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Review

The role of vitamin D in human fracture healing: a systematic review of the literature

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ABSTRACT

Introduction: Vitamin D is essential for bone mineralization and for the subsequent maintenance of bone quality. Mineralization is part of hard callus formation and bone remodelling, processes, which are part of fracture healing. We provide a comprehensive review of the literature to summarize and clarify if possible, the cellular effects of vitamin D and its clinical involvement in the process of fracture healing in human.
Material and methods: We conducted a literature search in PubMed, Embase (OVID version), and Web of Science.
Results: A total of 75 *in vitro* and 30 *in vivo* studies were found with inconsistent results about the cellular effect of vitamin D on fracture involved inflammatory cells, cytokines, growth factors, osteoblasts, osteoclasts and on the process of mineralization. With only five *in vitro* studies performed on material derived from a fracture site and one *in vivo* study in fracture patients, the exact cellular role remains unclear.
Seven studies investigated the circulating vitamin D metabolites in fracture healing. Although it appears that 25(OH)D and $24,25(OH)_2D_3$ are not affected by the occurrence of a fracture, this might not be the case with serum concentrations of $1,25(OH)_2D_3$. The potential clinical effect of vitamin D deficiency is only described in one case series and three case controlled studies, where the results tend to show no effect of a vitamin D deficiency.
No clinical studies were found investigating solely vitamin D supplementation. Two clinical studies found a positive effect of vitamin D supplementation and calcium, of increased bone mineral density or respectively increased fracture callus area at the fracture site. One study found indirect evidence that vitamin D and calcium promoted fracture healing.
Conclusion: Despite these results, and the presumed beneficial effect of vitamin D supplementation in deficient patients, clinical studies that address the effects of vitamin D deficiency or supplementation on fracture healing are scarce and remain inconclusive. We conclude that vitamin D has a role in fracture healing, but the available data are too inconsistent to elucidate how and in what manner.

Contents

Abbreviations: 25(OH)D, Calcidiol; $1,25(OH)_2D_3$, Calcitriol; IL, Interleukin; TNF-α, Tumor Necrosis Factor-alpha; TGF-β, Transforming Growth Factor beta; IGF, Insulin-Like Growth Factor; BMP's, Bone Morphogenetic Proteins; VEGF, Vascular Endothelial Growth Factor; FGF, Fibroblast Growth Factor; PDGF, Platelet-Derived Growth Factor; TNAP, Tissue-Nonspecific Alkaline Phosphatase.

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Introduction

Fracture healing is a complex biological process that starts directly after a fracture has occurred. Fracture healing processes can be divided into four overlapping stages: the inflammation stage, the soft callus formation stage, the hard callus formation stage and the bone remodelling stage [1]. In case of an anatomical reduction and rigidly stable conditions no soft callus formation occurs [2]. Each stage of the fracture healing process is characterized by specific cellular and molecular processes [1,3–5]. Normal fracture healing requires the coordinated action of signaling molecules, growth factors, osteoprogenitor cells and an extracellular matrix scaffold, as well as preservation of mechanical stability of the fracture [1,3–6]. Many of these basic requirements for bone growth are in turn influenced by a number of physiological, cellular, and molecular/genetic factors [1].

Vitamin D is essential for bone mineralization and for the subsequent maintenance of bone quality through its vital role in the regulation of calcium and skeletal homeostasis. Bone mineralization is part of hard callus formation and bone remodeling. Vitamin D is thus likely to play a key role in fracture healing in at least these stages. The classical endocrine function of vitamin D is the maintenance of calcium homeostasis through binding of $1,25(OH)_2D_3$ to vitamin D receptors in the intestine, kidney, parathyroid glands and bone (Fig. 1). The non-classical function of vitamin D is in most human tissue mediated through the intracellular expression of the Cyp27b1 gene (encoding for the 1α-hydroxylase enzyme), Cyp24a1 gene (encoding for the 24-hydroxylase enzyme) and vitamin D receptors [7].

Whereas the classical calcium regulating actions of vitamin D are well documented, much less is known about the role of vitamin D in fracture healing in humans. The aim of this systematic review of the literature on the role of vitamin D in fracture healing is to evaluate and summarize what is known about the cellular effects of vitamin D during fracture healing and of its clinical involvement in the process of fracture healing in human.

Methods

We conducted a literature search in PubMed, Embase (OVID version), and Web of Science. A systematic review was performed according to the PRISMA checklist. The search consisted of two subject queries taking into account the terminological and technical differences between databases (Table 1).

The first query, *the cellular effects of vitamin D in fracture healing*, concerned vitamin D and specific cytokines [interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α)], growth factors [transforming growth factor beta (TGF-β), insulin-like growth factor I and II (IGFI/II), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF)], osteoblasts and osteoclasts [1,3–5]. The second query, *the clinical involvement of vitamin D in fracture healing*, included vitamin D, vitamin D deficiency and fracture healing. Various synonyms and related terms were used for all topics (Table 1). The searches were conducted at the end of September 2013.

For both queries the results were limited to English language articles and conference abstracts were excluded. For the first query, both *in vitro* and *in vivo* human clinical studies were included. Articles were only included when describing an effect of vitamin D on osteoblasts or osteoclasts in the context of fracture healing or when they described an

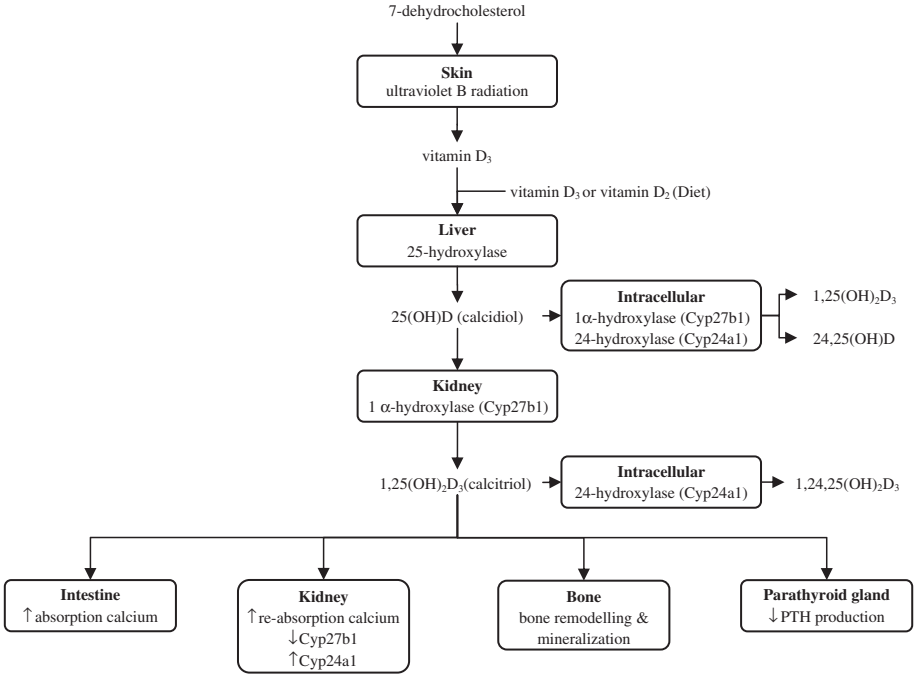

Fig. 1. Flow chart of vitamin D metabolism and its classical function in the maintenance of calcium homeostasis and skeletal homeostasis.

Table 1
Search strategies.

Query	Database	Search strategy
1	PubMed	(vitamin d[ti] OR vitamin d1[ti] OR vitamin d2[ti] OR vitamin d3[ti] OR "vitamin d"[ti] OR Cholecalciferol[ti] OR Hydroxycholecalciferols[ti] OR Ergocalciferol[ti] OR "25-Hydroxyvitamin D 2"[ti] OR Dihydrotachysterol[ti] OR Calcifediol[ti] OR Dihydroxycholecalciferols[ti] OR "24,25-Dihydroxyvitamin D 3"[ti] OR Calcitriol[ti] OR vitamin d[ti] OR "Vitamin D"[Majr]) **AND** ("Interleukin-1"[mesh] OR "Interleukin-6"[mesh] OR "Tumor Necrosis Factor-alpha"[mesh] OR "Transforming Growth Factor beta"[mesh] OR "Insulin-Like Growth Factor II"[mesh] OR "Insulin-Like Growth Factor I"[mesh] OR "Bone Morphogenetic Proteins"[mesh] OR "Vascular Endothelial Growth Factor A"[mesh] OR "Fibroblast Growth Factor "[mesh] OR "Platelet-Derived Growth Factor"[mesh] OR "Interleukin-1"[ti] OR "Interleukin-6"[ti] OR "Tumor Necrosis Factor-alpha"[ti] OR tnf alpha[ti] OR "Transforming Growth Factor beta"[ti] OR tgf beta[ti] OR "Insulin-Like Growth Factor II"[ti] OR "Insulin-Like Growth Factor I"[ti] OR "Bone Morphogenetic Proteins"[ti] OR "Vascular Endothelial Growth Factor A"[ti] OR "Fibroblast Growth Factor"[ti] OR "Platelet-Derived Growth Factor"[ti] OR "Osteoblasts"[Mesh] OR osteoblasts[ti] OR osteoblast[ti] OR osteoblastic[ti] OR "Osteoclasts"[Mesh] OR osteoclasts[ti] OR Osteoclast[ti] OR osteoclastic[ti]) **AND** english[la] NOT "Case Reports"[Publication Type], filter: Human
	Embase (OVID-version)	(exp *Vitamin D/OR vitamin d.ti OR vitamin d1.ti OR vitamin d2.ti OR vitamin d3.ti OR "vitamin d".ti OR Cholecalciferol.ti OR Hydroxycholecalciferols.ti OR Ergocalciferols.ti OR "25-Hydroxyvitamin D 2".ti OR Dihydrotachysterol.ti OR Calcifediol.ti OR Dihydroxycholecalciferols.ti OR "24,25-Dihydroxyvitamin D 3".ti OR Calcitriol.ti OR vitamin d.ti) **AND** (*Interleukin 1/OR "Interleukin-1".ti OR *Interleukin 6/OR "Interleukin-6".ti OR *tumor necrosis factor alpha/OR "Tumor Necrosis Factor-alpha".ti OR tnf alpha.ti OR *Transforming Growth Factor beta/OR "Transforming Growth Factor beta".ti OR tgf beta.ti OR *somatomedin B/OR "Insulin-Like Growth Factor II".ti OR "Insulin-Like Growth Factor 2".ti OR "Insulin-Like Growth Factor I".ti OR "Insulin-Like Growth Factor 1".ti OR *somatomedin C/OR *Bone Morphogenetic Protein/OR "Bone Morphogenetic Proteins".ti OR Bone Morphogenetic Protein.ti OR *vasculotropin/OR "Vascular Endothelial Growth Factor A".ti OR *fibroblast growth factor/OR "Fibroblast Growth Factor".ti OR "Fibroblast Growth Factor II".ti OR *Platelet Derived Growth Factor/OR "Platelet-Derived Growth Factor".ti OR Osteoblast/OR osteoblasts.ti OR osteoblast.ti OR osteoblastic.ti OR Osteoclast/OR osteoclasts.ti OR Osteoclast.ti OR osteoclastic.ti) **AND** english.la NOT (Journal: Conference Abstract.pt OR case report/), filter Human
	Web of Science	((TI = ("vitamin d" OR "Cholecalciferol" OR "Hydroxycholecalciferols" OR "Ergocalciferols" OR "25-Hydroxyvitamin D 2" OR "Dihydrotachysterol" OR "Calcifediol" OR "Dihydroxycholecalciferols" OR "24,25-Dihydroxyvitamin D 3" OR "Calcitriol" OR "vitamin d") **AND** TS = ("Interleukin-1" OR "Interleukin-6" OR "Tumor Necrosis Factor-alpha" OR "Transforming Growth Factor beta" OR "Insulin-Like Growth Factor II" OR "Insulin-Like Growth Factor I" OR "Bone Morphogenetic Proteins" OR "Vascular Endothelial Growth Factor A" OR "Fibroblast Growth Factor 2" OR "Platelet-Derived Growth Factor" OR "osteoblast" OR "osteoclast" OR "osteoblasts" OR "osteoclasts" OR "osteoblastic" OR "osteoclastic")) OR (TS = ("vitamin d" OR "Cholecalciferol" OR "Hydroxycholecalciferols" OR "Ergocalciferols" OR "25-Hydroxyvitamin D 2" OR "Dihydrotachysterol" OR "Calcifediol" OR "Dihydroxycholecalciferols" OR "24,25-Dihydroxyvitamin D 3" OR "Calcitriol" OR "vitamin d") AND TI = ("Interleukin-1" OR "Interleukin-6" OR "Tumor Necrosis Factor-alpha" OR "Transforming Growth Factor beta" OR "Insulin-Like Growth Factor II" OR "Insulin-Like Growth Factor I" OR "Bone Morphogenetic Proteins" OR "Vascular Endothelial Growth Factor A" OR "Fibroblast Growth Factor " OR "Platelet-Derived Growth Factor" OR "osteoblast" OR "osteoclast" OR "osteoblasts" OR "osteoclasts" OR "osteoblastic" OR "osteoclastic"))) **AND** Human AND LA = (English) NOT TI = ("case report*"); Refined by: [excluding] Document Types = (MEETING ABSTRACT)
2	PubMed	(vitamin d OR vitamin d1 OR vitamin d2 OR vitamin d3 OR "Vitamin D/administration and dosage"[Mesh] OR "vitamin d" OR "Vitamin D"[mesh] OR Cholecalciferol OR Hydroxycholecalciferols OR Ergocalciferols OR "25-Hydroxyvitamin D 2" OR Dihydrotachysterol OR Calcifediol OR Dihydroxycholecalciferols OR "24,25-Dihydroxyvitamin D 3" OR Calcitriol OR "Vitamin D Deficiency"[Mesh] OR vitamin D insufficiency OR vitamin D deficiency OR "vitamin d deficient" OR "vitamin d insufficient" OR Osteomalacia OR Rickets) **AND** ("Fracture Healing"[Mesh] OR "fracture healing" OR ((fracture[ti] OR fractures[ti]) AND healing[ti]) OR ("Fractures, Bone"[Mesh] AND "Wound Healing"[mesh]) OR fracture healing OR fractures healing OR "bone healing" OR ((fracture OR fractures OR fractured) AND (healing OR heal OR healed))) **AND** English[la]
	Embase (OVID-version)	(vitamin D deficiency/OR vitamin D deficiency.mp OR vitamin D insufficiency.mp OR "vitamin d deficient".mp OR "vitamin d insufficient".mp OR Osteomalacia/OR exp Rickets/OR Osteomalacia.mp OR Rickets.mp) OR ((vitamin d OR vitamin d1 OR vitamin d2 OR vitamin d3).af OR exp vitamin D/OR ("vitamin d" OR Cholecalciferol OR Hydroxycholecalciferols OR Ergocalciferols OR "25-Hydroxyvitamin D 2" OR Dihydrotachysterol OR Calcifediol OR Dihydroxycholecalciferols OR "24,25-Dihydroxyvitamin D 3" OR Calcitriol OR vitamin d).mp) **AND** (exp fracture healing/OR "fracture healing".mp OR ((fracture.ti OR fractures.ti) AND healing.ti) OR (exp Fracture/AND exp Healing/) OR (fracture* AND healing).mp OR "bone healing".mp OR ((fracture OR fractures OR fractured) AND (healing OR heal OR healed)).mp) **AND** English.la NOT (Journal: Conference Abstract.pt)
	Web of Science	TS = (("vitamin d" OR "vitamin d1" OR "vitamin d2" OR "vitamin d3"OR Cholecalciferol OR Hydroxycholecalciferols OR Ergocalciferols OR "25-Hydroxyvitamin D 2" OR Dihydrotachysterol OR Calcifediol OR Dihydroxycholecalciferols OR "24,25-Dihydroxyvitamin D 3" OR Calcitriol OR vitamin d OR Vitamin D Deficiency OR vitamin D insufficiency OR vitamin d deficient OR vitamin d insufficient OR Osteomalacia OR Rickets)) **AND** (TS = fracture healing OR TS = ((Fracture* OR bone*) AND Heali*) OR TS = bone healing) **AND** LA = (English); Refined by: [excluding] Document Types = (MEETING ABSTRACT)

effect of vitamin D on the expression of interleukins or growth factors by bone cells or by cells involved in fracture healing, or an effect of vitamin D on circulating concentrations of these interleukins or growth factors. No case reports were included. We first screened titles and abstracts to ascertain whether the articles contained information pertaining to the subject of the review and whether they met the eligibility criteria. The full article was then read before including it in the analysis.

Results

The query on *cellular effects of vitamin D in fracture healing* resulted in 2243 articles, including 816 duplicates and 160 in vivo or in vitro animal studies. A total of 153 articles were selected and read, after screening of titles and abstracts. A total of 105 papers, 75 with in vitro data and 30 with in vivo data, were found to fulfill the criteria of the search and were included in the analysis (Fig. 2). The query on *the clinical involvement of vitamin D in fracture healing* resulted in 927 articles. After excluding duplicate publications, animal studies, case report and in vitro studies, 431 titles and abstracts were screened. Only 14 articles were found to contain relevant information, and were subdivided based on the information provided on vitamin D metabolites during fracture healing, the effect of vitamin D deficiency on fracture healing and the effect of vitamin D supplementation on fracture healing (Fig. 2).

The cellular effect of vitamin D in fracture healing

The fracture healing process can be divided into four overlapping stages: the inflammation stage, the soft callus formation stage, the hard callus formation stage and the bone remodeling stage (Fig. 3). Each stage is characterized by specific cellular and molecular processes [1].

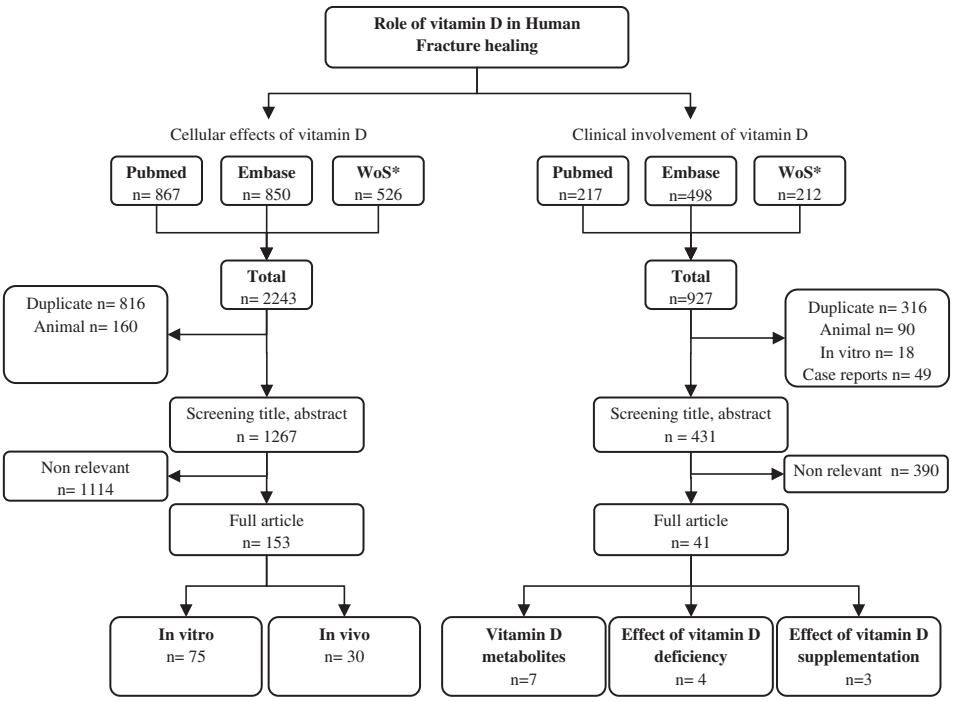

Fig. 2. Number and type of studies resulting from the literature search *WoS: Web of Science.

The inflammatory stage

Local disruption of bone and soft tissue leads to a fracture hematoma and to an associated inflammatory response. This results in the formation of a well vascularized precursor granulation tissue as a result of the infiltration of the hematoma by degranulated platelets, macrophages, granulocytes, lymphocytes, and monocytes, in the presence of a wide range of cytokines and growth factors.

The assumed effects of vitamin D on the *in vitro* expression of IL-1, IL-6 or TNF-α and their serum levels vary between studies. Vitamin D has been reported to reduce [8–22], increase [13,23–30] or to have no influence[12,13,22,26,31–34] on the expression of IL-1, IL-6 or TNF-α. Similar variability in results has been reported on the effect of vitamin D on serum levels of IL-1 [17,35–39], IL-6 [36–48] and TNF-α [17,35–37,39,42–44,49–53]. Patients with a hip fracture and a vitamin D deficiency at the time of fracture have been shown to have a higher serum level of IL-6 in the year after the fracture [54].

In vitro studies show that vitamin D increased the production and release of TGF-β [55], and up-regulated the synthesis of TGF-β receptor I and II [31,55–57] in osteoblasts. In contrast, Torricelli et al. [32] showed a decreased expression of TGF-β by osteoblasts after $1,25(OH)_2D_3$ stimulation and Sugimoto et al. [58] found no effect of vitamin D on the TGF-β production by osteoblasts. *In vivo*, Mahon et al. [59] showed an increased serum level of TGF-β1 in patients with multiple sclerosis after vitamin D supplementation, but Isik et al. [60] showed significantly higher serum levels of TGF-β1 in patients with severe vitamin D deficiency. In relation to the bone morphogenetic proteins (BMPs), vitamin D has been shown to down-regulate BMP-2 and BMP-4 mRNA expression [61], and to up-regulate BMP-3 mRNA expression in osteoblasts [55,62].

In osteoblastic cell cultures vitamin D has shown to enhance the expression of VEGF [31,55,63–67], although the exact mechanism by which this occurs is unclear. In human lumbar annulus tissue $1,25(OH)_2D_3$ decreased and $24,25(OH)_2D_3$ increased the production of VEGF [68].

The secretion of platelet-derived growth factor (PDGF), which stimulates the proliferation and migration of mesenchymal stem cells and osteoblasts, was shown to be enhanced by vitamin D in osteoblastic cell cultures [69,70].

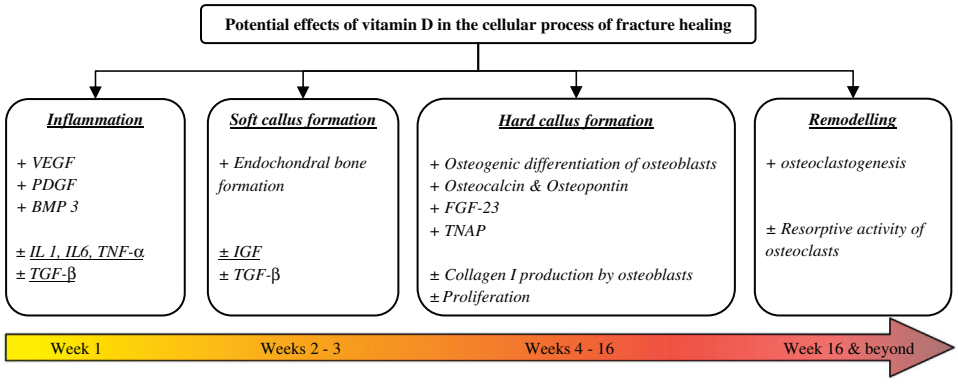

Fig. 3. Cellular effects of vitamin D during the four stages of fracture healing. + positive effect, − negative effect, ± unclear effect (both + and –, or no effects described) *in vitro* or in vivo studies.

The soft callus formation stage

In this second stage of fracture healing chondrocytes produce a cartilaginous matrix until all the granulation tissue is replaced by cartilage. Where cartilage production is deficient, fibroblasts fill the area with fibrous tissue, forming a semi rigid fibrous cartilaginous matrix called soft callus.

The proliferation and differentiation of chondrocytes and fibroblasts are stimulated by growth factors including TGF-β, PDGF and IGF [1]. These growth factors appear to be at least partly dependent on the presence of vitamin D [31,55,57,69], although Saggese et al. [18] showed that 1,25(OH)2D3 inhibited the proliferation of chondrocytes.

Various in vitro studies (human bone marrow or osteoblastic cells) have addressed the effect of vitamin D on IGF-I (promotes bone matrix formation [3]), IGF-II (stimulates type I collagen production, cartilage matrix synthesis, and cellular proliferation [3]) and the IGF-binding proteins. Results from these studies are conflicting regarding the effect of vitamin D on the expression of IGF-I, IGF II or IGF-binding proteins [31,55,71–75]. A positive correlation was found between the serum concentration of IGF-I and vitamin D was found in in vivo studies [76–78], and a vitamin D deficiency was found to be associated with lower circulating IGF-I levels in healthy human subjects [79]. However vitamin D supplementation did not appear to influence the IGF I levels in a clinical trial in 318 overweight or obese subjects [80].

The hard callus formation stage

This third stage of fracture healing is also known as the stage of primary bone formation, where a high level of osteoblast activity results in the formation of a mineralized bone matrix.

Osteogenic differentiation has found to be promoted by $1,25(OH)_2D_3$ during the early stages of osteoblastogenesis [55,81], and osteoblast proliferation was found to be either positively or negatively influenced by vitamin D [55,82–84]. Vitamin D in combination with vitamin K was found to modulate the differentiation of human mesenchymal stem cells derived from fracture sites towards the osteoblastic phenotype. Zhou et al. [85] showed that in bone marrow cells from vitamin D deficient patients vitamin D supplementation resulted in a greater stimulation of osteoblastogenesis than in bone marrow cells from vitamin D sufficient patients. The production of collagen I, the major component of the extracellular matrix, by osteoblastic cell cultures is stimulated by vitamin D [55,82,86], although no effect was found in other *in vitro* studies [55,87,88]. The production of osteocalcin and osteopontin (noncollagenous matrix proteins) by osteoblasts is stimulated by vitamin D [55]. In human the presence of vitamin D resulted in more osteocalcin production in late than in early stage fracture callus [89]. The Cyp27B1 gene and Cyp24 gene enable osteoblasts to metabolize 25(OH)D [90], which in turn exerts autocrine and paracrine actions [74,83,91–93].

Vitamin D is essential for the maintenance of mineralization of the skeleton [94]. Vitamin D promotes tissue-nonspecific alkaline phosphatase (TNAP) expression by chondrocytes and osteoblasts. TNAP hydrolyses pyrophosphate, which inhibits hydroxyapatite formation, into inorganic phosphatase. This allows the formation of hydroxyapatite, which fills the space between collagen fibrils in the skeletal matrices [95]. In cultures of osteoblasts [96–98] vitamin D stimulates mineralization of the extracellular matrix [55,93]. Van Driel et al. [99] showed that $1,25(OH)_2D_3$ directly stimulated mineralization by activation of vitamin D receptors in the osteoblast, and that the mineralization process was enhanced by the catabolic products of 25(OH)D and $1,25(OH)_2D_3$ ($24R,25(OH)_2D_3$ and $1,24R,25(OH)_3D_3$ respectively). The addition of vitamin D to cultures of human osteocytes has been shown to release FGF-23 [100,101], which plays a central role in skeletal mineralization and bone metabolism [100–102]. In vivo, vitamin D supplementation is however associated to either higher [103] or lower [104] circulating FGF-23 levels. Treatment with high dose vitamin D resulted in no effect on the mineralization process or vitamin D was shown to actually inhibit the mineralization [55]. In cultured human osteoblasts derived from ethmoidal bones, vitamin D had no effect on the extent of the area of mineralization [58]. In human bone marrow stromal cells vitamin D negatively influenced extracellular mineralization, by reduced calcium incorporation and calcium content of the matrix [87].

The stage of hard callus remodeling

The fourth stage of fracture healing, the callus remodeling process, starts with the resorption of the hard callus.

Kogawa et al. [105] showed that vitamin D may be an important regulator of osteoclastogenesis, because of the expression of vitamin D receptors [106] and of the Cyp27b1 [93] gene by osteoclasts. $1,25(OH)_2D_3$ stimulated osteoclast formation from human bone marrow cells [107,108]. Vitamin D stimulated osteoclast-mediated bone resorption [109] by increasing the expression of RANKL on the surface of the osteoblast [110]. However, Kudo et al. [111] found no stimulating effect on osteoclast bone resorbing activity. On the other hand vitamin D may also inhibit osteoclastogenesis by down-regulation of RANK expression [112]. Braun et al. [113] found that supplementation with a vitamin D analog, 1 alpha-(OH)D3, decreased bone resorption in iliac crest biopsies from patients on chronic glucocorticoid treatment. Increasing vitamin D concentrations dose-dependently inhibited the resorptive activity of osteoclast cell cultures, with a maximal inhibitory effect observed at 50 nmol calcidiol, which corresponds to the cut-off level below which vitamin D deficiency is defined in humans [114].

The clinical involvement of vitamin D in fracture healing

Vitamin D metabolites during fracture healing

Alkalay et al. [115] measured the serum and intra osseous levels of 25(OH)D, $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ in 28 patients after fracture surgery and in 27 patients after elective joint surgery (Table 2). They found a significantly reduced level of serum $1,25(OH)_2D_3$ in fracture patients compared to serum levels in electively operated patients. In the 7 patients with a trochanteric femur fracture they showed that the local intra osseous $24,25(OH)_2D_3$ was 4.6 times higher and $1,25(OH)_2D_3$ was 5.9 times higher than the serum concentrations and, respectively, 4.4 and 5.6 times higher compared to the local intra osseous levels in elective treated patients [115].

Results from a number of studies on serum levels of vitamin D metabolites after a recent fracture are not concordant (Table 2). Briggs et al. [116] measured the serum concentration of 25(OH)D, $24R,25(OH)_2D_3$ and $1,25(OH)_2D_3$ during 6 weeks in 28 patients with a long bone diaphyseal fracture. Only serum $1,25(OH)_2D_3$ concentrations showed a significant change over time; in the form of a 21% decrease after 6 weeks. Meller et al. [117] measured the serum levels of 25(OH)D and $24,25(OH)_2D_3$ in 13 young patients (mean age 24.5 years) with a fracture and in a healthy control group. They found that the level of $24,25(OH)_2D_3$ had significantly increased after 6 weeks in fracture patients, with no significant difference observed in the level of 25(OH)D. In 41 geriatric patients no significant difference was found however in case of $24,25(OH)_2D_3$ serum levels after a hip fracture [118]. In these 41 patients 25(OH)D levels were significantly lower at base line compared to controls and it remained low at 6 weeks, whereas $1,25(OH)_2D_3$ was significant increased at baseline compared to controls and remained increased despite a significant decrease at 6 weeks [118]. Wölfl et al. [119] found no significant difference in 25(OH)D levels in 15 fracture patients with a normal bone mineral density (BMD) compared to 15 paired fracture patients with a low BMD during an 8 week follow-up period. In 11 patients with a hip fracture there were no significant differences in serum levels of 25(OH)D measured during a 6 month follow-up period [120]. Another study, in 205 patients with a hip fracture, showed a significant decrease of the serum concentration $1,25(OH)_2D_3$ after 10 days followed by a gradually increase one year later [121].

Table 2
Overview of the results on clinical involvement of vitamin D in fracture healing.

Study	Year	Type of study	Study design	Result
Meller [117]	1984	Case controlled	Serum concentrations 25(OH)D and 24,25$(OH)_2D_3$ were measured in; 13 patients with a fracture (at baseline and after 6 weeks), healthy control group (at baseline).	- 25(OH)D no sign. change - 24,25$(OH)_2D_3$ sign. increased after 6 wks.
Meller [118]	1985	Case controlled	Serum levels 25(OH)D, 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were measured in; 41 geriatric patients with a fracture(s) (36/43 were hip fractures) (at baseline and after 6 weeks), healthy control group (at baseline).	- 25(OH)D was sign. lower at the time of fracture, and remained lower (no sign. change). - 24,25$(OH)_2D_3$ no sign. change. - 1,25$(OH)_2D_3$ was sign higher initially and decreased significantly 6 wks later but remained increased compared to the control.
Alkalay [115]	1989	Case controlled	Serum and bone levels of 25(OH)D, 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were measured once in; 28 patients operated on a fracture, 27 control patients undergoing an elective joint procedure.	- Serum 25(OH)D and 24,25$(OH)_2D_3$ no sign. difference - Serum 1,25$(OH)_2D_3$ sign. lower Sub analysis of 7 patients with a pertrochanteric femur fracture; bone levels of 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were higher compared to the serum level of those patients and compared to the bone levels in the elective treated patients.
Yu-Yahiro [121]	2001	Case series	Serum levels 1,25$(OH)_2D_3$ were measured in; 205 patients with a hip fracture during 1 year.	- Sign. decreased after 10 days, followed by a sign. gradually increase after 1 year..
Sakuma [120]	2006	Case series	Serum levels of 25(OH)D were measured in; 11 patients with a hip fracture at admission, operation, 2 weeks, 4 weeks, 3 months and 6 months after surgery.	- No sign. changes.
Briggs [116]	2013	Case series	Serum levels of 25(OH)D, 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were measured in; 28 patients with a long bone diaphyseal fracture two days, 1 week and 6 weeks post-fracture.	- 25$(OH)_2D_3$ sign. decreased (21%) after 6 wks. - 24,25$(OH)_2D_3$ no sign. change. - 1,25$(OH)_2D_3$ no sign. change.
Wölfl [119]	2013	Case controlled	Serum levels of 25(OH)D were measured in; 15 fracture patients with a normal BMD, 15 paired fracture patients with a low BMD over a 8 wk period	- 25(OH)D no sign. differences was found between the two groups and no sign. changes were found within the two groups.
Haining [125]	1986	Case controlled	Compared the serum concentrations of 25(OH)D, 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ from 15 patients with an established non-union of a fracture to 15 healthy controls	- No significant differences.
Tauber [123]	1990	Case controlled	Serum levels of 25(OH)D, 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were measured in 4 patients with a delayed- or non-union. Mean serum metabolites were compared with those from 15 patients with coxarthrosis.	- 4/4 had a 25(OH)D level <75 nmol/L. - 3/4 had a 25(OH)D level <50 nmol/L. - The levels of 25(OH)D and 24R,25$(OH)_2D_3$ were significant lower compared with those of the control group.
Brinker [122]	2007	Case series	37/683 patients with a non-union were evaluated for metabolic and endocrine abnormalities	- 21/37 had a 25(OH)D level <75 nmol/L (57%). - 18/37 had a 25(OH)D level <50 nmol/L (49%).
Boszcyk [124]	2013	Case controlled	Compared 35 patients with a non-union of a operated long bone diaphyseal fracture with 35 patients with a long bone diaphyseal fracture.	- In both groups 30/35 (86%) had a 25(OH)D level <75 nmol/L.
Hoikka [127]	1980	Double-blind comparative study	37 patients with an osteoporotic hip fracture received daily vitamin D and calcium or placebo an calcium during 4 months.	- The treatment did not change the BMD or the muscular force in either group. Placebo treatment resulted in a significant higer level of alkaline phosphatase.
Doetsch [126]	2004	Randomised controlled trial	30 women with an osteoporotic or osteopenic proximal humerus fracture were double-blind randomly assigned to either oral 800 IU vitamin D3 plus 1 gram calcium or placebo.	- Significantly more increased bone mineral density of the proximal humerus in the medication group after 6 weeks.
Kolb [128]	2013	Case series	94 postmenopausal females with a distal radius fracture received daily supplements of 1000 mg calcium and 880 IU 25-[OH]-cholecalciferol during 6 weeks.	- Callus area correlated significantly with postoperative normal range calcium levels on the fractured site.

The effect of vitamin D deficiency on fracture healing

In a case series, Brinker et al. [122] reported 25(OH)D levels of <75 nmol/L in 21 out of 37 (57%) patients with non-union following a fracture, with 18/37 (49%) having a level <50 nmol/L (Table 2). The authors proposed that vitamin D deficiency may account for the elevated alkaline phosphatase, elevated parathyroid hormone, and decreased calcium that they also observed in some of the patients studied; parameters that have been shown to be associated with impaired fracture healing. They also suggested that vitamin D deficiency may be responsible for inadequate calcium availability for the process of fracture healing or increased resorption due to chronically increased parathyroid hormone concentrations. In a case control study of seven patients, four patients who suffered delayed fracture healing had a 25(OH)D level <75 nmol/L, three with a level <50 nmol/L (Table 2) [123]. The levels of 25(OH)D and 24,25$(OH)_2D_3$ of these patients were significantly lower compared with levels measured in 15 otherwise healthy patients with coxarthrosis (Table 2) [123]. Tauber et al. [123] argue that these findings support the observation that a delayed union probably consumes more vitamin D metabolites.

In contrast, a retrospective case-controlled study of 35 patients with non-union of a diaphyseal fracture showed no difference in terms of prevalence of vitamin D deficiency compared to controls with normal healing of a similar fracture (Table 2) [124]. Haining et al. [125] also found no significant difference in serum concentrations of 25(OH)D, 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ in 15 patients with an established non-union of a fracture compared to 15 healthy controls (Table 2). The authors concluded that their findings suggested that patients with non-union of a fracture have normal bone turnover, with no evidence

of disturbed production or utilization of vitamin D metabolites, and that disturbance in vitamin D metabolism is therefore unlikely to significantly contribute to the persistence of non-union of a fracture [125].

The effect of vitamin D supplementation on fracture healing

In a placebo controlled randomized study supplementation of vitamin D in combination with calcium was found to significantly enhance callus formation in women with an osteoporotic or osteopenic proximal humerus fractures compared to placebo users (Table 2) [126]. Both the active and the placebo group showed an increased BMD of the proximal humerus with a peak level in week 6, but the increase in the vitamin D and calcium group was significantly higher than in the placebo group [126]. In a double-blind comparative study Hoikka et al. [127] treated 37 patients with osteoporotic hip fractures with 1-alpha-hydroxycholecalciferol and calcium carbonate or with calcium carbonate alone for a duration of 4 months. Alkaline phosphatase level rose significantly and the level of calcium remained the same in the calcium carbonate group, whereas alkaline phosphatase level remained the same and the level of calcium rose significantly in the vitamin D and calcium carbonate group (Table 2). Based on these results, the authors postulated that vitamin D may have an effect on fracture healing. Similarly, Kolb et al. [128] investigated the effect of vitamin D and calcium supplementation on callus formation after a distal radius fracture in 94 postmenopausal patients (Table 2). They found that patients suffering from significant vitamin D deficiency did not show significantly different fracture callus area parameters at the sixth week of control indicating regular callus formation and fracture healing patterns in this group once calcium and vitamin D are substituted. They concluded that adequate calcium homeostasis is required for appropriate callus formation indicating a need for timely supplementation of calcium and vitamin D.

Discussion

The aim of this systematic review of the literature on the role of vitamin D in fracture healing is to evaluate and summarize what is known about the cellular effects of vitamin D during fracture healing and of its clinical involvement in the process of fracture healing in human.

At a cellular level, vitamin D is involved in every stage of the complex process of fracture healing through its effects on inflammatory cells, cytokines, growth factors, osteoblasts, osteoclasts and through its effect on the process of mineralization (Fig. 3). This finding is based on results from in vivo, and in vitro studies predominantly. With the results not always concordant, which tend to weaken the premise of the cellular involvement of vitamin D in the process. Only 6 studies (5 in vitro and 1 in vivo) were performed on material derived from a fracture site or in fracture patients. This too makes it also more difficult to evaluate the exact cellular role of vitamin D in human fracture healing.

Four case controlled studies and three case series studied the vitamin D metabolites in fracture healing. Although it appears that circulating serum concentrations of 25(OH)D and $24,25(OH)_2D_3$ are not affected by the occurrence of a fracture, this might not be the case with serum concentrations of $1,25(OH)_2D_3$. Whether local intraosseous accumulation results in a decrease in circulating $1,25(OH)_2D_3$ remains unclear. Only one study [115] found a significantly higher level of $1,25(OH)_2D_3$ at the fracture site in a sub-analysis of the data. In contrast, studies in animal models did find evidence for increased levels of $24,25(OH)_2D_3$, $1,25(OH)_2D_3$ [129–131] and their receptors [129,132,133] in callus tissue.

A possible influence of vitamin D deficiency on fracture healing in humans remains controversial as only based on retrospective indirect evidence and causality remains elusive. One case series [122] did document that about 47% of patients with non-union had a 25(OH)D deficiency, a prevalence which is in keeping however with that found in the general population [134,135]. One case controlled study, which included only four patients, showed a significantly lower serum level of 25(OH)D and $24,25(OH)_2D_3$. Two case controlled studies showed no difference in the prevalence of vitamin D deficiency between patients with delayed- or non-union of a fracture and controls. There has recently been increased awareness of the potential negative impact of vitamin D deficiency on the occurrence of a fracture. In the elderly, it is indeed recommended that a sufficient serum concentration of 25(OH)D is maintained in order to decrease the risk of falls and fracture [134,136–138]. Although results of studies on the vitamin D deficiency effect on fracture healing remain inconclusive in humans, in rats vitamin D deficiency has been clearly shown to be associated with impaired fracture healing compared to normal fracture healing in a vitamin D sufficient control group [139]; less resistance to torsional stress [140,141] delayed union, increased bone fragility, smaller amount of callus and undermineralized bone [142], although Melhus et al. [143] did not find this negative impact.

Only two of the three human studies quantified the impact of supplementation of vitamin D on the process of fracture healing. Both found a positive effect in the form of increased bone mineral density or increased fracture callus area at the fracture site. Notwithstanding data on the effect of vitamin D supplementation on fracture healing in humans are scarce, with mostly data available based on combination treatment with calcium in patients with osteoporosis. In contrast, data from animal studies do suggest that vitamin D supplementation improved callus structure [144–149], improved biomechanical properties of the callus [140,146,150–154], increased the energy required for inducing a refracture [147,155,156], and improved consolidation rate and mineralization [139,149,157–159]. However, these data could not be confirmed in other animal studies showing no histological [154] or biomechanical effect [141,159], or showing even negative effects of vitamin D supplementation on callus remodeling [160], breaking strength [161], and impaired fracture healing [158].

In conclusion, published results of studies in humans or studies using human tissue provide evidence that vitamin D affects the cellular process of fracture healing, although the exact cellular role of vitamin D in human fracture healing remains unclear. Despite these intriguing results and the presumed beneficial effect of vitamin D supplementation in deficient patients, clinical studies that address the clinical effects of vitamin D deficiency or supplementation on fracture healing are scarce and remain inconclusive. We conclude that vitamin D has a role in fracture healing, but the available data are too inconsistent to elucidate how and in what manner. Future research should focus on the clinical effects of vitamin D deficiency and vitamin D supplementation on fracture healing.

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