



Review

Vitamin D and skeletal muscle tissue and function

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ABSTRACT

This review aims to summarize current knowledge on the role of vitamin D in skeletal muscle tissue and function. Vitamin D deficiency can cause a myopathy of varying severity. Clinical studies have indicated that vitamin D status is positively associated with muscle strength and physical performance and inversely associated with risk of falling. Vitamin D supplementation has shown to improve tests of muscle function, reduce falls, and possibly impact on muscle fiber composition and morphology in vitamin D deficient older adults. Molecular mechanisms of vitamin D action on muscle tissue include genomic and non-genomic effects via a receptor present in muscle cells. Genomic effects are initiated by binding of 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] to its nuclear receptor, which results in changes in gene transcription of mRNA and subsequent protein synthesis. Non-genomic effects of vitamin D are rapid and mediated through a cell surface receptor. Knockout mouse models of the vitamin D receptor provide insight into understanding the direct effects of vitamin D on muscle tissue. Recently, VDR polymorphisms have been described to affect muscle function. Parathyroid hormone which is strongly linked with vitamin D status also may play a role in muscle function; however, distinguishing its role from that of vitamin D has yet to be fully clarified. Despite the enormous advances in recent decades, further research is needed to fully characterize the exact underlying mechanisms of vitamin D action on muscle tissue and to understand how these cellular changes translate into clinical improvements in physical performance.

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1. Introduction

It has been well-established that vitamin D plays an essential role in the regulation of calcium and phosphate homeostasis and in bone development and maintenance (DeLuca, 2004). Classically, vitamin D is known to exert its actions on target organs, such as the intestine, the kidney, the parathyroid glands, and bone. Over the last two decades, however, there has been increasing evidence that vitamin D plays an important role in many other tissues including skeletal muscle. Early clinical descriptions of a reversible myopathy associated with vitamin D deficiency and/or chronic renal failure recognized a potential association between vitamin D and muscle (Boland, 1986). The identification of the vitamin D receptor (VDR) on muscle cells (Zanello et al., 1997; Bischoff et al., 2001) provided further support for a direct effect of vitamin D on muscle tissue. Recent investigations in cell culture and animals have advanced our understanding of some of the molecular mechanisms through which vitamin D targets skeletal muscle; however, much remains to be characterized. This review summarizes the clinical evidence of an association between vitamin D status and muscle function, describes how vitamin D affects muscle tissue morphology, considers the molecular mechanisms of vitamin D activity in normal muscle tissue, outlines the lessons learned from the VDR knockout mouse model, discusses potential VDR polymorphisms and their relationship to muscle function, and touches on parathyroid hormone's (PTH) effects on muscle.

2. Clinical evidence

2.1. Vitamin D deficient myopathy

The first associations between vitamin D and muscle function were made from clinical observations of muscle weakness in osteomalacia from vitamin D deficiency. In infants this myopathy is classically characterized by muscle weakness and hypotonia (Prineas et al., 1965). In adults it may present as predominantly proximal muscle weakness with difficulty in walking up stairs, in rising from a sitting or squatting position, and in lifting objects. However, the muscle weakness can present without any specific pattern. Other typical clinical features include a waddling gait and uniform generalized muscle wasting with preservation of sensation or deep tendon reflexes (Schott and Wills, 1976). There may also be symptoms of bone pain. Aside from a low serum 25-hydroxyvitamin D [25(OH)D] level, laboratory studies may be normal. The condition may develop independently of metabolic abnormalities such as hypocalcemia, hypophosphatemia, and hyperparathyroidism (Boland, 1986). Electromyographic abnormalities, such as polyphasic motor unit potentials with shortened duration and decreased amplitude consistent with a myopathy, have also been reported. However, these findings are nonspecific and are, in fact, seen in other muscular diseases, such as polymyositis (Boland, 1986). Nerve conduction velocity can also be reduced. This myopathy has been described in patients with end stage renal disease or malabsorption syndromes which are usually associated with severe vitamin D deficiency. The symptoms respond to treatment with vitamin D suggesting that vitamin D plays an etiological role (Ekblom et al., 1964; Smith and Stern, 1967).

2.2. Vitamin D and physical performance

Evidence from observational studies has shown an association between vitamin D status and physical performance. In an analysis of men and women age 60 and over who participated in the cross-sectional NHANES III survey, individuals with higher serum 25(OH)D levels up to 94 nmol/l were able to walk faster (8-foot walk test) and to get out of a chair faster (sit-to-stand test) than subjects with lower levels (Bischoff-Ferrari et al., 2004), particularly in the subset with 25(OH)D levels under 60 nmol/l. This association was not influenced by physical activity level. In the prospective Longitudinal Study of Aging Amsterdam, lower serum 25(OH)D levels predicted decreased grip strength and appendicular muscle mass in elderly men and women over the subsequent three years (Visser et al., 2003).

A few studies have examined the effect of vitamin D supplementation on balance and gait performance. Specifically, vitamin D with calcium, compared to calcium alone, improved body sway by 9% in ambulatory elderly women with serum 25(OH)D levels less than 50 nmol/L within 8 weeks (Pfeifer et al., 2000) and improved musculoskeletal function in institutionalized elders with serum 25(OH)D levels less than 50 nmol/L by 4–11% within 12 weeks (Bischoff et al., 2003). Similarly, in another study among elders with low serum 25(OH)D levels less than 30 nmol/L, compared to placebo, vitamin D

supplementation significantly improved choice reaction time, aggregate functional performance time and postural sway (Dhesi et al., 2004).

2.3. Vitamin D and falls

In view of the association between serum 25(OH)D level and physical performance, one would expect a similar relationship between vitamin D status and fall risk. In the Longitudinal Aging Study Amsterdam, low 25(OH)D levels (less than 25 nmol/L) were associated with an increased risk of repeated falling over the subsequent year, particularly in persons under 75 years of age (Snijder et al., 2006). In a randomized, controlled trial, Bischoff et al. showed that treatment with vitamin D₃ and calcium (800 IU and 1200 mg per day) for 3 months reduced the risk of falls by 49% in comparison to calcium alone (Bischoff et al., 2003). Similarly in an Australian study, treatment with vitamin D₂ (initially 10,000 IU per week then 1000 IU per day) and calcium (600 mg per day) for 2 years reduced the risk of falls in the compliant group by 30% compared to calcium alone (Flicker et al., 2005). Further evidence of an effect of vitamin D supplementation on muscle is found in a meta-analysis of five randomized controlled trials, including over 1200 ambulatory and institutionalized subjects (Bischoff-Ferrari et al., 2004). In this analysis, vitamin D supplementation lowered the risk of falling by 22% (Bischoff-Ferrari et al., 2004). Other experimental studies using vitamin D in various doses did not observe significant effects on falls, but falls were not the primary outcomes in these studies and assessment of fall frequency in them was not always optimal (Graafmans et al., 1996; Grant et al., 2005; Porthouse et al., 2005; Latham et al., 2003).

3. Muscle morphology

3.1. Vitamin D deficiency and muscle histology

Muscle biopsies in adults with profound vitamin D deficiency have shown predominantly type II muscle fiber atrophy. Of note, type II muscle fibers are fast-twitch and are the first to be recruited to prevent a fall. Thus, the fact that primarily type II fibers are affected by vitamin D deficiency may help explain the falling tendency of vitamin D deficient elderly individuals (Snijder et al., 2006). Histological sections of vitamin D deficient individuals also reveal enlarged interfibrillar spaces and infiltration of fat, fibrosis and glycogen granules (Yoshikawa et al., 1979). The morphological features of the myopathy associated with chronic renal failure are identical to those seen in vitamin D deficient osteomalacia with type II muscle fiber atrophy (Floyd et al., 1974; Lazaro and Kirshner, 1980).

3.2. Vitamin D supplementation and muscle morphology

To date, only two studies have examined whether vitamin D supplementation may have an impact on muscle fiber composition. In a small uncontrolled study, Sorenson et al. (Sorensen et al., 1979) obtained muscle biopsies from elderly women after treatment with 1- α -hydroxyvitamin D and calcium for 3–6 months. Results showed an increase in relative fiber composition and in fiber area of type IIa muscle fibers. More recently, a randomized controlled study found that treatment of elderly stroke survivors with 1000 IU of vitamin D₂ daily significantly increased mean type II muscle fiber diameter and percentage of type II fibers over a 2 year period (Sato et al., 2005). There was also a correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after two years of follow-up. It remains unclear, however, if the increase in type II muscle fiber number is caused by new formation of type II fibers or a transition of already existing fibers from type I to type II.

4. Molecular mechanisms of vitamin D activity

4.1. VDR in muscle tissue

The biologically active form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], exerts its principal actions by binding to a vitamin D receptor (VDR).

VDRs are expressed in muscle tissue at particular stages of differentiation from myoblasts (mononucleated myogenic cells) to myotubes (multinucleated cells). In 1985, Simpson et al. identified a binding protein consistent with the 1,25(OH)₂D receptor in rodent skeletal muscle cell lines (Simpson et al., 1985). At the same time, other reports demonstrated evidence of the VDR in chick monolayers of myoblasts (Boland et al., 1985), and in cloned human skeletal muscle cells (Costa et al., 1986). Two different 1,25(OH)₂D receptors have been described, one acting as a nuclear receptor and the other located at the cell membrane.

4.2. Genomic effects of 1,25(OH)₂D in muscle

The nuclear VDR is a ligand-dependent nuclear transcription factor which belongs to the steroid–thyroid hormone receptor gene superfamily (DeLuca, 1988; Pike, 1991). Using immunohistochemical methods to analyze tissue from adult females,

Bischoff et al. reported the first in situ detection of the VDR in human skeletal muscle tissue (Bischoff et al., 2001). The data demonstrated intranuclear staining for the VDR providing evidence for the presence of the nuclear $1,25(\text{OH})_2\text{D}$ receptor.

Once transported to the nucleus by an intracellular binding protein, $1,25(\text{OH})_2\text{D}$ binds to its nuclear receptor which results in changes in the gene transcription of mRNA and subsequent de novo protein synthesis (Freedman, 1999). At the nuclear level, the activation of VDR induces the heterodimerization between the active VDR and an orphan steroid receptor known as retinoic receptor (RXR). The formation of this heterodimer facilitates the interaction between the receptor's zinc finger region with DNA activating the protein transcription process (McCary et al., 1999). This genomic pathway has been found to influence muscle calcium uptake, phosphate transport across the cell membrane, phospholipid metabolism, and muscle cell proliferation and differentiation.

In vitro and in vivo experiments in chick skeletal muscle have shown that $1,25(\text{OH})_2\text{D}$ regulates muscle calcium uptake by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma. It also regulates the calcium influx via voltage-sensitive calcium channels thereby altering intracellular calcium (Boland, 1986). Modifications in intracellular calcium levels control contraction and relaxation of muscle, thus impacting muscle function (Ebashi and Endo, 1968; Boland et al., 1995). Other experiments in cultured myoblasts and myocytes further supported these findings by demonstrating increased ^{45}Ca uptake in cells exposed to physiological levels of $1,25(\text{OH})_2\text{D}$ (de Boland and Boland, 1985; Walters et al., 1987). These longer-term effects of $1,25(\text{OH})_2\text{D}$ are dependent on de novo RNA and protein synthesis via the activation of the nuclear VDR. Calcium binding proteins, such as calbindin D-9K, are examples of newly synthesized myoblast proteins expressed by activation of the nuclear VDR (Zanello et al., 1995).

Prolonged treatment with $1,25(\text{OH})_2\text{D}$ has been shown to affect the synthesis of certain muscle cytoskeletal proteins which are important in controlling muscle cell surface properties (Boland et al., 1995). One of these $1,25(\text{OH})_2\text{D}$ -dependent proteins has been described as a calmodulin-binding component of the myoblast cytoskeleton (Brunner and de Boland, 1990). Calmodulin is a calcium-binding protein that regulates several cellular processes including muscle contraction. Experiments in mitotic myoblasts treated with $1,25(\text{OH})_2\text{D}$ revealed increased synthesis of calmodulin (Drittanti et al., 1990).

$1,25(\text{OH})_2\text{D}$ appears to play a role in the regulation of phosphate metabolism in myoblasts (Boland et al., 1995). In skeletal muscle cells as in other cell types, phosphate in the form of ATP or inorganic phosphate, is necessary for structural and metabolic needs of the cell. Exposure to $1,25(\text{OH})_2\text{D}$ stimulates accelerated phosphate uptake and accumulation in the cells. This effect is thought to be mediated through the nuclear VDR resulting in de novo protein synthesis (Boland et al., 1995).

Finally, via its genomic pathway, $1,25(\text{OH})_2\text{D}$ appears to have a role in the regulation of muscle cell proliferation and differentiation. Studies in cultured chick embryo myoblasts demonstrated that up to 40 h treatment of $1,25(\text{OH})_2\text{D}$ at physiological levels increased both cell density and fusion (Giuliani and Boland, 1984). $1,25(\text{OH})_2\text{D}$ was found to exert a biphasic effect on DNA synthesis (Drittanti et al., 1989). Specifically, the hormone had a mitogenic effect in proliferating myoblasts followed by an inhibitory effect during the subsequent differentiation phase (Drittanti et al., 1989). Additionally, expression of cell cycle genes, such as *c-myc* and *c-fos*, and other skeletal muscle cell proteins were altered during $1,25(\text{OH})_2\text{D}$'s stimulatory and inhibitory effects on proliferation (Drittanti et al., 1989).

4.3. Non-genomic effects of $1,25(\text{OH})_2\text{D}$ in muscle

$1,25(\text{OH})_2\text{D}$ elicits rapid non-transcriptional responses that cannot be explained by a slow genetic pathway. There is extensive evidence supporting the presence of a cell surface receptor which mediates $1,25(\text{OH})_2\text{D}$'s rapid effects. The nature of the cell surface receptor remains somewhat controversial. Thus far, it has been proposed that the initiation of the fast $1,25(\text{OH})_2\text{D}$ signal may involve binding to a novel membrane receptor (Nemere et al., 1994) and/or the VDR itself which is translocated from the nucleus to the cell surface (Capiati et al., 2002). On binding to the membrane receptor, $1,25(\text{OH})_2\text{D}$ activates several interacting second-messenger pathways resulting in cellular effects within seconds to minutes.

$1,25(\text{OH})_2\text{D}$'s role in modulating muscle contractility also appears to involve a non-genomic mechanism. In vitro studies in vitamin D deficient chicks showed that $1,25(\text{OH})_2\text{D}$ added to skeletal muscle cells had a rapid (1–15 min) effect on calcium uptake (de Boland and Boland, 1987; Selles and Boland, 1991). Inhibitors of RNA and protein synthesis did not inhibit these rapid effects suggesting no involvement of the nuclear VDR. However, calcium channel blockers did suppress these effects indicating that $1,25(\text{OH})_2\text{D}$ was acting at the membrane level affecting calcium entry into the cell. Additional experiments have suggested that this pathway involves G-protein-mediated activation of phospholipase C (Morelli et al., 1996), thereby producing diacylglycerol and inositol 1,4,5-triphosphate (IP_3), and adenylyl cyclase with the simultaneous acute increase in cyclic AMP levels (Vazquez et al., 1995), leading to the activation of protein kinase A and C (Vazquez and de Boland, 1996; Vazquez et al., 1997; Capiati et al., 2000), release of calcium from intracellular stores (Vazquez et al., 1997), and activation of voltage-gated and store-operated calcium channels (Vazquez and de Boland, 1993; Vazquez et al., 1998).

Recent data indicate that downstream responses to $1,25(\text{OH})_2\text{D}$ depend on fast activation of mitogen-activated protein kinase (MAPK) signaling pathways. These pathways transmit extracellular signals to their intracellular targets which ultimately result in initiation of myogenesis, cell proliferation, differentiation, or apoptosis (Wu et al., 2000). In mammalian cells, the MAPK family has four different subgroups: extracellular signal-regulated kinases (ERKs 1/2), c-Jun N-terminal kinases (JNK), ERK5, and p38 MAPK (Widmann et al., 1999). When activated, these MAPKs regulate cell processes through phosphorylation of other kinases, proteins, and transcription factors. The ERKs are key components of the signal transduction pathways in growth and differentiation responses (Cobb et al., 1991; Sugden and Clerk, 1997). In proliferating cultured myoblasts, $1,25(\text{OH})_2\text{D}$ rapidly (within 1 min) activates ERK-1/2, phospholipase C γ and the *c-myc* (Morelli et al., 2001). The ERK

pathway is activated by $1,25(\text{OH})_2\text{D}$ through phosphorylation by several kinases, such as c-Src, Raf-1, Ras, and MAPKK (Buitrago et al., 2001; Buitrago et al., 2003). Through these mechanisms, $1,25(\text{OH})_2\text{D}$ causes the translocation of ERK-1/2 from the cytoplasm to the nucleus in an active phosphorylated form and induces the synthesis of the growth-related protein, *c-myc*, and stimulation of muscle cell proliferation (Buitrago et al., 2001). The hormone also stimulates tyrosine phosphorylation and membrane translocation of phospholipase C γ in myoblasts (Buitrago et al., 2002). Although considerable progress has been made in characterizing the metabolic pathways involved in $1,25(\text{OH})_2\text{D}$'s action on skeletal muscle cells, more research is needed to clarify how $1,25(\text{OH})_2\text{D}$ is affecting these pathways and what the mechanisms are.

5. Studies in VDR knockout mouse model

The VDR knockout mouse model has provided strong evidence for a direct effect of vitamin D and its receptor on skeletal muscle tissue. VDR null mutant mice are characterized by alopecia, reductions in both body size and weight and impaired motor coordination (Burne et al., 2005). Another feature of the VDR knockout behavioral phenotype is poor swimming ability (as assessed by the forced swimming test) (Kalueff et al., 2004). Studies in VDR null mutant mice show that they grow normally until weaning and thereafter develop various metabolic abnormalities including hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, and bone deformity similar to the typical features of rickets (Song et al., 2003). Given the similar features to the human myopathy associated with profound vitamin D deficiency, the VDR knockout mouse model has contributed to clarifying whether the VDR, as opposed to the metabolic abnormalities, is primarily responsible for the symptoms and clinical findings of this condition. Independent of the systemic metabolic changes, VDR null mutant mice were found to have muscle fiber diameters that were approximately 20% smaller and more variable in size than those of the wild type mice at 3 weeks of age (prior to weaning) (Endo et al., 2003). By 8 weeks of age, these muscle fiber changes were more prominent in the VDR null mutant mice compared to the wild type suggesting either that these abnormalities progress over time or that as these mice age the metabolic alterations that occur contribute to the morphological changes (Endo et al., 2003). The muscle fiber abnormalities were noted diffusely without any preference for type I or II fibers, differing from the hypovitaminosis D myopathy histology. Interestingly, there was no evidence of degeneration or necrosis in the VDR null mice (Endo et al., 2003). Such morphological changes indicate that the VDR plays an important role in skeletal muscle fiber development and its maturation.

In addition, studies in VDR null mutant mice at 3 weeks of age demonstrate abnormally high expression of myogenic differentiation factors compared to wild type mice (Endo et al., 2003). Myf5, E2A, and myogenin – factors that are minimally expressed in the wild type – were found to have increased expression in the VDR null mutant mice. Embryonic and neonatal myosin heavy chain (MHC) isoforms were also noted to have increased expression, whereas the type II (adult fast twitch) MHC expression was similar to the wild type mice (Endo et al., 2003). The abnormal levels in these differentiation factors may in part explain some of the morphological abnormalities seen in the VDR null mutant mice. As the differentiation pathways are altered, so are muscle fiber development and maturation.

6. VDR polymorphisms and muscle strength

Several VDR polymorphisms, which are defined as subtle variations in DNA sequence of the VDR gene, exist that are associated with a range of biological characteristics including muscle strength. For example, one well-described polymorphism, *FokI*, is a polymorphism involving a T/C transition in exon 2 of the VDR gene (Hopkinson et al., 2008). Individuals with the C allele ("F") have a shorter 424-amino acid VDR than do those with the T ("f") allele, the former having been associated with enhanced VDR transactivation capacity as a transcription factor (Whitfield et al., 2001). This would suggest that greater VDR activity would result in improved muscle strength in light of the clinical data reporting a positive association between vitamin D status and muscle strength. On the contrary, the C allele has been associated with reduced fat-free mass and quadriceps strength in healthy elderly men (Roth et al., 2004) and elderly individuals with COPD (Hopkinson et al., 2008).

BsmI, a restriction fragment length polymorphism at the 3' end of the VDR gene, has also been associated with skeletal muscle function. The 3' end is known to play an important role in regulating gene expression. Young healthy women with the bb allele, which may be associated with higher VDR activity in combination with the C allele of *FokI*, were found to have lower fat-free mass and hamstring (but not quadriceps) strength compared to those with the BB allele (Grundberg et al., 2004). In non-obese older women aged 70 and older, those with the bb genotype were found to have a 7% higher grip strength and a 23% higher quadriceps strength than those with BB genotype (Geusens et al., 1997). Once again, it remains somewhat unclear as to why the allele associated with higher VDR activity would be found to have reduced muscle strength.

7. PTH effects on muscle

Clinically, patients with PTH excess (as in hyperparathyroidism) share similar symptoms of muscle weakness and fatigue (Kristoffersson et al., 1992) and muscle biopsies demonstrate atrophy of type II muscle fibers as in vitamin D deficiency (Patten et al., 1974). Furthermore, PTH has been shown to predict falls (Stein et al., 1999) and muscle strength independent of $25(\text{OH})\text{D}$, age, and BMI (Dhesi et al., 2002). The question of whether vitamin D deficiency itself or secondary hyperparathyroidism is the primary cause of muscle tissue and functional abnormalities still has not been fully answered. Low vitamin

D levels stimulate PTH production and PTH may have direct effects on skeletal muscle. Studies in rats have demonstrated that PTH induces muscle catabolism (Garber, 1983), reductions in calcium transport (calcium-ATPase activity) and impairment of energy availability (reduction in intracellular phosphate and mitochondrial oxygen consumption) and metabolism (reduction in creatinine phosphokinases and oxidation of long-chain fatty acids) in skeletal muscle (Smogorzewski et al., 1988).

8. Conclusion

The link between vitamin D and skeletal muscle health has been well-described in clinical studies. There is a broad range of muscle dysfunction associated with varying degrees of vitamin D insufficiency, and supplementation with various forms of vitamin D has mostly shown beneficial effects. The identification of the VDR in skeletal muscle tissue provides solid evidence for a direct role of the vitamin. Recent research studies in the last two decades have begun to identify genomic effects of $1,25(\text{OH})_2\text{D}$ leading to the synthesis of new proteins that affect muscle cell contractility, proliferation, and differentiation. In addition, scientists are gradually constructing non-genomic pathways of $1,25(\text{OH})_2\text{D}$ activity in muscle cells that also impact on muscle contraction and possibly muscle cell development. The VDR knockout mouse model has provided insights into the $1,25(\text{OH})_2\text{D}$ -VDR specific actions and will continue to help improve our understanding of the underlying mechanisms. Other incoming research, such as the studies in VDR polymorphisms and PTH effects on muscle, are somewhat inconsistent with the clinical hypothesis that vitamin D, VDR, and optimal muscle health are directly linked; however, they are raising important questions that need to be investigated.

Further research is needed to clarify the underlying mechanisms of $1,25(\text{OH})_2\text{D}$ on skeletal muscle. From a clinical perspective, it would be important to know whether the beneficial effects of vitamin D treatment occur via direct or indirect VDR actions on skeletal muscle cells. This information will be clinically relevant for the musculoskeletal health of individuals at high risk of acquiring vitamin D deficiency, such as the elderly who are known to have age-related loss of muscle mass and strength and an increased rate of falls.

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The author has nothing to disclose.

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