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Review

Vitamin D, muscle and bone: Integrating effects in development, aging and injury

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ABSTRACT

Beyond the established effects of muscle loading on bone, a complex network of hormones and growth factors integrates these adjacent tissues. One such hormone, vitamin D, exerts broad-ranging effects in muscle and bone calcium handling, differentiation and development. Vitamin D also modulates muscle and bone-derived hormones, potentially facilitating cross-talk between these tissues. In the clinical setting, vitamin D deficiency or mutations of the vitamin D receptor result in generalized atrophy of muscle and bone, suggesting coordinated effects of vitamin D at these sites. In this review, we discuss emerging evidence that vitamin D exerts specific effects throughout the life of the musculoskeletal system – in development, aging and injury. From this holistic viewpoint, we offer new insights into an old debate: whether vitamin D's effects in the musculoskeletal system are *direct* via local VDR signals or *indirect* via its systemic effects in calcium and phosphate homeostasis.

Contents

Introduction

The link between vitamin D and musculoskeletal function is long-standing. In the first description of rickets four centuries ago, Whistler reported the combination of "flexible, waxy" bones and "flabby, toneless" muscles in young children (Whistler, 1645). Adults with vitamin D deficiency also display concurrent defects in bone and muscle, characterized by osteomalacia (*i.e.* reduced bone mineral) and type 2 muscle fiber atrophy (Girgis et al., 2013). These conditions are also seen in subjects with mutations of the vitamin D receptor (VDR), suggesting local genomic effects of vitamin D in the muscle–bone unit.

Studies have elucidated a range of effects of vitamin D in bone and muscle cells and transgenic mouse models have shed light on potential interconnected vitamin D signals between these tissues (Garcia et al., 2011; Girgis et al., 2014b; Lam et al., 2014; van Driel et al., 2006). Concurrently, we are becoming increasingly aware that muscle and bone interact to form a finely coordinated unit (DiGirolamo et al., 2013). This notion is supported not only by effects of muscle loading on bone function but also the emergence of multiple paracrine/endocrine factors that facilitate cross-talk between these tissues. Bone and muscle arise together from embryonic mesoderm, grow together throughout puberty and atrophy during aging.

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The search for biomechanical and endocrine cues responsible for this life-long synchrony of muscle and bone is underway.

Vitamin D is one endocrine system that may integrate bone and muscle function. Clinically, vitamin D levels predict the rate of functional decline, fracture risk and bone mass in young and old individuals (Sohl et al., 2013; Ward et al., 2010). At a molecular level, muscle and bone both express the 1-alpha-hydroxylase enzyme, encoded by Cyp27b1, facilitating the local synthesis of the bioactive hormone, $1,25(OH)_2D$ (Anderson et al., 2005; Girgis et al., 2014b; Srikuea et al., 2012). Regulated by local and systemic vitamin D signals, bone is the major depot for calcium, an essential mineral in muscle contraction, morphology and plasticity. Conversely, skeletal muscle is emerging as a major storage site for vitamin D from where it may diffuse back into the circulation or possibly, into adjacent bone, following specific signals (Abboud et al., 2013; Girgis et al., 2014d). Thus, vitamin D may have complementary effects in bone and muscle, further supporting the integration of these two tissues.

In this review, we will discuss key events in the life of the musculoskeletal system – development, aging and injury – and evidence for integrated effects of vitamin D at these times. We will cover areas of controversy, particularly whether vitamin D's effects in the musculoskeletal system are mainly indirect via systemic calcium and phosphate levels or direct via local VDR, the presence of which is debated in muscle. Finally, potential therapeutic implications of this field and outstanding questions will be raised.

Vitamin D and musculoskeletal development

The VDR makes its first appearance in the fetal rat at day 13 gestation (Johnson et al., 1996). At this stage, it resides within the condensing mesenchyme of the vertebral column and by day 17, within osteoblasts and proliferating, hypertrophic chondrocytes. Mesenchymal stem cells (MSCs) express VDR (Artaza and Norris, 2009). In various studies, $1,25(OH)_2D$ results in nuclear translocation of VDR in MSCs and induction of myogenic and osteogenic pathways by effects in TGF-beta and bone matrix proteins ALP, bone sialoprotein (BSP) and osteopontin (Artaza and Norris, 2009; Honda et al., 2013; Mostafa et al., 2012). MSCs also express 1-alpha-hydroxylase (Cyp27b1). Inhibition of this enzyme reverses the effects of 25OHD in human MSCs including its anti-proliferative, pro-differentiation and anti-apoptotic activity (Geng et al., 2011b). Therefore, effects of vitamin D in MSCs and the presence of vitamin D signaling components in these primordial cells provide *prima facie* evidence for vitamin D's role in musculoskeletal development.

Vitamin D exerts a range of *in vitro* effects in committed myoblasts and osteoblasts. These effects vary widely depending on the particular cell model used (van Driel et al., 2006; Yamaguchi and Weitzmann, 2012), the stage of cellular differentiation (Tanaka et al., 2014), origin of primary cells (Yang et al., 2013) and species (Thomas et al., 2000; van Driel and van Leeuwen, 2014). In general, $1,25(OH)_2D$ stimulates bone formation and mineralization in human osteoblasts by effects in Wnt and B-catenin pathways (Fretz et al., 2007) and the subsequent expression of RUNX2, osteocalcin, osteopontin and matrix Gla protein (Prince et al., 2001; van Driel et al., 2006; Zhang et al., 1997). By contrast, $1,25(OH)_2D$ inhibits bone formation and mineralization in cultures of murine osteoblasts due to suppression of the same osteogenic markers (Shi et al., 2007; Yamaguchi and Weitzmann, 2012) and leads to increases in the mineralization inhibitor pyrophosphate (Drissi et al., 2002; Lieben et al., 2012). This discrepancy between responses of murine and human osteoblasts to vitamin D may result from different extracellular conditions including phosphate or cytokine levels or species-specific VDR cistromic interactions (Pike et al., 2014).

C2C12 muscle cells also respond to vitamin D by increased expression and nuclear translocation of VDR (Garcia et al., 2011; Girgis et al., 2014b). In addition to anti-proliferative effects due to altered cell cycle activity, vitamin D intriguingly leads to a doubling in the size of C2C12 myotubes by profound inhibition of myostatin (Garcia et al., 2011; Girgis et al., 2014b). Conversely, knockdown of VDR and CYP27B1 results in increased proliferation, impaired differentiation and myotube maturation in C212 and G8 cells (Srikuea et al., 2012; Tanaka et al., 2014). Together, these studies support a direct role for vitamin D signaling in bone and muscle cells but present a mixed picture of its effects. Under certain conditions, vitamin D has an anabolic effect in bone and muscle cells, promoting mineralization and myotube growth. However, the physiologic relevance of these findings is unclear and a limitation inherent to these studies is the isolated assessment of bone and muscle cells without accounting for interactions. Cross-culture studies would be helpful in assessing integrated effects of vitamin D in bone and muscle cell differentiation.

Despite the early presence of VDR in the developing embryo, the clinical features of rickets develop after weaning (Li et al., 1997; Yoshizawa et al., 1997). These features including growth plate defects, increased osteoid (*i.e.* unmineralized bone), and generalized atrophy of type I and II muscle fibers are seen in VDRKO mice at day 35 (Endo et al., 2003; Li et al., 1997). However, subtle changes are seen prior to weaning including expansion of the growth plate (Li et al., 1997), smaller muscle fibers and altered expression of myogenic regulatory factors at day 15 (Endo et al., 2003). Interestingly, another VDRKO model – generated by ablation of exon 2 – showed no musculoskeletal defects prior to weaning (Yoshizawa et al., 1997), possibly due to the presence of a truncated form of VDR with potential biologic activity in these mice (Bula et al., 2005). This suggests non-classical signaling may be responsible for pre-weaning musculoskeletal effects of VDR.

In humans, subtle differences in fetal bone development are observed in association with maternal vitamin D deficiency (Ioannou et al., 2012; Mahon et al., 2010). Maternal vitamin D status is indirectly associated with fetal bone mineral content via methylation at 4 sites of the RXRA in umbilical cord tissue (Harvey et al., 2014; Jaenisch and Bird, 2003). This indicates a novel epigenetic role for vitamin D in embryonic skeletal development. VDR may also prepare bone during embryogenesis for post-natal mineralization by enhancing production of mature matrix vesicles rather than directly increasing mineral deposition (Woeckel et al., 2010).

Vitamin D may also regulate muscle fiber size during development. In humans, maternal vitamin D deficiency is associated with reduced arm-muscle area in newborn offspring (Krishnaveni et al., 2011). In rats born to vitamin D deficient dams, smaller muscle fibers were seen in association with altered expression of genes involved in protein catabolism, differentiation and the cytoskeleton (Max et al., 2013). These effects are not restricted to mammals as European sea bass treated with dietary 25OHD after hatching also demonstrate dose-dependent increases in muscle fiber size and myogenic gene expression (Alami-Durante et al., 2011). Growth-promoting effects of vitamin D in muscle may potentially enhance loading on bone and stimulate bone mass. In addition, skeletal muscle may represent a reservoir for vitamin D from where it may diffuse to adjacent bone during embryogenesis. In support of this, a study from 1986 reported the *in utero* transfer of radiolabeled 25OHD across the placenta and into the skeletal muscle of rat embryos (Clements and Fraser, 1988). More recently, we reported a direct effect of VDR in the uptake of 25OHD into muscle fibers (Girgis et al., 2014d). Human studies also support the notion that vitamin D may be taken up and utilized by the musculoskeletal system during growth. Reductions in serum vitamin D levels concurrent with increases in lean muscle mass and bone mineral content during puberty have been reported (Breen et al., 2011; Willis et al., 2007).

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Vitamin D directly alters growth plate fusion. Cyp27b1-KO mice display persistent abnormalities in long bone growth *even after* correcting their mineral levels (Dardenne et al., 2003). Chondrocyte-specific VDR knockout mice displayed an intriguing phenotype with transient impairment in osteoclastogenesis, reduced vascular invasion of the growth plate and increased serum phosphate (Masuyama et al., 2006). This elegantly demonstrates the role of VDR in regulating interactions between chondrocytes, osteoblasts and endothelial cells in growth plate fusion and systemic mineral levels. Mechanical loading is also important for growth plate fusion (Mirtz et al., 2011) and effects of vitamin D in muscle fiber size and mass may thereby play an indirect role in growth plate physiology.

In summary, evidence suggests that vitamin D plays a subtle role in prenatal musculoskeletal development that has perhaps been obscured by overwhelming evidence in support of a predominantly post-natal effect. VDR appears at an early stage in the developing embryo and is expressed by MSCs, osteoblasts and myoblasts, all of which are responsive to vitamin D (Artaza and Norris, 2009). *In vitro* studies report growth-promoting effects of vitamin D in myotubes and maturation of matrix vesicles in osteoblasts (Girgis et al., 2014b; Woeckel et al., 2010). *In vivo* studies support subtle musculoskeletal changes in pre-weaned VDRKO mice (Li et al., 1997). However, pre-weaning effects of VDRKO are not consistently found (Yoshizawa et al., 1997) and VDR activity in muscle/bone precursor cells is confounded by its increased expression following cell isolation (Girgis et al., 2014d). Therefore further research is required to clarify these questions. If confirmed, a role for VDR in musculoskeletal development would be consistent with its known pleiotropic effects in other tissues (Lin et al., 2002). For further discussion on development effects of vitamin D on bone, we refer readers to the following review (van Driel and van Leeuwen, 2014).

Vitamin D and musculoskeletal involution

Vitamin D deficiency is common in elderly and institutionalized subjects and may contribute to age-related involution of bone (osteoporosis) and muscle (sarcopenia) (Sohl et al., 2013; Visser et al., 2003). Vitamin D levels predict the risk of functional decline, loss of muscle mass and osteoporotic fracture in elderly subjects, all of which have devastating effects by increasing morbidity, the need for assisted-care and mortality (Sohl et al., 2013). Elderly individuals are also at risk of vitamin D deficiency due to intrinsic age-related changes in vitamin D synthesis, reduced exposure to sunlight and malnutrition, which is rife among those living in institutions (Girgis, 2014).

VDR expression in muscle and bone declines with age (Bischoff et al., 2001; Montero-Odasso and Duque, 2005), rendering the musculoskeletal system more vulnerable to low vitamin D levels in the elderly. In addition, Cyp27b1 levels drop in aged bone cells and MSCs, blunting their responses to 25OHD (Anderson et al., 2005; Geng et al., 2011a). Clinically, 25OHD levels correlate more tightly with bone parameters in the elderly than $1,25(OH)_2D$, also supporting an effect of Cyp27b1 in skeletal aging (Anderson et al., 2013). By contrast, muscle mass correlates tighter with $1,25(OH)_2D$ (Marantes et al., 2011), suggesting a lesser role for Cyp27b1 in this tissue.

Vitamin D also affects age-related musculoskeletal atrophy, a complex process that involves involution and adipose infiltration of the muscle–bone unit (Schellinger et al., 2001; Visser et al., 2005). Vitamin D deficiency activates pathways responsible for muscle atrophy by increasing protein turnover via activation of the ubiquitin-proteasome system, ubiquitin ligases (MAFBx and MuRF1), FoxO signaling and TGF-beta signaling (Bhat et al., 2013; Bonaldo and Sandri, 2013). These features are only partly reversible following correction of serum calcium, suggesting a direct VDR effect. Conversely, treatment of muscle cells with $1,25(OH)_2D$ increases myotube size by effects on TGF-beta signaling (Garcia et al., 2011; Girgis et al., 2014b) and prevents free fatty acid (FFA)-induced atrophy by effects on Akt/c-Jun N-terminal kinase (cJNK) (Zhou et al., 2008). However, at supra-physiological doses, vitamin D may induce muscle protein breakdown by activating calpain proteolytic pathways, a mechanism that has been studied for its potential to tenderize beef from aged cattle (Montgomery et al., 2004).

Age-related decline in bone mineral density and mass involves increased osteoclastogenesis, mismatch in RANKL:OPG activity and proteasomal degradation of bone morphogenetic signaling proteins (Cao et al., 2005; Guo et al., 2008). $1,25(OH)_2D$ exerts anti-aging effects in bone marrow pluripotent stem cells by inducing osteogenesis (Duque et al., 2004), delaying replicative senescence (Klotz et al., 2012) and modulating cell survival proteins FoxO, Sirt1 and sestrins (An et al., 2010; Eelen et al., 2013). In osteoblasts, $1,25(OH)_2D$ upregulates cystathionine beta-synthase (CBS) (Kriebitzsch et al., 2011), a key enzyme that catalyzes homocysteine and thereby exerts positive effects on bone strength and collagen cross-linking in aging (van Meurs et al., 2004). This effect is supported by an inverse relationship between serum vitamin D and homocysteine levels in human subjects (Amer and Qayyum, 2014) and importantly, the discovery of a VDRE in the CBS gene in pre-osteoblastic MC3T3-E1 cells (Kriebitzsch et al., 2011).

Adipose infiltration is another pertinent factor in muscle and bone aging. Clinical studies report an inverse correlation between serum vitamin D levels, muscle adiposity and poor functional outcomes (Gilsanz et al., 2010; Redzic et al., 2014; Tagliafico et al., 2010). In muscle cells, low-doses of $1,25(OH)_2D$ (10^{-11} and 10^{-13} M) mimicking deficiency result in upregulation of PPARγ2 and formation of lipid droplets while higher doses (10^{-7} and 10^{-9} M) inhibited transdifferentiation (Ryan et al., 2013). Similarly in bone, $1,25(OH)_2D$ inhibits marrow adipogenesis by reducing expression of PPARγ2 and has concomitant pro-osteogenic effects on bone marrow stem cells (Duque et al., 2004; Kelly and Gimble, 1998). Therefore vitamin D and PPARγ2 signaling are related in muscle and bone and this link requires further evaluation for its tissue-modulatory effects.

Clinical studies show that vitamin D supplementation reduces (Bischoff-Ferrari et al., 2005; Broe et al., 2007), increases (Sanders et al., 2010) or has no effect on the risk of falls and fractures (Girgis et al., 2014c), functional endpoints of musculoskeletal aging. The musculoskeletal system may respond dose-dependently to vitamin D with a U-shaped curve. Individuals with high and low serum vitamin D levels were found to have the highest risk of fracture and frailty compared to those with intermediate levels (60–70 nmol/l) (Bleicher et al., 2014; Ensrud et al., 2010). Likewise, single mega-doses of vitamin D (500,000 IU) increased the risk of falls and fractures (Sanders et al., 2010) while daily supplementation at lower doses had favorable effects in bone mineral density and muscle fiber size in older individuals (Ceglia et al., 2013; Reid et al., 2013). However, these effects did *not* translate to improvements in muscle function or fracture incidence possibly due to the dose-limiting calcemic effect of vitamin D. By contrast, a $1,25(OH)_2D$ analog with lower calcemic potency, eldecalcitol increased bone mineral density, reduced falls/fractures and improved lower limb muscle power in older individuals (Iwamoto and Sato, 2014; Matsumoto et al., 2011). These findings may result from tissue-specific activation of VDR in bone and muscle, an effect that has been recently observed following vitamin D supplementation (Ceglia et al., 2013).

In summary, serum vitamin D and musculoskeletal VDR decline with age concurrent with the physiological involution of muscle and bone. Effects of vitamin D deficiency overlap with those of aging, including adipose-tissue infiltration, activation of proteolytic pathways in muscle, osteoclastogenesis and increased mineral turnover in bone. Evidence also suggests that musculoskeletal aging responds to vitamin D at a cellular level and in human clinical studies. These findings raise an important question for future consideration: can therapies targeting VDR reverse musculoskeletal aging?

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Vitamin D and musculoskeletal injury

We are becoming increasingly aware of the interconnected responses of muscle and bone to injury. Fractures associated with muscle injury are more likely to undergo non-union (Reverte et al., 2011) and conversely, the use of muscle flaps to treat open fractures leads to improved bone healing (Harry et al., 2008). In this regard, skeletal muscle may represent a kind of "second periosteum", providing trophic factors, morphogens and cells to aid bone repair.

Vitamin D may also integrate the combined response to injury. Serum 25OHD levels drop in the curative phase of a fracture and increased 24,25OHD levels correlate with fracture healing (Ettehad et al., 2014; Seo and Norman, 1997). This suggests that vitamin D is being utilized and metabolized by healing bone. To support this, fracture callus displays increased Cyp24a1 activity and 24,25$(OH)_2$D notably improves fracture repair in mice and chickens (Seo and Norman, 1997). This may relate to known effects of 24,25$(OH)_2$D in the differentiation and maturation of growth plate chondrocytes via a putative membrane receptor in fracture callus (St-Arnaud and Naja, 2011). 1,25$(OH)_2$D may also play an independent role following fracture via regulation of a range of genes involved in bone remodeling (Ormsby et al., 2013) and neovascularization (Wang et al., 1996). Although polymorphisms in the vitamin D-binding protein (DBP) are associated with osteoporosis (Al-oanzi et al., 2008), local administration of DBP did not alter healing in a rodent model of fracture (Sun et al., 2009).

Similar to bone, reductions in serum 25OHD levels are seen in humans directly following muscle injury and baseline vitamin D levels correlate with faster recovery in muscle function (Barker et al., 2013). Studies in mice and rats demonstrate substantial increases in the expression of VDR and Cyp27b1 following injury (Girgis et al., 2014e; Srikuea et al., 2012; Stratos et al., 2013). This has been reported in various muscle injury models including intramuscular $BaCl_2$ or Notexin administration (Girgis et al., 2014e; Srikuea et al., 2012) and freeze-crush injury (Stratos et al., 2013). Importantly, increased VDR is localized to regenerating muscle fibers and not dependent on other cell types such as inflammatory or satellite cells seen in injury (Srikuea et al., 2012). Vitamin D supplementation also reduces muscle injury due to high-intensity exercise or crush injury in rats (Choi et al., 2013; Stratos et al., 2013). Mechanisms include reduced activation of stress-related proteins (p38 MAPK, ERK1/2, IKK, IkappaB), reduced expression of inflammatory cytokines (TNF-alpha, IL-6) and increased muscle fiber turnover (Choi et al., 2013). As such, vitamin D leads to faster recovery of contractile force in the injured muscle. At a cellular level, increases in VEGF and FGF-1 – classic factors involved in tissue regeneration and neovascularization – were seen in C2C12 myoblasts treated with 1,25$(OH)_2$D, suggesting additional pathways by which vitamin D modulates muscle regeneration (Garcia et al., 2013). 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-beta/SMAD) (Ding et al., 2013), but is yet to be addressed in this tissue.

These intriguing effects of vitamin D in bone and muscle injury highlight the need to examine models that integrate these components. In particular, potential effects of vitamin D on paracrine factors and morphogens crossing between muscle and bone during injury and the common activation of regeneration pathways at both sites need to be addressed.

Effects on muscle and bone: direct or indirect?

The extent to which vitamin D *directly* affects the musculoskeletal system *versus* its *indirect* actions via calcium and phosphate homeostasis is a matter of ongoing debate. This is further complicated by controversy regarding the presence of VDR in muscle. Overwhelming evidence supports a *predominantly* indirect effect. This includes the observation that musculoskeletal defects in vitamin D deficiency or VDR ablation coincide with altered serum mineral levels and are reversed by correcting these levels (Bhat et al., 2013; Li et al., 1998; Schubert and DeLuca, 2010). In addition, sole expression of VDR in the intestine reverses the skeletal phenotype of VDRKO mice, highlighting its central role in the enteric absorption of calcium and phosphate (Xue and Fleet, 2009). However, there is evidence to suggest direct effects of vitamin D in muscle and bone, potentially working in parallel or complementary to its systemic effects.

The first, perhaps weakest, line of evidence is that vitamin D exerts a range of effects in cultured bone and muscle cells. Genomic effects of 1,25$(OH)_2$D-VDR alter muscle and bone cell proliferation, differentiation, remodeling and regeneration (Girgis et al., 2014b; Tanaka et al., 2014; van Driel et al., 2006; Yang et al., 2013). Rapid effects in calcium handling and cellular proliferation occur via diverse signaling pathways including c-Src, MAPK, protein kinase C, phospholipases and voltage-gated ion channels (Buitrago et al., 2003; Morelli et al., 2001; Wali et al., 2003). Receptors modulating these effects include membrane-bound VDR and non-classical proteins such as protein-disulfide isomerase-associated 3 protein (Pdia3) and membrane associated rapid response-binding protein (MARSS) (Nemere et al., 1998). However the physiological relevance of these *in vitro* studies is questionable and VDR levels increase during cell isolation, possibly augmenting its activity in culture (Bhalla et al., 1987; Girgis et al., 2014d).

Mice with aberrant vitamin D signaling continue to display subtle musculoskeletal defects even after correction of mineral defects. Smaller muscle fibers and changes in osteoblast number, mineral apposition rate and bone volume persist in VDRKO mice on rescue diets (Endo et al., 2003; Panda et al., 2004). However, upon transplanting femora from VDRKO bone mice into WT mice, they *paradoxically* form more bone (Tanaka and Seino, 2004). This raises the possibility that extra-skeletal signals, potentially originating from muscle, may modulate bone effects of VDR. Interestingly, lack of VDR in bone protects VDRKO mice from the more severe skeletal phenotype seen in intestine-specific VDRKO mice (Lieben et al., 2012). However, intestine-specific VDRKO mice showed normal calcium and phosphate levels, presumably at the expense of bone. These observations support complex interactions in local and systemic VDR signaling in modulating bone and calcium levels.

Transgenic mouse models have shed further light on local effects of VDR in bone. These effects vary depending on the differentiation stage of osteoblasts, systemic calcium levels and the particular promoter-based Cre model used (*e.g.* osteoalcin, collagen 1-promoter) (Eisman and Bouillon, 2014). VDR overexpression in mature osteoblasts (OSVDR model, osteoalcin promoter) enhances bone formation, reduces bone resorption and protects against bone loss due to vitamin D deficiency (Baldock et al., 2006; Gardiner et al., 2000; Lam et al., 2014). These mice respond to mechanical loading by increasing bone formation and mineralization (Anderson et al., 2013), suggesting an intriguing interaction between muscle and bone through osteoblast VDR. This effect may occur by activation of Wnt co-receptor LRP5, a transcription factor with a central role in bone mechano-transduction (Bonewald, 2007; Shi et al., 2007), supported by the presence of a putative VDRE in the LRP5 gene (Fretz et al., 2007). Alternatively, 1,25$(OH)_2$D may alter mechano-transduction via non-genomic mechanisms, suggested by its VDR-independent effect on nitric oxide (NO) production by osteoblasts (Willems et al., 2012).

In immature osteoblasts, VDR signaling may have the *opposite* effect in stimulating bone resorption and reducing bone mass, as suggested by a knockout model using the collagen 1-promoter

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(Yamamoto et al., 2013). In osteocytes (terminally-differentiated osteoblasts), VDR plays a more systemic role in inhibiting mineralization to preserve normal serum calcium levels (Lieben et al., 2012).

By contrast, whether VDR is expressed in muscle remains controversial (Bischoff et al., 2001; Ceglia et al., 2010, 2013; Srikuea et al., 2012; Wang and DeLuca, 2011). Differences in experimental conditions, the presence of problematic VDR antibodies and altered expression of VDR throughout muscle differentiation/aging explain this controversy (Girgis et al., 2014d). We have recently reported that age is a major determinant in VDR detection in muscle (Girgis et al., 2014d). Significantly higher levels of VDR are found in muscle of young mice. In adult mice, marked increases in VDR following muscle injury are also seen, supporting a predominantly pleiotropic role in this tissue (Choi et al., 2013; Srikuea et al., 2012; Stratos et al., 2013).

Although effects of vitamin D in the musculoskeletal system are mainly indirect, VDR acts locally to regulate bone mass, mineralization and responses to muscle loading. These effects may complement systemic vitamin D activity and may be either anabolic or catabolic depending on the calcemic status and stage of bone cell differentiation. Further research is needed to characterize the complex nature of these interactions and therapeutic implications.

Vitamin D and muscle-bone signals

Under various stimuli, muscle and bone produce a range of hormones that facilitate cross-talk between these tissues. Vitamin D may regulate such factors, thereby indirectly affecting muscle–bone interactions (Fig. 1).

One such factor, osteocalcin is produced by osteoblasts, regulated by vitamin D and its gene contains a well-established VDRE (Morrison et al., 1989; Terpening et al., 1991). Apart from being a primary marker of bone formation, osteocalcin in its undecarboxylated form participates in glucose homeostasis and forms the basis of a putative bone–pancreas endocrine loop (Clemens and Karsenty, 2011). Osteocalcin also has potential effects in skeletal muscle by altering insulin sensitivity (Clemens and Karsenty, 2011), muscle mitochondrial function (Clemens and Karsenty, 2011) and lower limb strength in older women (Levinger et al., 2014).

Sclerostin is another potential link. This factor, secreted by osteocytes, regulates the osteogenic response to muscle loading and, interestingly, responds to vitamin D supplementation (Dawson-Hughes et al., 2014; Tu et al., 2012). Similarly, FGF23 is a vitamin D-responsive phosphaturic hormone produced by bone cells that has effects on cardiac and smooth muscle, suggesting potential effects in skeletal muscle (Six et al., 2014; Touchberry et al., 2013).

Vitamin D supplementation has been shown to alter IL-6 (Schleithoff et al., 2006), an inflammatory cytokine and hormone produced by skeletal muscle following contraction and exercise (Pedersen and Febbraio, 2008). In addition to its role in glucose homeostasis via fat and liver, IL6 stimulates bone resorption and IL6-related cytokines, Oncostatin M and ciliary neurotrophic factor (CNTF), alters osteoblast differentiation and bone strength (Johnson et al., 2013, 2014). Alterations in IL6 may also explain age-related inflammation and skeletal muscle defects in vitamin D-deficient older individuals (Sanders et al., 2014).

Myostatin, a member of the TGF-beta superfamily and a muscle-derived hormone, has been linked to vitamin D in cultured muscle cells and in a human study (Girgis et al., 2014b; Szulc et al., 2012). Myostatin negatively regulates muscle mass by effects on Smad family proteins and the ubiquitin–proteasome system and affects bone mass directly (via activin receptors) and indirectly (by muscle loading) (Bowser et al., 2013; Elkasrawy and Hamrick, 2010). Vitamin D has a profound inhibitory effect on myostatin expression in cultured muscle cells, an intriguing connection which explains the doubling in myotube size in response to $1,25(OH)_2D$ (Girgis et al., 2014b). Another member of the TGF-beta family, follistatin enhances $1,25(OH)_2D$-simtulated mineralization in osteoblasts (Woeckel et al., 2013) and is also regulated by vitamin D in muscle cells (Garcia et al., 2011).

Therefore, vitamin D is associated with several bone and muscle-derived hormones, suggesting indirect links in muscle–bone cross-talk. While these links are tenuous, they require further evaluation and present interesting lines of enquiry. These links have been summarized in Fig. 1.

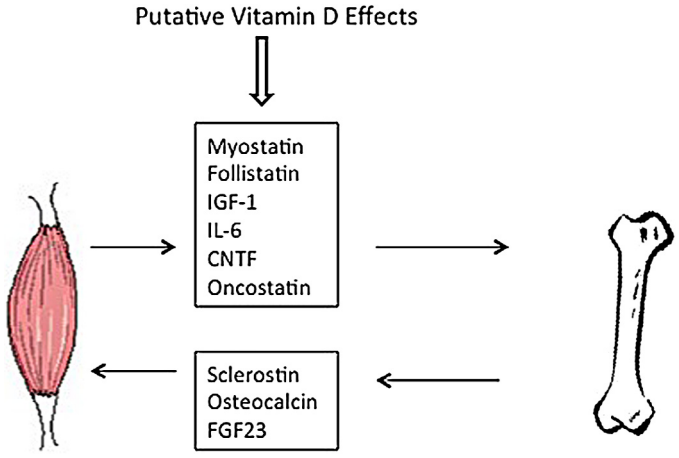

Fig. 1. Putative mechanisms by which vitamin D modulates bone–muscle cross-talk.

Conclusions

In his seminal publication on the cure of rickets by sunlight, the American physician Alfred Hess remarked that "although we have realized the importance of sunlight in the growth of plant life, we have [until now] accorded it too little significance in the development of animal life" (Hess, 1922). Since this time, we have made major advances in understanding the biologic activity of the "sunshine hormone", $1,25(OH)_2D$, and its system-wide effects in mineral homeostasis, organ development, fibrosis and cell cycle regulation. We are also becoming increasingly aware of the interconnected biology of muscle and bone and the search for unifying factors to explain this connection has clear implications to future therapies (Girgis et al., 2014a).

Vitamin D is one such factor that potentially integrates bone and muscle. To support this contention, *in vitro* studies demonstrate a range of rapid and genomic effects of vitamin D in differentiation, bone mineralization and muscle fiber size (Girgis et al., 2014b; Tanaka et al., 2014; van Driel et al., 2006; Yang et al., 2013). Following injury, the muscle–bone unit displays heightened sensitivity to vitamin D with local up-regulation of Cyp27b1, VDR and associated reductions in serum 25OHD levels (Ettehad et al., 2014; Seo and Norman, 1997; Srikuea et al., 2012). In contrast, the aging muscle–bone unit displays reduction in local VDR in association with activated proteolytic pathways in muscle, increased bone resorption and adipose-tissue infiltration by common effects on PPARγ2 (Bhat et al., 2013; Duque et al., 2004; Ryan et al., 2013).

A central question that has perplexed this field for many years is whether effects of vitamin D in bone and muscle are entirely indirect – via calcium and phosphate homeostasis – or partly direct by local VDR. This is especially controversial in muscle where the presence of VDR is debated and in bone, where transgenic mouse models have *not* presented a clear picture of VDR's local effects (Eisman and Bouillon, 2014). The latter highlights the complexity

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of local and inter-system VDR signals in modulating tissue responses. Another question is whether vitamin D's effects in bone and muscle are truly integrated or rather independent. In support of the former, osteoblast VDR modulates the effect of muscle loading on bone (Anderson et al., 2013) and vitamin D, sequestered within muscle fibers, may diffuse to adjacent bone under the influence of local stimuli (Abboud et al., 2013). Furthermore, vitamin D regulates paracrine factors considered to facilitate bone–muscle crosstalk – osteocalcin and, possibly, IL6 and myostatin (Morrison et al., 1989; Schleithoff et al., 2006; Szulc et al., 2012). Perhaps the most compelling evidence of an inter-connected response is seen in elderly patients receiving the vitamin D analog eldecalcitol. They demonstrated parallel improvements in muscle and bone parameters with associated functional outcomes (Iwamoto and Sato, 2014; Matsumoto et al., 2011).

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

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