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Review

Bone muscle interactions and vitamin D

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ABSTRACT

Beyond the established roles of vitamin D in bone and mineral homeostasis, we are becoming increasingly aware of its diverse effects in skeletal muscle. Subjects with severe vitamin D deficiency or mutations of the vitamin D receptor develop generalized atrophy of muscle and bone, suggesting coordinated effects of vitamin D in musculoskeletal physiology. At a mechanistic level, vitamin D exerts wide-ranging effects in muscle and bone calcium handling, differentiation and development. Vitamin D also modulates muscle and bone-derived hormones, facilitating cross-talk between these tissues. In this review, we discuss emerging evidence that vitamin D regulates bone and muscle in a direct, integrated fashion, positioning the vitamin D pathway as a potential therapeutic target for musculoskeletal diseases.

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Contents

Introduction

Bone and muscle serve obvious and critical functions in our structure, strength and motion. In addition, bone serves as the major reservoir for calcium which is vital for the regulation of blood calcium levels. This tight regulation of serum calcium is an absolute requirement for normal neurological function. Muscles are not usually thought of as a reservoir, but they contain up to 80% of stored carbohydrates in healthy individuals and may contain a surprising amount of lipid in trained athletes, and in the obese. Emerging evidence also suggests that skeletal muscle may be a storage site of 25(OH)vitamin D (25D), a process that relies on innate vitamin D signaling components and transport carriers within muscle cells [1,2].

Diseases of the musculoskeletal system are becoming more common with increasing longevity and adiposity of the population in many

Abbreviations: 1,25D, 1,25dihydroxyvitamin D; 25D, 25hydroxyvitamin D; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IL6, interleukin 6; TGF, transforming growth factors; VDR, vitamin D receptor; VDRKO, VDR knockout mouse.

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countries around the world. Loss of muscle mass (sarcopenia) and decreased bone density (osteopenia or osteoporosis) with subsequent fractures cause much of the frailty and disability of aging, with enormous societal and economic costs. This has been estimated at $850 billion dollars a year in the USA [3].

During fetal life, post-natally and in adulthood, muscle mass and bone mass are linked. This link is mediated in part via direct mechanical interactions, and also via tissue cross-talk between muscle and bone. Bones weaken with decreased muscle use in people and in animals. Examples occur in humans with congenital myotonic dystrophy and with other hereditary muscle disorders. These groups of people are born with thin long-bones, relating to severely impaired fetal muscle movements [4,5]. Bone mass and strength are rapidly lost in people with denervating neurological injuries, e.g. spinal cord lesions, and in astronauts who spend prolonged periods of time in a gravity-free environment.

In the opposite direction, bones may also regulate muscle function via a range of osteokines, such as fibroblast growth factor (FGF)-21 which is produced by osteocytes, and other factors [6]. Osteocytes are osteoblasts which have become completely surrounded by bone-matrix. Additionally, common pathways such as growth hormone/insulin-like growth factor (IGF)-1, sex steroids and Wnt-signaling may centrally coordinate the bone–muscle unit during development and adaptation to mechanical stimuli [7].

Vitamin D represents another pathway by which muscle and bone may interact. Individuals with vitamin D deficiency and rickets display concurrent defects in muscle and bone. Mutations in vitamin D signaling are also associated with generalized underdevelopment of the musculoskeletal system. These observations suggest an integrated role of vitamin D in muscle/bone health. Potential mechanisms may relate to effects of vitamin D in the expression of myokines and osteokines (i.e. muscle and bone-derived hormones) or regulation of mechanostimulatory processes.

This review presents evidence for a holistic role of vitamin D in the musculoskeletal system beyond its established roles in skeletal health alone. While evidence for such an integrated role is slowly emerging, there is hope that the vitamin D pathway may present therapeutic targets for the treatment of musculoskeletal disease [8].

Development and regulation of bone and muscle

Bone and skeletal muscle both develop during early fetal life from the somatic mesoderm in close physical association with each other. This has been the subject of excellent reviews, including [6,9].

Bone forms sequentially, initially by the condensation of mesenchymal precursors at future skeletal sites and then by differentiation into chondrocytes to form a cartilage anlage (i.e. endochondral bone) or directly into osteoblasts (i.e. intramembranous bone). This early process relies on positional and mechanical cues [6]. Once formed, bone undergoes continual remodelling throughout life in response to mechanical loads and in order to repair microdamage and adapt to changing mechanical requirements. This relies on the coordinated action of bone cells: bone resorption by osteoclasts, then subsequent bone matrix formation and mineralization by osteoblasts. Osteocytes are sensitive to mechanical forces and may initiate adaptation through regulation of both bone resorption and formation.

During embryogenesis, myogenic differentiation occurs adjacent and concurrent to skeletal development. Myogenesis is specified by the master regulator Pax3 in fetal and neonatal life and by Pax7 in later life. In the trunk and limbs, mesodermal precursor cells under the control of Pax3 become committed to myogenic lineage and differentiate and fuse to form multinucleated syncytia under the control of external signals such as MyoD and Myf5 [10]. These syncytia grow with fusion of additional myoblasts and ultimately, nascent myotubes develop into mature, multinucleated muscle fibers. Not all myoblasts fuse. A subset of myogenic precursors instead form a reservoir of Myf5/MyoD-expressing cells that remain on the periphery of myofibers as a source of new muscle cells during postnatal growth and regeneration [11]. These are known as satellite cells [12–15].

The linked developmental origins of muscle and bone suggest the possibility that common signaling pathways or networks may regulate their mass. To support this, mice with genetic defects in muscle development (MyoD knockout) display profound impairments in bone development and mineralization [16]. Signals responsible for coordinated muscle/bone development include FGFs, transforming growth factors (TGFs), IGF-1 and other morphogens [17].

Muscle and bone mass are both increased by anabolic exercise and are both decreased by disuse. The changes in mass with exercise or with disuse are mediated by some of the same factors. As well as the direct mechanical interactions between muscle and bone, there is further evidence that muscles may indirectly regulate bone repair following fracture. Covering fractures with muscle flaps, even with immobilization is well recognized to improve healing of traumatic factures [18,19]. Conversely, injury to overlying muscle impairs fracture healing [20].

The vitamin D receptor (VDR) makes an early appearance during musculoskeletal development. In the fetal rat, it is found at day 13 of gestation [21] within the condensing mesenchyme of the vertebral column. By day 17, the VDR resides within osteoblasts and proliferating chondrocytes. Despite the early embryologic presence of the VDR, defects in vitamin D signaling do not lead to clinical features of rickets until after birth [22]. The skeletal phenotype is most manifest after weaning, coinciding with development of hypocalcemia and hyperparathyroidism in VDR knockout (VDRKO) mice. Abnormal growth plate development, impaired bone mineralization and muscle fiber atrophy are seen at day 35 [22,23]. However, subtle changes *are* noted in pre-weaned VDRKO mice. These include growth plate enlargement [22] and smaller muscle fibers at day 15 [23]. These effects occur prior to the onset of abnormal calcium and phosphate levels, suggesting a direct albeit subtle role for VDR in musculoskeletal growth.

Vitamin D

Vitamin D synthesis and signaling are the subject of many reviews [24]. Briefly, vitamin D may be synthesized in the body by conversion of 7-dehydrocholesterol in the skin upon ultraviolet (UV) light exposure, or obtained from the diet, where it is mainly in fatty fish or in supplemented foods. Because humans can synthesize vitamin D, it is actually not a classic vitamin, but has retained the name for historical reasons.

Vitamin D is then hydroxylated in the liver into 25-hydroxyvitaminD (25D). This is the main form of circulating vitamin D and it is measured to assess adequacy of overall vitamin D status. However, the major active hormone is 1,25-dihydroxyvitaminD (1,25D). This binds to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor superfamily. VDR usually heterodimerizes with the retinoid X receptor (RXR) although it can homodimerize with a second VDR. The receptor dimer, when ligand activated, classically translocates to the nucleus, binds to vitamin D response elements (VDRE) in the DNA and regulates transcription [25].

There are hundreds of well-characterized targets for activated VDR in the genome. Recent use of RNA-sequencing combined with chromatin immunoprecipitation (ChIP) has given new power to examine genes regulated by vitamin D in non-classic target tissues such as liver [26] and bone cells [27] although this technology has not yet been applied to muscle.

Vitamin D in bone

Vitamin D has long-recognized actions in both bone and muscle [28–30]. The classical consequence of vitamin D deficiency is a defect of bone mineralization, causing rickets in children and osteomalacia in adults. However, vitamin D has been linked more to active calcium

absorption from the intestine than to locally stimulate mineralization of the newly formed bone matrix, the osteoid. The active vitamin D metabolite, 1,25D binds to VDR in intestinal cells and stimulates formation of calbindin which binds calcium and influences the calcium channels. It is generally assumed that bone mineralization is mostly a passive process when sufficient calcium and phosphate are available. Besides active calcium absorption, passive diffusion through the intestinal wall also is possible, depending on the calcium gradient.

The study of knockout mice has largely confirmed this concept. Mice with genetic deletion of VDR may display reversal of rickets/osteomalacia when supplied with a diet very high in calcium and lactose; "rescue diet". Similarly, rickets can be healed in children with a genetic defect in the VDR; vitamin D dependent rickets type 2. However, VDRKO mice have atrophic muscle fibers and changes in osteoblast number, mineral apposition rate and bone volume despite rescue diet, resulting in lower longitudinal growth and a smaller skeleton [23,31].

In recent years, our understanding of vitamin D signaling in bone has expanded to include direct vitamin D signaling in bone cells. Mouse models have enabled the delineation of direct effects of vitamin D. In mice, overexpression of VDR restricted to mature osteoblasts increases bone formation, reduces bone resorption and inhibits bone loss related to vitamin D deficiency [32,33]. Recent data suggests that these mice respond more actively to mechanical loading by increasing bone formation and mineralization [34], a finding consistent with greater cortical bone mass and size in mice with elevated osteoblastic VDR levels, under normal or vitamin D-deficient conditions [32,33]. The muscle phenotype of these mice has not been reported.

The direct effects of vitamin D in bone may be differentiation stage specific. VDR signaling in immature osteoblasts may have the *opposite* effect, stimulating bone resorption and reducing bone mass, as demonstrated in a knockout model using the collagen 1-promoter [35]. Moreover, vitamin D treatment of early committed osteoblastic cells is inhibitory, reducing cell activity and number [36]. In contrast, vitamin D levels have been reported to be inversely related to serum sclerostin levels. Sclerostin is a marker of osteocyte mechano-sensing, and an active suppressor of WNT-mediated mechanical stimulation of bone formation by terminally differentiated osteocytes [37]. These stage-specific effects may relate in part to vitamin D's regulation of WNT signaling in the osteoblastic lineage [36]. The osteoblast-specific VDR-null mouse suggests that VDR plays a more systemic role in inhibiting mineralization in order to maintain serum calcium levels [38]. The direct role of VDR in osteocytes appears to be regulation of their development by effects on osteoprotegerin and maintaining calcium levels during deficiency [39]. Therefore, local effects of vitamin D in bone may complement systemic vitamin D effects and may be anabolic or catabolic depending on the calcemic status and stage of bone cell differentiation.

Vitamin D in muscle and potential effects on bone mass

The link between the effects of vitamin D signaling in muscle/bone has long been appreciated. There are many potential factors which may play a role in mediating this interaction, some of which are shown in Fig. 1.

Indeed, one of the major components of vitamin-D deficiency-related increases in fracture rates is due to muscle weakness and falls. The greatest effects are evident in those with lowest vitamin D levels [40,41]. In addition, vitamin D status is associated with physical performance in epidemiological studies. Vitamin D deficiency can predict a decline in physical performance after 3 years [42,43]. According to the study of Need et al. [44], the calcium absorption starts to decrease and the alkaline phosphatase as a sign of deficient mineralization starts to rise when serum 25D is below 20 nmol/l. Physical performance decreases when serum 25D falls below 50 nmol/l, but the decrease accelerates when 25D is below 30 nmol/l [42].

A meta-analysis of randomized clinical trials has confirmed that vitamin D supplementation can decrease the incidence of falls [45],

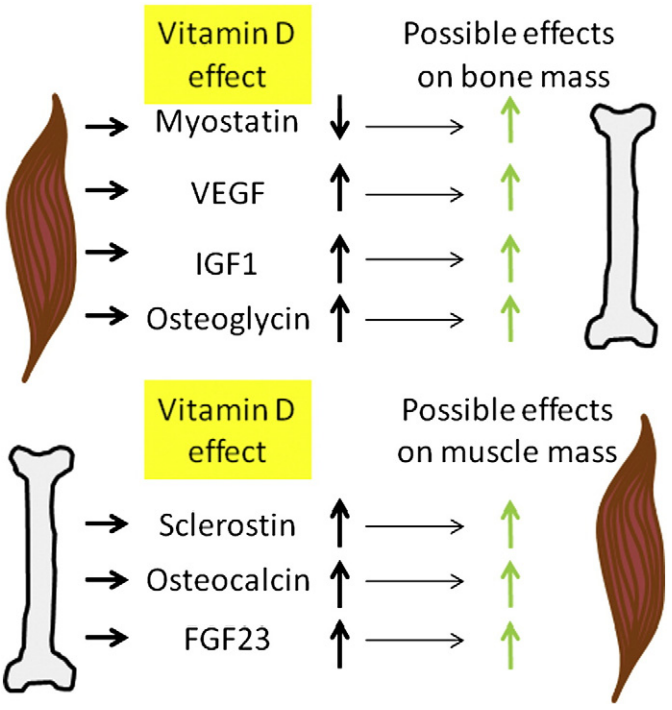

Fig. 1. Potential vitamin D mediated mechanisms of bone–muscle cross-talk.

although this remains controversial. However, the appreciation of muscle-bone cross-talk continues to increase, and evidence is emerging of a role for vitamin D in this axis [46]. Co-regulation of bone and muscle by vitamin D is evident in co-existence of osteoporosis and sarcopenia in the elderly, vitamin D deficient population [40,47–49].

Interestingly, the notion of direct humoral interactions between bone and muscle has also been supported by studies such as those of myostatin, a powerful inhibitor of muscle growth (reviewed in [50]), which also has powerful effects upon bone mass and fracture healing, independent of the changes in muscle strength [51,52]. Vitamin D treatment decreases myostatin in cultured muscle cells, suggesting a potential relationship between these pathways in vivo.

Vitamin D-related signals have also been implicated in muscle bone interactions [53]. Osteoglycin, produced by muscle cells under the control of vitamin D, regulates osteoblastic activity [53].

Vitamin D in muscle

We reviewed the roles of vitamin D in muscle in 2013 and 2014 [40,41] and others have published excellent reviews on this area [28,54]. Whether vitamin D receptor is expressed in muscle has been a controversial question, made more difficult by the low level of expression of VDR in myocytes after neonatal life. However, by RNA, Western immunoblot and by immunohistochemistry, VDR is present at low levels in cultured C2C12 myotubes [55] and normal murine muscle [56]. VDR expression is typically much higher in cell lines and isolated myocytes than in primary muscle. VDR expression is also significantly higher in neonatal muscle and declines with age at this site. This further supports developmental roles of VDR in muscle.

It has been long-recognized that vitamin D deficiency is associated with muscle weakness, particularly proximal muscle weakness, and that this resolves with correction of deficiency. There are non-specific electromyographic (EMG) features which resolve with correction of deficiency (reviewed in [40]). In biopsy studies, most of which are non-randomized, people with deficiency have preferential loss of type 2 muscle fibers which improves with supplementation [57,58]. In

children with rickets and adults with osteomalacia, muscle weakness can be extreme [40]. In the people with vitamin D pathway mutations, weakness is also a clinical feature prior to treatment. However, in these cases, and in severe vitamin D deficiency, the situation is frequently confounded by other factors such as hypocalcemia or hypophosphatemia [59].

In addition, in people, it is challenging to control for sunlight and diet exposure; 'healthier' individuals may have better diets and undertake more outdoor activities, and have correspondingly better muscle and bone function. For this reason, it is useful to turn to animal models to examine the question of bone–muscle interactions and vitamin D.

Mice with mutations in vitamin D receptor are weak and can have low bone mass and less longitudinal growth. However, these findings are confounded by the altered calcium and phosphate status of the mice. Studies of VDR-null mice where phosphate and calcium are aggressively replaced with a 'rescue diet' may result in near-normal bone phenotype [60,61]. However, there can be more subtle residual effects, suggesting persistent and local effects of VDR ablation in bone, including in heterozygous VDR-null mice [61]. Epidemiological studies show associations between vitamin D deficiency and poor physical performance, assessed by a walking test, 5 chair stands, and a balance test [42].

Potential muscle to bone vitamin D cross-talk

Even with use of rescue diet, there is decrease in the size of muscle fibers in mice lacking VDR [23]. This suggests that the effect on muscle is more likely to be a VDR effect rather than a calcium or phosphate effect. On 'rescue diet' the bone mass changes in VDR null mice are smaller than those changes seen in muscle mass and strength measures. This suggests that there may be a muscle-related or hormonal factor that is stimulating bone mass in VDR-null muscle.

Myostatin is a hormone secreted from muscle. Its role is to inhibit increased muscle mass, hence its name. It is a member of the TGFβ superfamily which acts on activin receptors and SMADs. Vitamin D inhibits myostatin production from muscle cells, for example C2C12 cells [62], and vitamin D treatment doubles myotube size in these cells [55]. Decreased myostatin is associated with greater bone mass [63] (Fig. 1).

Vitamin D may regulate responses to muscle injury and regeneration. Serum 25D levels drop directly following muscle injury, which may partly be due to the fall in vitamin D binding protein after trauma [64]. Baseline levels correlate directly with muscle recovery [65]. Studies in rodents demonstrate substantial increases in the expression of *Vdr* and *Cyp27B1* following injury [66–68]. This has been found both in models of chemical injury [66,68], and a freeze–crush model of physical injury [67]. Increased VDR is directly localized to regenerating muscle fibers [66]. Vitamin D supplementation also reduces muscle injury due to high-intensity exercise in rats and humans [69,70]. At a cellular level, it increases VEGF and FGF1; both factors involved in tissue regeneration and neovascularization. This was seen in C2C12 myoblasts treated with 1,25D, and VEGF and FGF1 are potential pathways by which vitamin D may modulate muscle regeneration [71]. An important question is whether vitamin D also affects muscle fibrosis following injury. This is suggested by recently described effects of VDR in hepatic stellate cells and liver injury via pathways relevant to muscle (TGF-B/SMAD) [72], but is yet to be addressed in this tissue.

Vitamin D is known to stimulate local vascular endothelial growth factor (VEGF) and IGF-1 (Fig. 1) production in muscle. Both factors are well known to have potential beneficial effects in bone. How large a contribution muscle vitamin D action makes to circulating levels of either hormone is not clear.

Another potential muscle factor which is regulated by vitamin D is IL-6 (interleukin-6). It is produced following exercise or contraction. It stimulates bone resorption, and may alter bone strength. It is decreased by vitamin D [73].

Potential bone to muscle vitamin D cross-talk

The osteocyte cell line MLO-Y4 expresses muscle anabolic factors IGF-1, MGF and VEGF after mechanical loading [74]. Osteocalcin which is produced by osteoblasts has recently come to a new light for its role in regulating beta-cell function [75]. Its classic use is as a marker of bone formation. It is regulated by vitamin D and its gene contains a vitamin D response element (VDRE) indicating direct regulation by vitamin D [2]. It has potential effects in muscle, in which it alters mitochondrial function, insulin sensitivity [75], and possibly strength in women.

Sclerostin is secreted by mature osteocytes during completion of osteon formation. It inhibits bone formation. Mutations in the SOST gene, coding for sclerostin, cause sclerosteosis with undetectable or low sclerostin levels, increased bone formation, very high bone mass and neurological impairments due to entrapment of nerves [37]. Sclerostin antibodies have a potential for exciting use in the treatment of osteoporosis. Sclerostin secretion by osteocytes increases in response to bedrest and decreases with muscle loading [76]. Serum levels increase in people treated with vitamin D [77]. FGF23 is another vitamin D responsive hormone produced by bone which may have positive effects on cardiac and smooth muscle [78]. The effects on skeletal muscle are the subject of current investigation.

Conclusions

In addition to established roles in calcium and phosphate homeostasis, vitamin D plays a vital role in musculoskeletal health. People with severe vitamin D deficiency display the combination of reduced bone mineral density and muscle wasting. Mechanistic studies demonstrate a range of rapid and genomic effects of vitamin D on cell differentiation, bone mineralization and muscle fiber size [53,55,79,80]. Vitamin D may also exert effects in bone and muscle injury. Following injury, these tissues display heightened sensitivity to vitamin D with local upregulation of Cyp27b1, VDR and associated reductions in serum 25D levels [66,81,82]. We are also becoming increasingly aware of the interconnected biology of muscle and bone. The search for unifying factors to explain this connection has clear implications to future therapies [8]. Vitamin D appears to be one of these factors integrating bone and muscle. To support this, vitamin D exerts effects in a range of bone and muscle-derived hormones, including osteocalcin, sclerostin, IL-6 and myostatin, and may thereby modulate bone–muscle cross-talk.

Taken together, these studies confirm the intimate relation between bone and muscle and add to vitamin D's increasing repertoire of extra-skeletal effects. Future studies are needed to unravel mechanisms involved in vitamin D's tissue-modulatory, pleiotropic effects and explore the therapeutic potential of targeting this pathway in musculoskeletal disease.

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