussed. It is proposed that chronic fatiguing illnesses might be result from mineral deregulations that are barely detected by routine laboratory workups because of compensatory changes in bone mineral stores.

### Keywords

Vitamin D3-deficiency, Mineral regulation, Calcium-sensing receptor, Fibroblast-growth-factor-23, Alpha-klotho, Parathormone, Chronic fatigue

### Objective

The metabolite of vitamin D3, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], regulates bone minerals calcium and phosphate [1-6]. Less well known, however, are the mechanisms by which 1,25(OH)2D3 regulates many minerals and ion channels. More recent research data add to the understanding how mineral deregulation induced by vitamin D3-deficiency could be responsible for chronic symptomatic chronic fatigue and the accompanying functional and depression-like symptoms. The objective is to attempt shedding some light on such possible interactions of calcium metabolism and uncommon clinical features, and thus adding to a potential differential diagnosis of chronic fatigue disorders, depression, and others.

### Vitamin D3 regulates most parts of mineral metabolism in a highly complex manner

Well-known is that 1,25(OH)2D3 induces gene expression of its own nuclear receptor, called vitamin D receptor (VDR), and its own deactivating enzyme, named 1,25-dihydroxyvitamin D3 24-hydroxylase (CYP24A1) [7,8]. It also down-regulates the gene expression of its own activating enzyme, 1-alpha-hydroxylase (alternatively, cytochrome 27B1; CYP27B1) in renal tubular cells [7]. In contrast, gene expression of CYP27B1 in many other cells occurs

by any kind of cell stress as long as sufficient 25-hydroxyvitamin D3 (25OHD3) is available [8-11].

1,25(OH)2D3 induces in addition the gene expression of following important mineral regulators such as calcium-sensing-receptor (CaSR), Fibroblast Growth Factor-23 (FGF23) and its co-receptor  $\alpha$ -Klotho ( $\alpha$ KL, also FGF23/ $\alpha$ KL in this paper), yet represses the gene expression of parathormone (PTH) [2,6,12-16]. These mineral regulators, like 1,25(OH)2D3 itself, act not only via gene expression, but also modulate cell functions directly by rapid non-genomic actions. This contributes substantially to the high complexity and adaptability of mineral regulation [2,6,12,17-21].

### Low vitamin D3, low dietary calcium and high phosphate intake occur frequently

Vitamin D3-insufficiency/deficiency is a common condition arising from prevalent indoor activities and use of sunscreens with high protection factor. Lacking sunlight is frequent in patients suffering from chronic fatigue syndrome (CFS), fibromyalgia (FMS), and myalgic encephalopathy (ME) probably because of fatigue-related reduction of outdoor activity, high pre-morbid engagement in professional or caring activities, and preceding stressful life events [22]. Vitamin D3-deficiency may develop also as side effect of certain drugs (anti-epileptics, phenobarbital, hyperforin, carbamazepine, and rifampin) and is due to induction of the CYP3A4 gene [23].

As enteral calcium absorption is enhanced by 1,25(OH)2D3, and calcium, like 1,25(OH)2D3, is engaged in gene expression and cell signaling, knowledge about the impact of calcium deficiency on global mineral balance is important as well. Western diets commonly are poor in milk and milk products, nuts and calcium-rich vegetables, but are rich in phosphate supply (soft drinks, processed foods). They thus favor calcium deficiency and deficient bone mineralization [16,24-27], as well as "phosphate toxicity" [16,26-29]. Calcium deficiency is also promoted by long-standing therapy with proton-pump inhibitors by reducing enteral calcium absorption substantially [30].

### Impact of 1,25(OH)2D3 on enteral mineral absorption and renal reabsorption

Binding of 1,25(OH)2D3 to VDR induces gene expression of calcium transporting channels, pumps, exchangers and binding proteins such as transient potential protein, type 6 or type 5, calbindin

9 and calbindin 28, ATP-dependent plasma membrane calcium-ATPase, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger, and the intercellular proteins claudin 2 and 12 [2-6,16,30,31]. In addition, enteral and renal phosphate transporters are also induced by 1,25(OH)<sub>2</sub>D<sub>3</sub> [2,6], as are enteral magnesium uptake proteins [32-35].

However, renal magnesium re-uptake is inhibited by claudin 16 which is induced by both 1,25(OH)<sub>2</sub>D<sub>3</sub> and CaSR [33-36]. Such a potent 1,25(OH)<sub>2</sub>D<sub>3</sub>-induced antagonism against mineral overload is not only known with respect to magnesium, but exists also for calcium and phosphate. Such 1,25(OH)<sub>2</sub>D<sub>3</sub>-induced antagonists are CaSR which regulates calcium transport [6,37-40], and FGF23/αKl which regulates phosphate transport [2,6,12,15,16].

### **CaSR prevents calcium overload, and in addition, modifies cellular signal transduction**

CaSR is ubiquitously expressed and is a nutrient sensing, G protein-coupled receptor, activated by extracellular calcium binding [3,37-40]. Transcription of CaSR and VDR genes is enhanced by Ca<sup>2+</sup> and 1,25(OH)<sub>2</sub>D<sub>3</sub> [37,40], and by the pro-inflammatory cytokines interleukin 1β and interleukin 6 [38,40]. In addition to calcium, CaSR becomes activated by other di- and trivalent ions, such as magnesium, strontium, gadolinium, and by organic poly-cations like polyamines and neomycine, as well as by amino acids. In contrast, protons and high sodium concentrations rather suppress CaSR activity [27,38,39]. Thus, depending upon the given biological and metabolic status, CaSR activity may vary substantially. This is further complicated as activating and deactivating antibodies may interfere in this balance [37,41], and different CaSR ligands can elicit different cell responses, called biased agonism [42,43].

Activated CaSR reduces enteral and renal transport of calcium, Na<sup>+</sup> and Mg<sup>2+</sup>, as well as protons and water. It thus serves as a potent vitamin D<sub>3</sub> antagonist with respect to mineral metabolism [13,15,16]. On the other hand, CaSR co-localizes and cooperates with many cell membrane and intracellular receptors, translating extracellular calcium levels into intracellular signal modulation [37-39,44,45]. Thus it also serves as a cellular calcium agonist.

In the parathyroid glands, activated CaSR represses excessive parathyroid hormone synthesis and secretion when serum calcium is above its set-point [37-40]. It also supports bone mineralization in osteocyte cells by a feed forward mechanism with enhancement of VDR and CaSR expression, while inhibiting osteoclast activity [16,37].

### **FGF23/αKl prevents phosphate overload, and controls calcium x phosphate product**

Binding of αKl, which is a co-receptor for FGF23, mediates effective FGF23 signaling which represses 1,25(OH)<sub>2</sub>D<sub>3</sub>-synthesis and increases renal phosphate excretion. The latter is mediated by reduced gene expression and augmented membrane internalization of renal sodium-phosphate co-transporters [16,25,27,46,47]. FGF23 gene expression in osteoblasts and osteocytes is induced by 1,25(OH)<sub>2</sub>D<sub>3</sub>, and by a rise in the calcium x phosphate product. Conversely, a decrease of either calcium or phosphate inhibits the effectiveness of FGF23 levels [16,27]. With FGF23/αKl also promoting calcium re-uptake in the distal renal tubule [16,48,49], a delicate build-in control system appears in line for a balanced metabolic activity.

As αKl also interferes with the function of other transport proteins, such as, for instance, various ion channels, carriers and pumps, it as well serves as a key protein for mineral transport in general and for cell function [50].

It is worth mentioning at this place that a high phosphate diet decreases enteral calcium absorption, and, vice versa, high dietetic calcium contents inhibit enteral phosphate uptake. This mutual absorption inhibition becomes mitigated by PTH and CaSR which both assist in keeping a physiological mineral balance in spite of enteric uptake variations [27].

Because FGF23, αKl and FGFR1 must cooperate to induce FGF23 signaling, reduced αKl gene expression is expected to compromise FGF23 signaling. It is reported that already in early stages of chronic kidney disease reduced αKl might occur, whereas in advanced renal insufficiency increased FGF23 levels were found consisting of truncated proteins with reduced biologic activity [16,17,25,27,46,47].

### **Low serum calcium induces PTH rise and represses FGF23**

Persistently low serum calcium fails to activate CaSR resulting in inhibition of repression of PTH gene and parathormone secretion, and also resulting in inhibition of FGF23 gene expression [1,6,14,51]. Both events have negative effects on bone mineralization [27,46]. A persistent PTH elevation enhances calcium transport in gut and kidney, thus mitigating serum calcium deficits secondary of vitamin D<sub>3</sub>-deficiency or low calcium intake, on the one hand, but compromises bone mineral content on the other hand [1,6,14,51]. Yet, PTH elevation may be blunted by magnesium deficiency, resulting in inadequately low PTH compared to serum calcium [52]. While transient PTH surges are essential for normal bone turnover, a persistent PTH elevation induces inflammatory cytokines and rather increases bone resorption [19,53]. This increased bone resorption keeps serum calcium and phosphate at normal levels for longer times, although bone stores become reduced; in fact, serum levels of bone minerals and phosphate may be even temporarily increased [19] with transient activation of CaSR, thus aggravating global mineral loss.

In addition, persistent PTH elevations will cause renal phosphate wasting [14,29], secondary to PTH-mediated gene induction of FGF23 [54], and also through PTH-enhanced internalization of renal sodium/phosphate co-transporters [2,6]. The resulting high renal phosphate loss will impair bone formation and renal tubular function in its own way [2,29,55,56].

Interestingly, a set-point shift of the Ca<sup>2+</sup>/PTH curve in aged calcium-deficient mice prevented excessive PTH rise, as long as CaSR activity and gene expression remained intact, possibly pointing to an adaptive phosphate-saving mechanism [57].

### **Bone health depends also on sufficient dietary supply of minerals and energy sources**

Bone turnover and mineral balance are important for bone health by providing bone tissue renewal, repair and physical stability. Vitamin D<sub>3</sub> sufficiency and the correct functional interplay of all known factors which regulate mineral metabolism clearly are essential for bone health, but the optimal enteral uptake of calcium, phosphate, other minerals, proteins, and other essential nutrients, and in addition, sufficient mitochondrial ATP production are important as well [1,5,14,15,27-29,31,52,55,58-69]. Due to mineral deregulation not only bone metabolism becomes negatively affected, but energy and cell metabolism as well [70-77].

### **Mineral regulation influences intracellular function and signaling**

Although extra- and intracellular calcium and phosphate levels are regulated by different mechanisms, extra- and intracellular compartments of these ions are bound to each other by multiple complex mechanisms [69,75,77-83]. The role of CaSR in connecting extra- with intracellular calcium events has been already mentioned. Another mechanism is mediated by connexin hemichannels [80,83,84]. Furthermore, cell signaling cascades use preferentially calcium signaling cascades. These cascades are modified by the amount of primary calcium inflow from the extracellular milieu. Signaling depends upon the cellular membrane potential and transmembrane physicochemical conditions such as voltage, pH, and gradients in extracellular and intracellular concentrations of ions [85]. Functions of intracellular proteins depend widely upon calcium and phosphate binding, whereas persistent and excessive elevation of free calcium and phosphate is toxic to cells [30,69,75,77,78,81,86-95].

Distinct intracellular organelles need distinct levels of calcium concentration for proper functioning; they then contribute to storage,

signaling, and homeostasis of calcium [95]. Mitochondrial energy production [87-90,96,97], endoplasmic reticulum stress-response [76,86,91,94,95,97,98], and proteasome and autophagolysosome functions [92,94] depend upon optimal calcium binding conditions which are also essential for both cell structure and function [79,80,82,85].

### Low serum calcium initiates a $\alpha$ -Klotho-mediated compensatory mechanism

When serum calcium drops below a critical threshold, neuromuscular hyper-excitability will occur with muscle spasms and tetany [81,99-101]. The reduced transmembrane calcium gradient affects all membrane receptors, channels, transporters, exchangers and pumps by a sodium dependent mechanism, and finally results in cellular calcium overload [79,80,82,85,102].

Due to low serum calcium, CaSR activity should be reduced resulting in attenuation or failing of the modulating interaction between CaSR and intracellular cell signaling [44]. However, since multiple additional activators of CaSR are known, the real in-vivo effects of CaSR in hypocalcemia still need elucidation.

A particular  $\alpha$ KI-dependent compensatory process is active in hypocalcemia. Low serum calcium levels induces  $\alpha$ KI gene expression. Binding of  $\alpha$ KI to the intracellular  $\text{Na}^+/\text{K}^+$ -ATPase enhances recruitment of the complex to the cell membrane where its ATPase activity becomes substantially enhanced. This results in membrane hyperpolarization [25,58,102]. This hyperpolarization enhances the calcium transport across renal tubular cells, and across epithelia of the plexus chorioideus and of the parathyroid gland, thus protecting renal calcium re-uptake, liquor calcium levels, and parathyroid response. However, this only occurs at the expense of ATP production [25,58,102]. This implies that in case of failing mitochondrial calcium optimum, this compensatory mechanism might become compromised due to blunted ATP production.

### Intracellular calcium and phosphate deregulation induces endoplasmic reticulum stress

The endoplasmic reticulum (ER) is the key organelle when it comes to calcium storage, release and re-uptake. Most of its effector proteins are calcium-binding, some with low affinity, but with high capacity,

while others show high affinity and low capacity [76,87,94,95,98]. Deregulated calcium balance will affect ER-resident proteins which in particular act as sensors for extra- and intracellular stress [95]. Such a condition will initiate a cellular rescue response, but only if optimal calcium re-uptake in the ER is available [76,87,103]. Otherwise, compromised function and stability of ER effector proteins such as chaperones will ensue followed by premature and augmented protein decay, interrupted new protein synthesis, and augmented protein malformations [103]. Calcium re-uptake into ER is dependent upon ATP-consuming ATPase activity, while ATP synthesis is thought to be compromised by a suboptimal level of mitochondrial calcium [96]. This failure, though, may be compensated by augmented gene expression of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger [104]. This exchanger does not consume ATP and may partly alleviate the problems in calcium re-uptake. At this place it must be taken into account, though, that a process called "store-operated calcium entry" replenishing ER calcium stores by calcium inflow from the extracellular compartment will be negatively affected by low extracellular calcium, as well [93,105,106]. In addition, in cell stress, ER-chaperones such as calreticulin are leaking into the extracellular compartment [107] which appears to be linked to the activation of pro-inflammatory reactions by mediators such as the interleukins-1, -6, -12, -23 and tumor necrosis factor - $\alpha$  [76]. This underpins the close link between Ca metabolism, ER-stress response and inflammatory reactions.

### Discussion

Common vitamin D3 deficiency with unbalanced calcium and phosphate metabolism are easily overlooked since progressive bone resorption quasi "normalizes" serum mineral levels. Routine medical check-ups thus remain inconclusive at this stage. Only at late stages, pathological calcium levels become evident as being too low or too high. Therefore, in early stages of calcium metabolic derangements only clinical signs such as chronic fatigue, post-exertional malaise and wide-spread organ dysfunctions without detectable structural damage should prompt the care-taking physician to also check vitamin D3 levels and respective mineral changes.

Symptoms of generalized muscle weakness and widespread pain, severe headaches, intolerance of drugs, nutrients, pollutions, and generalized stress intolerance, besides non-refreshing sleep or other sleeping disorders, and failing recovery after rest are some of the most

**Table 1:** Summary of observed disease stages, the corresponding key events, expected markers, presumed pathophysiology and distinct clinical diagnoses of chronic fatiguing illnesses.

| Stage | Corresponding key stage events   | Expected markers   | Presumed pathophysiologic changes  | Correlated clinical diagnosis  |
|-------|--|--|--|--|
| I     | Lack of sunlight.<br><br>Low calcium diet.<br>High phosphate diet.   | 25OHD <sub>3</sub> low.<br>P <sub>i</sub> /Crea and Ca <sup>2+</sup> /Crea ratio dependent on diet and 25OHD <sub>3</sub> ?  | Calcium and/or phosphate deficiency, according to underlying cause.<br><br>Phosphate toxicity due to high phosphate diet.  | Idiopathic fatigue   |
| II    | Calcium body stores low, but serum Ca <sup>2+</sup> and P <sub>i</sub> normal due to accelerated bone loss.  | 1,25(OH) <sub>2</sub> D <sub>3</sub> high, though 25(OH)D <sub>3</sub> low.<br>P <sub>i</sub> /Crea ratio very low or variable?<br>Ca <sup>2+</sup> /Crea ratio variable dependent on diet, PTH <sub>i</sub> and bone loss.<br>PTH <sub>i</sub> low in magnesium deficiency. | FGF23 low due to low calcium x phosphate product and due to low 25OHD <sub>3</sub> ?<br><br>$\alpha$ KL low due to low 25OHD <sub>3</sub> ?<br>CaSR gene induction reduced due to low 25OHD <sub>3</sub> ?<br><br>CaSR activity variable due to bone loss and diet.    | Chronic fatigue syndrome   |
| III   | Calcium body stores very low. Extracellular Ca <sup>2+</sup> low normal. Yet paradoxical hypercalcaemia. Global renal and bone mineral loss.                                     | PTH <sub>i</sub> inappropriate to serum and urine Ca <sup>2+</sup> ?<br><br>P <sub>i</sub> /Crea- and Ca <sup>2+</sup> /Crea-ratio inappropriate to serum levels?  | $\alpha$ KL very low?<br>Ca <sup>2+</sup> low normal due to set-point change of Ca <sup>2+</sup> /PTH <sub>i</sub> curve?<br>Hypercalcaemia due to set-point change or hyperactive CaSR?   | Vitamin D <sub>3</sub> -resistant chronic fatigue syndrome<br><br>Resistance reversible by multi-minerals and phosphate?         |
| IV    | Ca x P <sub>i</sub> body stores extremely low. Multiple mineral deficits variably and intermittently measurable. Ca <sup>2+</sup> /P <sub>i</sub> deposits at unwanted location. | Obviously elevated P <sub>i</sub> /Crea and Ca <sup>2+</sup> /Crea ratio?<br>CaSR activity activated by other agonists?<br><br>Unsuspected laboratory results? Truncated FGF23 and PTH?  | Tubular epithelial damage with multi-mineral depletion?<br><br>Reduced renal NH <sub>4</sub> <sup>+</sup> excretion??<br>Global channelopathy?<br>Hormone resistance against CaSR, FGF23/ $\alpha$ KL, PTH?<br>At least osteoporosis as clinical hint to the disorder. | Myalgic encephalopathy (younger patients).<br><br>Fibromyalgia (older patients).<br><br>Somatic irreversible secondary diseases. |

**Abbreviations:** 25OHD<sub>3</sub>: 25-hydroxyvitamin D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>: 1,25-dihydroxyvitamin D<sub>3</sub>,  $\alpha$ KI:  $\alpha$ -Klotho, Ca<sup>2+</sup>: calcium, CaSR: Calcium sensing receptor, FGF23: Fibroblast Growth Factor-23, P<sub>i</sub>: Phosphate, PTH<sub>i</sub>: Parathormone intact

prominent symptoms. In general, patients complain of symptoms mimicking a viral infection. They report clear deterioration after physical and mental stress. Due to cognitive and emotional impairment physicians are poised to diagnose a depression. But the symptoms differ substantially from major depression.

All these dysfunctional symptoms could arise from endoplasmic reticulum stress and reduced endo- and sarcoplasmic calcium-re-uptake. Additionally, the inflammatory response induced by derangement of mineral metabolism would fit well to the few research reports about measurable inflammatory changes in chronic fatiguing illnesses [108-114].

Four stages in the respective development of clinical disease are observed corresponding to the key events in metabolic derangement. These are summarized in [table 1](#).

It is important to consider that calcium and phosphate deficiency with resulting endocrine, metabolic and mineral disorders will persist in spite of vitamin D3-substitution, if calcium, phosphate and multi-minerals are not substituted at the same time. Even then, a transient phase of low normal calcium with inadequate low PTH levels might occur due to a set-point shift of the Ca<sup>2+</sup>/PTH curve. This might arise as compensation for excessive renal phosphate loss. In addition, concurrent magnesium deficiency can also induce inadequate low PTH levels. The inadequate PTH level will then compromise PTH-dependent calcium rescue mechanisms resulting in additional reduction of calcium stores. Inadequate renal calcium loss in spite of low serum calcium might be caused by low PTH, by inappropriately augmented CaSR activity, presumably due to aberrant polyamine synthesis, or by inflammatory cytokines. Another cause of inadequate renal calcium loss is tubular damage induced by continuously impaired mineral regulation and accompanying vitamin D-deficiency. In particular severe phosphate deficiency is linked to tubular damage with secondary renal calcium leak.

Disordered mineral regulation can be suspected if 1,25(OH)<sub>2</sub>D<sub>3</sub> levels are inadequately high compared to low 25OHD<sub>3</sub> levels. This suggests low stores of calcium and/or phosphate. In severe phosphate depletion, no renal phosphate excretion should be detectable. In contrast, a certain amount of urinary calcium is lost inevitably and physiologically.

Deficiencies of vitamin D<sub>3</sub>, as well as deficiency of calcium and phosphate, induce renal tubular epithelial damage which then affects also renal reabsorption of all minerals. These deficiencies induce compromised tubular sodium re-uptake which is followed by a compromised counter-current mechanism. Decreased α-Klotho expression and function, as well as lowered expression of vitamin D-dependent proteins like claudins which manage passive calcium transport contribute to reduced enteral uptake and renal re-uptake of minerals. All these events can explain the generalized mineral disturbance following vitamin D-deficiency. Plasma mineral overflow due to increased global bone mineral release will contribute as well.

A scientific pilot study of coincident testing of 25OHD<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, PTHi, αKl and FGF23, possibly also CaSR gene expression, might promise clarification. However, disturbance of these parameters are not supposed to be specific for chronic fatigue syndrome and related disorders. Yet, pathologic results would help to convince that real disease is going on in these patients.

Routine screening in everyday clinical setting should be restricted to investigation of 25OHD<sub>3</sub> and PTHi, as well as calcium/creatinine (Ca<sup>2+</sup>/Crea) ratio in the second fasting morning urine. Only in special cases, such as increased urinary calcium and/or phosphate loss, and inappropriate PTHi levels, or in obvious resistance against vitamin D<sub>3</sub>-treatment, investigation of 1,25(OH)<sub>2</sub>D<sub>3</sub> is indicated. Inadequately high levels suggest deficiency of calcium and/or phosphate. As long-standing vitamin D-deficiency is reported to affect tubular phosphate reabsorption earlier than calcium reabsorption, investigation of the phosphate/creatinine (Pi/Crea) ratio might be indicated as well.

Specific and early therapy with calcium and other bone minerals,

combined with vitamin D<sub>2</sub> or D<sub>3</sub> compounds presumably might prevent progression from chronic fatigue and functional disorder to more debilitating stages of fatiguing illnesses such as CFS, FMS, and ME.

Interestingly, a subgroup of patients with progressive chronic fatigue syndrome and obvious vitamin D<sub>3</sub> resistance showed abnormal laboratory results suggesting indeed underlying mineral derangement. They showed low normal serum calcium, inadequately augmented Ca<sup>2+</sup>/Crea ratios, sometimes low serum phosphate, lowered or low normal 25OHD<sub>3</sub>, high normal or elevated 1,25(OH)<sub>2</sub>D<sub>3</sub>, inappropriately low PTH, and urinary anion gap results suggestive of reduced renal ammonium excretion (unpublished clinical observations of the author).

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