Will vitamin D supplementation ameliorate diseases characterized by chronic inflammation and fatigue?

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S U M M A R Y

Chronic NF-κB activation has been supposed as a key event in chronic fatigue syndrome (CFS) and many other better-defined pro-inflammatory diseases. Knowledge about the impact of deficiency vitamin D on chronic NF-κB activation could open a new disease approach.

Whereas NF-κB activation leads at first to a pro-inflammatory immune response, later on a vitamin D-dependent anti-inflammatory response ensues. Binding of the active vitamin D metabolite 1,25(OH)2D3 to vitamin D receptor (VDR) yields a transcription factor which represses NF-κB activation, and additionally modulates and down-regulates adaptive, but enhances innate immune responses, and improves redox balance, thus counterbalancing inflammation on multiple levels. However, this built-in late counterbalance against inflammation works only when stores of calcium and 25(OH)D3 are abundant.

Therefore a connection between lowered vitamin D-metabolism and persistent NF-κB activation, augmented nitrosative-oxidative stress, redox imbalance, chronic inflammation, and concomitant fatigue can be postulated.

In order to confirm this hypothesis, randomized controlled clinical studies about the clinical effects of supplementation of calcium and vitamin D3 would be necessary in diseases characterized by persistent NF-κB activation and chronic inflammation and fatigue.

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Initial stress response is pro-inflammatory and self-amplifying

In chronic inflammatory illnesses, like for instance rheumatoid arthritis, chronic fatigue is a well accepted symptom accompanying or even preceding clinical relapse. Well known causes inducing fatigue are pro-inflammatory cytokines and lowered ATP pool (energy loss). Meanwhile, chronic inflammatory diseases, but also chronic fatigue syndrome (CFS) and related disorders, are presumed to be connected with persistent activation of nuclear factor-kappa B (NF-κB) resulting in augmented nitrosative-oxidative stress, lowered ATP pools, and chronic inflammation [1–3].

On the other hand, a stress-induced vicious up-regulation of nitric oxide/peroxynitrite (NO/ONOO-) cycle, as a local event, and discussed extensively elsewhere, is presumed to lead in a multi-step manner to several diseases, also named multi-system illnesses [4–12].

However, NF-κB activation and augmented nitrosative-oxidative stress are key events in this cycle, as well. Therefore NF-κB activation might be a central topic of this disease mechanism.

NF-κB activation is an essential defence response mechanism in innate immune system [13–15] altering protein synthesis [16], gene expression before, during and after transcription [3,16–18], as well as growth, differentiation, and apoptosis [3,19]. NF-κB activation induces the production of high levels of reactive oxygen species (ROS), like superoxide (OO-), on the one hand, and of nitric oxide (NO), at the other hand, with resulting generation of peroxynitrite (ONOO-), thus leading to altered cellular structures and functions [10,20–28]. In particular, metabolic consequences will ensue like derangement of citric acid cycle and oxidative phosphorylation, resulting in lowered ATP [10,25,26] and NADH/NAD redox pool [10,23–26].

It is important to realize that NF-κB activation possesses an inherent self-amplifying potency by induction of interferon-γ (IFN-γ), pro-inflammatory cytokines like interleukin-1-beta (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and inducible NO synthase (iNOS) [28,29], with the net result of overproduction of ROS [29–34] which activate NF-κB once more. However, this is not the only positive back loop. INF-γ, IL-2, and TNF-α activate NF-κB directly [29], creating another feed-forward loop. Furthermore, ROS damage ion channels, in particular calcium pumps. By this way free intracellular calcium (Ca2+) levels become elevated...
stress, interferon (IFN), and the active metabolite 1,25(OH)2D3 it-

counterbalance of NF-

B activation ensues in late stress response (Fig. 1). Exactly this scenario of not properly opposed pro-inflamma-
yory immune and stress response, with chronic elevation of

os: tumor necrosis factor-alpha.

which, like ROS, are able to induce a new round of NF-kB activation [29].

This inherent trend towards initial augmentation of pro-inflamma-

tory stress response might play an essential pathogenic role in

chronic inflammatory and auto-immune diseases if no effective

counterbalance of NF-kB activation ensues in late stress response (Fig. 1). Exactly this scenario of not properly opposed pro-inflam-

atory immune and stress response, with chronic elevation of Ca2+ levels [23,43–45,47]. A calcium indepen-

dent manner by parathormone (PTH), and serves to control

The pleiotropic actions of vitamin D in metabolism

Vitamin D comprises vitamin D3 and vitamin D2. Vitamin D,

which may come either from suntanned skin, diet or supple-

ments, is synthesized in the liver to 25-hydroxy-cholecalciferol

[25(OH)D3] which is activated by the enzyme 25-hydroxyvitamin

D-1 alpha-hydroxylase, CYP1a: 25-hydroxyvitamin D-1- alpha-hydroxylase, IFN-γ: interferon gamma (type II interferon), IL1-β: interleukin-beta 1, IL-2: interleukin-2, IL-6:

interleukin-6, IL-8: interleukin-8, iNOS: inducible NO-synthase, NF-kB: nuclear factor-kappa B, NO: nitric oxide, NO/ONOO−: peroxyxinitrite, ROS: reactive oxygen species, TNF-

α: tumor necrosis factor-alpha.

[29–31] which, like ROS, are able to induce a new round of NF-kB activation [29].

Fig. 1. NF-kB activation induces a burst of stress responses [23,28,29,38]. Abbreviations: 1,25(OH)2D3: 1,25-dihydroxyvitamin D3, ATP: adenosine triphosphate, Ca2+: free intracellular calcium, CYP1a: 25-hydroxyvitamin D-1-alpha-hydroxylase, IFN-γ: interferon gamma (type II interferon), IL1-β: interleukin-beta 1, IL-2: interleukin-2, IL-6: interleukin-6, IL-8: interleukin-8, iNOS: inducible NO-synthase, NF-kB: nuclear factor-kappa B, NO: nitric oxide, NO/ONOO−: peroxyxinitrite, ROS: reactive oxygen species, TNF-

α: tumor necrosis factor-alpha.

The pleiotropic actions of vitamin D in metabolism

1,25(OH)2D3, designated as ''activated'' vitamin D3 [23,45,46]. A well known activation process from 25OHD3 to 1,25(OH)2D3 is mediated in the tubular cells of the kidney in a calcium depend-

gent manner by parathormone (PTH), and serves to control extra-cellular calcium levels [23,43–45,47]. A calcium independ-

ent, vitamin D3 stores dependent activation occurs in more than 10 tissues in order to ensure specific cellular needs, for instance control of cell growth, proliferation, differentiation, or apoptosis [37,43–45]. 1,25(OH)2D3 binds to a receptor protein, named vita-

min D receptor (VDR). This complex acts as transcription factor and regulates the expression of a host of different genes [46–53]. Besides the genomic actions of 1,25(OH)2D3, non-genomic actions operate on several signaling pathways, described elsewhere [43,47,49–50]. Genomic and non-genomic actions are interrelated by substantial cross-talk [43,50–52]. These pleiotropic actions are supposed to influence general health considerably. However, this paper concentrates on effects of 1,25(OH)2D3 on regulation of NF-kB, immune functions, Ca2+, and redox balance, which are all strongly linked to stress pathways and NO/ONOO− cycle.

1,25(OH)2D3 enhances early and calms down late stress response

Cell stress of any kind, like heat, inflammation, oxidative stress, interferon (IFN), and the active metabolite 1,25(OH)2D3 it-

self, are able to induce heat shock 70 (Hsp-70)-like proteins, called intracellular vitamin D-binding proteins (IDBP). They be-

long to a large family of ATP binding stress proteins acting as ATPases and chaperones [53]. Preferentially 25(OH)D3 has a great affinity to bind to these proteins, but 1,25(OH)2D3 can also bind [53,54]. The complex of Hsp-70 like proteins with 25OHD3 or 1,25(OH)2D3 interacts with toll-like receptor 2 (TLR2) leading to activation of NF-kB as part of an overall defence mechanism against cell stress [53–55]. Thus, vitamin D enhances early stress response [53–58].

However, activation of NF-kB induces as well gene expression of CYP27B1 [34–40,53,56–58], thus promoting augmented syn-

thesis of 1,25(OH)2D3 (Fig. 2) which inhibits gene expression of multiple products of NF-kB activation, like IFN-γ, IL-2, IL-6, iNOS, and TNF-α [40,59–63], and down-regulates NF-kB activation directly by inhibition of several NF-kB-related proteins [39,42,53], and more indirectly via numerous further mecha-

nisms, and in multiple cells [38–43,49,53,47–55,59–63]. The net result is potent inhibition of responses that will otherwise limit chronic inflammation. Thus, opposite to the early stress re-

response, 1,25(OH)2D3 is able to suppress the late pro-inflamma-

tory stress responses.

Regardless of the mentioned inhibitory effect on iNOS expres-

sion, there is coupled gene expression of the iNOS gene and 1,25(OH)2D3 synthesis in activated macrophages (Mø), dendritic cells (DCs) and T-cells [38,42,56–59]. This may lead to very high levels of 1,25(OH)2D3 even spilling over into blood [56,57]. This coupling economizes cellular redox balance in case of cell stress, as moderate amounts of NO, instead of NADPH, may be able to act as electron donator for the synthesis from 25(OH)D3 to 1,25(OH)2D3, thus saving the stores of NADPH [53,57] which con-

tributes to anti-inflammation. On the contrary, high concentrations of NO block the enzymatic activity of CYP27B1, thus lowering the synthesis of 1,25(OH)2D3 in this condition, now contributing to pro-inflammation [57].

1,25(OH)2D3 induces immune regulation and tolerance

1,25(OH)2D3 counteracts inflammation through many indirect immune mechanisms as well. For instance, it represses interleu-

kin-12 (IL-12) in macrophages [39,47,53,56,59,62] and myeloid dendritic cells (mDCs) [39,41,42,47,63] resulting in diminished
EARLY STRESS RESPONSE IS A POSITIVE INFLAMMATORY AMPLIFICATION LOOP

heat, stress, 1,25(OH)2D3 → hsp-70 like proteins (DBP) ↑ → 1,25(OH)2D3/DBP complex activates TL2
→ signal cascade → ACTIVATION of NF-κB → INFγ, IL-2, TNFα, LPS, Ca++, ROS ↑
→ new round of ACTIVATION of NF-κB → disposition to vicious local NO/ONOO− cycle

IN CASE OF VITAMIN D AND CALCIUM REPLETION,
LATE STRESS RESPONSE IS ABLE TO CREATE NEGATIVE FEED BACK LOOPS AGAINST INFLAMMATION

NF-κB → iNOS ↑ and CYP1a ↑ → NO ↑ and 1,25(OH)2D3 ↑
[NO− serves as electron donor for synthesis of 1,25(OH)2D3 (NADPH economy)]
1,25(OH)2D3 INHIBITS many NF-κB proteins (p105/p50, c-Rel, Rel-B) and products (IFNγ, IL-2,
IL-6, IL-12, iNOS, TNFα) and INHIBITS more indirectly activation of NF-κB, on several levels and in many cells

immune maturation with subsequent inhibition of auto-immune Th1 responses. As well, 1,25(OH)2D3 mitigates immune response in antigen presenting and T-cells by differentially influencing gene expression of ligands, like cytokines and chemokines [38–40], multiple immune receptors [38,62], and co-stimulatory membrane proteins, MHC II receptors, and other immune-regulatory proteins [38–42,59,62] (Table 1). Furthermore, 1,25(OH)2D3 inhibits in T-cells the expression of Fas-ligand (CD95L) [38] which is a co-stimulatory signal for immune maturation and polarization in CD8+ cells, Th1 cells and mDCs [38]. The result is enhanced development of less immune-matured and hence more immune-naïve regulatory CD24+CD25−T-lymphocytes (Treg) [39–42,62,63].

Table 1

1,25(OH)2D3 INDUCES IMMUNE REGULATION AND IMMUNE TOLERANCE [38–40,42,59,62].

| NF-κB | (m-DCs, CD4+T-cells) → iNOS | NO ↑ → inflammation ↓
| NF-κB | (APC, m-DCs) → IL-12 | maturation of m-DCs and T-cells ↓ → Treg ↑ → immune response ↑
| NF-κB | (APC, m-DCs) → IL-12 | Th1-cells ↓ (number and function), co-stimulatory molecules CD40, CD80, CD86 ↓ (m-DCs) → enhancement of Treg
| Adhesion molecules (CD54) | (m-DCs) → enhancement of Treg
| MHC-II-antigen receptors | (m-DCs) → enhancement of Treg
| Inhibitory membrane protein ILT3 | (APC) → enhancement of Treg
| CCR 4, CCR5, CCR 8, CCR10 | → enhancement of Treg
| CCL22, CCL17 | (APC) → enhanced recruitment of Treg
| IL-10 | (m-DCs) → (an anti-inflammatory cytokine) → inflammation ↓
| NK- and CTL-activity in cognate immune system ↓
| GM-CFS | (m-DCs) → monocytes ↓
| IFNγ | TNFα, IL-1, IL-2, IL-6, IL-8, IL-12, IL-17, IL-23 | in Ma

Abbreviations: APC, antigen presenting cells, CCL22, anti-inflammatory chemokine; CCL17, pro-inflammatory chemokine; CCR 4, CCR5, CCR 8, CCR10, chemokine receptors; COX-2, cyclooxygenase-2; CTL, cytotoxic T-cells; m-DCs, myeloid dendritic cells; IFN-γ, interferon gamma; IL-1, interleukin-1; IL-10, interleukin-10; IL-6, interleukin-6; IL-8, IL-10, interleukin-10; IL-12, interleukin-12; IL-17, interleukin-17; IL-23, interleukin 23; iNOS, inducible NO-synthase; Ma, macrophage; NF-κB, nuclear-factor-kappa B; NK, natural killer cells”; NO, nitric oxide; Th1-cells, T-helper cells type 1; TNFα, tumor necrosis factor-alpha.

1,25(OH)2D3 supports innate defence against microbes

1,25(OH)2D3 helps innate immune system in first line defence against microbial invasion [38,44,45,58,60,64] (Table 2). 1,25(OH)2D3 enhances monocyte maturation into macrophages [38,53,56,57], as well as macrophage antigen uptake by antigen presenting cells, antigen cell trafficking [38,53,56,57], expression of interleukin-1β (IL-1 β) [53,56,57], TNF-α [38,53,56,57], and NADPH-oxidase [38,39,53,56,64], thus augmenting pathogen killing ability and hydrogen peroxide (H2O2) production. Furthermore, 1,25(OH)2D3 enhances the synthesis of cathelicidin, an agent with inherent anti-microbial potency against viral, bacterial and fungal invasion, secreted locally in antigen presenting cells and keratinocytes [40,54,55]. As all these mechanisms serve as appropriate tools to shut down inflammation as early as possible, they contribute to NF-κB counterbalance.

1,25(OH)2D3 is essential for redox balance

1,25(OH)2D3 can act as a pro-oxidant in appropriate cases [22], but also as a potent anti-oxidant [22,47] (Table 3). Like cholesterol, 1,25(OH)2D3 is able to reduce the fluidity of the cell membrane and to prevent iron-dependent lipid peroxidation [65,66]. It increases
the activity of gamma-glutamyl transpeptidase (γGT) [22,66], supports glutathione synthesis [65–67], induces glutathione peroxidase (GPX) [65], and manganese dependent superoxide dismutase (Mn-SOD) [65]. Furthermore, 1,25(OH)2D3 induces the production of thiol-containing and metal-binding metallothioneins [68,69], thus preventing metal derived oxidative stress. By enhancing activity of glucose-6-phosphate-dehydrogenase (G6PD) [47,65] 1,25(OH)2D3 is able to act also against oxidative stress. All these redox balancing abilities serve as another contribution to down-regulation of NF-κB activation.

However, 1,25(OH)2D3 is able as well to lower glutathione reductase with a potentially pro-oxidant effect [47,65]. But being relatively modest and being found with all of the anti-oxidant responses, it is not likely to be a major influence.

Discussion

Calcium and vitamin D deficiency/insufficiency are nowadays “everyday” conditions in western world due to either living beyond the 40th degree of latitude, and/or frequent indoor working, and/or use of high protecting sun cream, for instance. Bone and connective tissue alteration, culminating in rickets [37], muscle weakness, wide spread pains [35–37], immune dysfunctions [35,54,55], and otherwise unexplainable chronic fatigue combined with functional disorders are meanwhile described as vitamin D dependent syndromes [35–37,75–77].

As long as 250HD3 is abundant, local production of 1,25(OH)2D3 will occur according to special cellular needs [22,37,45]. However, in case of low stores of 25(OH)D3, local production in tissues becomes diminished which deranges tissue homeostasis [22,37,45], generating high ROS and NO production especially in highly metabolically active body regions [21,22,27,43,46,47,69].

Whole body calcium deficiency is supposed to result from long-standing vitamin D deficiency, as levels of 25(OH)D3 lower than 40 ng/ml (100 nMol/L) are said to compromise adequate calcium absorption [43,71,73,74]. Due to calcium deficiency, compensatory mechanisms like PTH secretion, for instance, and other bone resolving mechanisms might contribute to pro-inflammation, even when vitamin D stores become replenished [70–78], and might explain why calcium optimizes vitamin D supplementation substantially [43,69,71,74].

Additionally, it should be of interest that a rise of PTH in the wake of calcium deficiency will provoke enhanced CYP27B1 activity, thus leading to increased turnover of 25(OH)D3 which means more rapid reduction of actual vitamin D stores [43,70–78].

In summary, raising 1,25(OH)2D3 levels subsequent to an initial inflammatory or unspecific stress response can act to lower pro-inflammatory immune responses by twelve mechanisms:

1. Down-regulating NF-kappa B activity
2. Lowering iNOS induction
3. Lowering IL-6
4. Lowering IL-2
5. Lowering IFN gamma
6. Lowering IL-12 synthesis
7. Increased G6PD
8. Increased metallothioneine synthesis
9. Increased Mn-SOD
10. Increased reduced glutathione synthesis
11. Increased gamma-glutamyl transpeptidase
12. Lowering iron-dependent lipid peroxidation

The depletion of vitamin D pools in the tissues that can be used to elevate local 1,25(OH)2D3 in response to local inflammation may be a very important limiting factor in preventing or reversing chronic inflammation. Therefore vitamin D supplementation is likely to be important both as a preventive agent or treatment of many chronic inflammatory diseases, whether these are caused by the NO/ONOO-cycle, auto-immune response or by other mechanisms.

Improvement of inflammatory disease course has been already described [77], yet no studies in case of full-blown CFS and related diseases have been done until now. With respect to recent papers connecting chronic fatigue syndrome with a possible opportunistic pathogen [79], and chronic NF-κB activation with reactivation of latent and opportunistic pathogens [80–82], future controlled, randomized, and double blind studies seem to be reasonable. Clinical effectiveness could be compared with respect to different calcium and vitamin D compounds and dosages.

Conflict of interest

The authors declared that no conflict of interest is existing.

References


