Calcif Tissue Int
DOI 10.1007/s00223-012-9645-y

ORIGINAL RESEARCH

Vitamin D and Its Role in Skeletal Muscle

Lisa Ceglia · Susan S. Harris

Received: 31 January 2012 / Accepted: 20 August 2012

Abstract This review discusses the clinical and laboratory studies that have examined a role of vitamin D in skeletal muscle. Many observational studies, mainly in older populations, indicate that vitamin D status is positively associated with muscle strength and physical performance and inversely associated with risk of falling. Clinical trials of vitamin D supplementation in older adults with low vitamin D status mostly report improvements in muscle performance and reductions in falls. The underlying mechanisms are probably both indirect via calcium and phosphate and direct via activation of the vitamin D receptor (VDR) on muscle cells by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. VDR activation at the genomic level regulates transcription of genes involved in calcium handling and muscle cell differentiation and proliferation. A putative membrane-associated VDR activates intracellular signaling pathways also involved in calcium handling and signaling and myogenesis. Additional evidence comes from VDR knockout mouse models with abnormal muscle morphology and physical function, and VDR polymorphisms which are associated with differences in muscle strength. Recent identification of *CYP27B1* bioactivity in skeletal muscle cells and in regenerating adult mouse muscle lends support to the direct action of both 25-hydroxyvitamin D and 1,25$(OH)_2$D in muscle. Despite these research advances, many questions remain. Further research is needed to fully characterize molecular mechanisms of vitamin D action on muscle cells downstream of the VDR, describe the effects on muscle morphology and contractility, and determine whether these molecular and cellular effects translate into clinical improvements in physical function.

Keywords Skeletal muscle · Vitamin D · Vitamin D receptor

Vitamin D is most classically known for its role in the regulation of calcium and phosphate homeostasis [1]. Its biologically active metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], stimulates calcium and phosphate absorption from the intestine, reabsorption from the kidney tubules, and calcium and phosphate mobilization from the skeleton [1]. Yet, over the last few decades, there is growing evidence that vitamin D regulates many other cell functions. Its receptor (VDR) has been identified in a large number of human tissues [2], indicating the potential for widespread effects. Furthermore, 1,25$(OH)_2$D-VDR binding influences the expression of genes involved in cell development, differentiation, and growth [3, 4].

The potential effect of vitamin D on skeletal muscle structure and function is receiving a great deal of attention on the basis of both clinical and basic science research. Early clinical case reports of a reversible myopathy associated with profound vitamin D deficiency and/or chronic renal failure identified an association between vitamin D and muscle [5]. There are reports of reduced muscle mass, strength, and performance and an increased risk of falls in

The authors have stated that they have no conflict of interest.

L. Ceglia (✉)
Division of Endocrinology, Diabetes, and Metabolism, Tufts Medical Center, 800 Washington Street, Box 268, Boston, MA 02111, USA
e-mail: lisa.ceglia@tufts.edu

L. Ceglia · S. S. Harris
Bone Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA

Published online: 12 September 2012

older individuals with low serum 25-hydroxyvitamin D [25(OH)D] levels [6, 7]. Although indirect mechanisms have been described [8], the localization of the VDR in skeletal muscle cells [9, 10] has provided evidence for a direct mechanism by which vitamin D acts in skeletal muscle. Despite these recent investigations in cell culture and animals, much remains to be characterized. This review investigates the role of vitamin D in muscle physiology, muscle strength, and physical performance and considers the molecular mechanisms for its actions in muscle tissue.

Vitamin D—Deficient Myopathy

Clinical Presentation

Muscle weakness or myopathy is a prominent feature described in states of severe vitamin D inadequacy. Case reports describe a link between hypovitaminosis D–induced osteomalacia and a proximal myopathy that could be reversed with vitamin D supplementation [11–14]. Some of these clinical descriptions reported that the myopathy could be independent of metabolic abnormalities such as hypocalcemia [13] and hypophosphatemia [15]. Others, however, reported a correlation between hypovitaminosis D–induced metabolic abnormalities and muscle myopathy [16].

Clinical characteristics of the muscle syndrome include muscle weakness and hypotonia in infants [13] and a proximal myopathy with diffuse skeletal or muscle pain in adults [17, 18]. Case reports describe difficulty in climbing stairs, rising from a sitting or squatting position, and lifting objects. Other features include a waddling gait and uniform generalized muscle atrophy with preservation of sensation or deep tendon reflexes. Electromyographic evaluation can reveal abnormalities, such as polyphasic motor unit potentials with shortened duration and decreased amplitude, consistent with a myopathy [19]. One uncontrolled study found that patients with hypovitaminosis D-induced osteomalacia had improvement in their electromyogram but not nerve conduction velocity during parenteral vitamin D treatment for several months, suggesting that vitamin D plays an etiological role [19].

Muscle Histological Characteristics

Histological analysis of muscle biopsy specimens in adults with profound vitamin D deficiency and proximal myopathy report a predominance of type II (fast twitch) muscle fiber atrophy [20, 21]. Type II fibers are efficient for short bursts of speed and power, are quicker to fatigue, and are also the first to be recruited to prevent a fall [22]. Muscle cross-sections of vitamin D–deficient individuals have also indicated enlarged interfibrillar spaces and infiltration of fat, fibrosis, and glycogen granules [19, 20]. A recent cross-sectional study in 90 young women reported a greater degree of fat infiltration in muscle by computed tomography scan in those with lower serum 25(OH)D levels [23]. Similar morphological features have been noted in the vitamin D–deficient myopathy associated with chronic renal failure [24, 25]. There are no human prospective studies on the effect of vitamin D depletion on skeletal muscle morphology. In a female ovariectomized Sprague Dawley rat model, the depletion of vitamin D stores over a 4-week period failed to show atrophy of muscle fibers in the hind limb compared to control [26]. Interestingly, this study found that significant alterations in balance on the basis of beam-walk test in animals with profound vitamin D deficiency, indicating effects on neuromuscular function independent of changes in muscle mass in this 4-week study.

It has been hypothesized, however, that individuals with low vitamin D status who are placed on supplementation with vitamin D improve muscle strength and performance in part by an increase in muscle mass. To date, there have been two reports examining whether supplementation with a form of vitamin D affects muscle fiber composition and size. These studies appear to support a selective effect on type II muscle fibers. More than three decades ago, a small uncontrolled study obtained biopsy muscle samples from 11 older women after treatment with 1–2 μg of 1-α-hydroxyvitamin D (vitamin D analog) and 1 g of calcium daily for 3–6 months [27]. These older women were noted to have an increase in the relative number and in the cross-sectional area of type IIa muscle fibers (a subtype of type II fibers). About 25 years later, a randomized controlled study in 96 older Japanese female stroke survivors found that treatment with 1000 IU of vitamin D_2 daily increased mean type II muscle fiber diameter by over 96 % in the non-paralyzed limb compared to placebo over a 2-year period [28]. There was also a positive correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after 2 years of follow-up. These surprisingly exuberant findings have not been replicated. Further research to investigate the effect of vitamin D supplementation on muscle fiber area and muscle mass are important to verify these earlier studies.

Vitamin D and Muscle Performance

Observational Studies

Several observational studies in community-dwelling older adults show a positive association between vitamin D

status and measures of physical performance [29–32]. A cross-sectional analysis of men and women age 60 and over who participated in the National Health and Nutrition Examination Survey (NHANES) III survey found that those individuals with higher serum 25(OH)D levels up to 94 nmol/L had faster gait speed and sit-to-stand speed than subjects with lower levels [29], particularly in the subset with 25(OH)D levels under 60 nmol/L. An analysis of the Longitudinal Study of Aging Amsterdam (LASA) indicated that lower serum 25(OH)D levels predicted decreased grip strength and appendicular muscle mass in older men and women over the subsequent 3 years [6]. The LASA study also found that there was an increased risk of a decline in physical performance over 3 years in those with serum 25(OH)D below 50 nmol/L as compared to those with levels of 75 nmol/L or greater [30]. A more recent longitudinal study, the Cardiovascular Health Study All Stars, corroborated these findings [32]. The study in 77–100-year-old adults reported that those with baseline 25(OH)D levels below 50 nmol/L had a greater risk of developing mobility disability over 3 years of follow-up than those with levels 75 nmol/L or greater [32]. Notably, observational studies of adults with higher baseline 25(OH)D levels report no significant association with muscle performance [33, 34]. Thus, a serum 25(OH)D concentration of 60 nmol/L has been proposed by some as the threshold level for improvement in physical performance [31].

The association between vitamin D status and muscle strength and performance may not be unique to older individuals. A positive relationship between 25(OH)D levels and muscle power, force, velocity, and jump height was noted in 99 postmenarchal girls aged 12–14 years with low 25(OH)D levels (mean 21.3 nmol/L) [35]. These analyses, however, were not adjusted for physical activity [35]. Serum 25(OH)D level was positively associated with handgrip strength after adjusting for physical activity in another study of 301 Chinese adolescent girls (mean age 15 years) with low serum 25(OH)D levels of 34 nmol/L [36]. Studies in a mixed-age population of younger and older women with higher baseline 25(OH)D levels $\sim$50 nmol/L [37] and in younger postmenopausal women (early 60s) with 25(OH)D levels <50 nmol/L did not find a correlation between vitamin D status and tests of muscle performance [38].

Randomized Controlled Studies

A large number of randomized clinical trials have examined the effect of vitamin D supplementation on upper and lower extremity muscle strength, balance, and physical function. Several intervention trials in older individuals with low baseline vitamin D status have reported that vitamin D supplementation improved measures of muscle strength, balance, and/or physical function [28, 32, 39–42]. The positive studies found that treated individuals achieved mean serum 25(OH)D levels of at least 66 nmol/L. However, not all studies have shown positive results [43–46]. In a healthy and highly fit sample of older adults with 25(OH)D levels below 50 nmol/L, vitamin D_3 8400 IU weekly, compared to placebo, did not show improvement in body sway or the short physical performance battery over 4 months [46]. One explanation for this null effect was that the study subjects had little room for improvement in physical function. A large multicenter study in New Zealand reported that a single oral dose of vitamin D_3 300,000 IU, compared with placebo, did not alter physical performance measures in adults age 65 and older over 6 months [44]. Yet, this study included older individuals in unstable health after a recent hospitalization for hip fracture. In addition, it did not report 25(OH)D levels achieved at 6 months, which would raise the question of whether levels were trending back down by the 6-month mark in light of the single dose given.

A meta-analysis of 17 randomized, controlled trials involving over 5000 individuals over the age of 18 years found that only in a subgroup analysis of studies in individuals with starting serum 25(OH)D levels of $\leq$25 nmol/L was vitamin D found to benefit lower and upper extremity muscle strength [47]. Muir and Montero-Odasso performed a more recent meta-analysis in adults age 60 and over [48]. This analysis considered vitamin D dose administered and type of muscle performance evaluated (i.e., balance, Timed Up & Go testing, and muscle strength); thus, each meta-analysis had just three studies in each category [48]. The analysis revealed that tests of balance and muscle strength improved with daily supplemental vitamin D doses of 800 to 1000 IU daily although the magnitude of the effect appeared to be small (standardized mean differences of approximately 0.20). No significant effect of vitamin D on gait speed was noted [48].

Vitamin D and Falls

Observational Studies

Muscle strength and physical performance are linked with risk of falls in older individuals. In view of substantial data demonstrating a positive association between serum 25(OH)D concentrations and physical performance in older vitamin D–deficient adults, a similar relationship between vitamin D status and fall risk would be expected in this population. In the LASA study, 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 [7]. A similar finding was shown in a large cohort study of older community-dwelling women where higher 25(OH)D levels were associated with a lower rate of falls over a 4-year period [49]. Other observational data in older adults have demonstrated similar results [50–53].

Randomized Controlled Studies

A recent meta-analysis by the U.S. Preventive Services Task Force reviewed 9 randomized controlled trials in community-dwelling elders and found a 17 % risk reduction in falling in the vitamin D compared with placebo group [54]. Another recent meta-analysis of 26 randomized controlled trials in over 45,000 participants mostly older and female, reported a similar 14 % reduction in fall risk with vitamin D compared with placebo [55]. Yet, not all meta-analyses have shown a beneficial effect. A Cochrane review noted that overall vitamin D did not reduce falls in 111 trials of over 55,000 participants; however, there was a probable beneficial effect in those with low vitamin D status [56]. On the other hand, the recent Institute of Medicine report reported no benefit on falls [57]. Another recent meta-analysis brought up concerns in variability in vitamin D dosing regimens in different trials as one key factor influencing falls risk. In this analysis, doses of vitamin D that ranged from 700 to 1000 IU/day reduced risk of falling by about 19 %, whereas lower doses showed no risk reduction [58].

The minimum level of 25(OH)D required for maximal fall-risk reduction remains uncertain. Concerning studies at higher levels of 25(OH)D above the 50 to 60 nmol/L, there may not be additional benefit as suggested by the Cochrane review [56] and a recent study comparing the effect of vitamin D_3 800 IU/d vs. 2000 IU/d on fall risk in hip fracture patients [59]. In the latter study, individuals on vitamin D_3 800 IU/d achieved 25(OH)D levels of 90 nmol/L and those on 2000 IU/d achieved levels of 118 nmol/L and first falls were similar in the two groups [59].

Uncertainty over the optimal 25(OH)D level is further highlighted by a recent study testing the effect of an annual oral dose of 500,000 IU of vitamin D_3 vs. placebo on falls in older Australians [60]. This single high dose of vitamin D actually increased risk of falling in the first 3 months after its administration. Although the reasons for these findings were not clear, it emphasizes the need for more studies to define the optimal levels for muscle outcomes.

Proposed Mechanisms for Vitamin D Effects in Muscle

More than 3 decades ago, attempts were made to investigate the underlying pathophysiology of proximal myopathy in vitamin D-deficient experimental animals. Rodman and Baker [61] used an in situ rat soleus neuromuscular preparation to study changes in muscle contraction kinetics in response to profound vitamin D depletion. Muscle analyses revealed prolongation of the relaxation phase of contraction. This research suggested that vitamin D affects calcium handling in muscle sarcoplasmic reticulum. Another study of the muscles of rabbits fed a vitamin D-deficient diet showed that their sarcoplasmic reticulum had decreased ability to bind calcium compared to that from vitamin D-replete animals [62]. Matthews et al. [63] provided evidence that $1,25(OH)_2D$ potentiates the ability of uremic rabbit sarcoplasmic reticulum to bind and store calcium. These studies suggested that an effect of vitamin D on muscle is to increase calcium accumulation in the sarcoplasmic reticulum either by increasing the number of calcium-binding sites or altering the efficiency of these sites for calcium uptake.

Other studies have investigated vitamin D's impact on muscle protein metabolism. Birge and Haddad [64] found that isolated diaphragm muscles from vitamin D-deficient rats given vitamin D_3 and $25(OH)D_3$ had increases in ATP content and leucine incorporation—changes consistent with net increases in muscle protein synthesis.

These early studies provide important preliminary contributions to the growing body of evidence that vitamin D status affects skeletal muscle metabolism and function; however, the mechanism of these effects is not entirely elucidated. Studies have shown effects of hypocalcemia, hypophosphatemia, and hyperparathyroidism on skeletal muscle strength and function [8, 12, 16]. Yet, there is also strong evidence for a direct effect on muscle with the localization of the VDR in skeletal muscle cells in vitro and in vivo [9, 10, 65–67].

Indirect Effects

A study in rachitic rats with vitamin D deficiency and phosphate deficiency found muscle weakness reversible when vitamin D treatment significantly raised serum phosphorus levels to the normal range [8]. However, rachitic rats with vitamin D deficiency but on a phosphorus-containing diet did not have muscle weakness suggesting that the muscle impairment was being mediated by phosphate imbalance rather than vitamin D status. Interestingly, rats with both calcium deficiency and vitamin D deficiency demonstrated no measureable change in muscle strength.

Direct Effect Via the VDR

$1,25(OH)_2D$ exerts its principal actions by binding to the VDR, which has been identified in many human tissues [2].

As described with other steroid receptors, VDR acts as a nuclear receptor and appears to also have a nonnuclear receptor mediating nongenomic actions [68]. The nuclear VDR is a ligand-dependent transcription factor, which belongs to the steroid–thyroid hormone receptor gene superfamily [69–72]. Once transported to the nucleus by an intracellular binding protein, $1,25(OH)_2D$ binds to its nuclear receptor which results in gene transcription and subsequent de novo protein synthesis [73]. At the nuclear level, the activation of VDR induces the heterodimerization between the active VDR and the retinoic receptor (RXR). The formation of this heterodimer facilitates the interaction between the receptor's zinc finger region with DNA activating the transcription process [74].

Rapid, nongenomic actions of $1,25(OH)_2D$ have been reported in several target tissues through a nonnuclear VDR. It has been proposed that $1,25(OH)_2D$ binds to the nonnuclear receptor and initiates the formation of a second-messenger or phosphorylation of intracellular proteins resulting in cellular effects within seconds to minutes. The characterization and mechanism of action of this putative nonnuclear receptor have not been definitively established. Some have proposed that the initiation of the fast $1,25(OH)_2D$ signal may involve binding to a novel membrane receptor [75], while others have suggested a membrane-associated calcium-binding protein that functions as a calcium-specific ion channel [76]. However, more recent studies have suggested that the nonnuclear receptor is the intranuclear VDR itself, which translocates from the nucleus to the plasma membrane [77]. More recent data from the same laboratory suggest that caveolae microdomains are involved [78]. Caveolae are cholesterol- and sphingolipid-rich microdomains or lipid rafts of the plasma membrane that concentrate components of certain signal transduction pathways. Caveolae microdomains are rich in its principle structural protein, caveolin, that functions both in protein trafficking and signal transduction, as well as in cholesterol homeostasis [79, 80].

More than 2 decades ago, VDR was identified in two established rodent skeletal myoblast cell lines and excised muscle cells rinsed of serum contamination [9]. Shortly thereafter, other investigators demonstrated evidence of the VDR in chick monolayers of myoblasts [81], and in cloned human skeletal muscle cells [65]. In 2001, by means of 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 [10]. The data demonstrated intranuclear staining for the VDR using VDR antibody 97A (Affinity BioReagents). A recent study has questioned the selectivity of this VDR antibody because it was reacting with proteins on Western blot not related to the VDR, even in a VDR knockout mouse model [82]. A monoclonal VDR antibody D-6 (Santa Cruz Biotechnology), which is reported to have the best specificity for VDR protein, was not detected in muscle of VDR knockout mice and C57BL/6 mice by immunohistochemistry and Western blot [82], thus calling into question the presence of the VDR in muscle. Yet, there have been two additional recent studies providing support for the presence of VDR in skeletal myocytes. First, a study in older women detected VDR by Western blot using multiple commercial antibodies to the VDR including the D-6 antibody in vastus lateralis muscle biopsies [83]. Most recently, a study by Srikuea et al. [67] combined the use of Western blot, immunocytochemistry, PCR cloning, and DNA sequencing to validate expression of the VDR in the C2C12 mouse cell line. Of note, this study found VDR protein primarily expressed in the nucleus of C2C12 myoblasts, and in the cytoplasm of C2C12 myotubes. In summary, this last study, which used multiple techniques, provides strong evidence for the presence of VDR in skeletal muscle cells.

VDR Knockout Mouse Model

The VDR knockout mouse model, in which animals are placed on normal or rescue diets, provides evidence that vitamin D has a direct effect on skeletal muscle mass, morphology and performance via its receptor [84]. The VDR null mutant mice are characterized by alopecia, reductions in both body size and weight and impaired motor coordination [85]. Studies in VDR null mutant mice show that these animals can grow normally until weaning and thereafter develop various metabolic abnormalities including hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, and bone deformities similar to those features typical of rickets [86]. Independent of the systemic metabolic changes, VDR null mutant mice have muscle fiber diameters that are approximately 20 % smaller and more variable in size than those of the wild type mice at 3 weeks of age (before weaning) [84]. By 8 weeks of age, these muscle fiber changes are more prominent in the VDR null mutant mice on rescue diet, as 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 [84]. The muscle fiber abnormalities are noted diffusely without any preference for type I or II fibers, differing from the human hypovitaminosis D myopathy that is characterized by type II muscle fiber atrophy on histological sections. Interestingly, there is no evidence of degeneration or necrosis in the VDR null mice [84]. Such morphological changes indicate that the VDR plays an important role in skeletal muscle fiber development and its maturation.

Studies in VDR null mutant mice at 3 weeks of age have also demonstrated abnormally high expression of myogenic differentiation factors compared to wild type mice [84].

Thus, Myf5, E2A, and myogenin, factors minimally expressed in wild type mice, were found to be increased 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 [84]. The abnormal levels in these differentiation factors may explain, in part, 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.

An additional feature of the VDR knockout behavioral phenotype is poor swimming ability (as assessed by the forced swimming test) [87]. This is a well-known method to assess motor and balance functions in rodents. This finding has been attributed to muscular or motor impairment in the mouse; however, a recent study [88] considers whether impaired vestibular function in these VDR null mutant mice may be an important contributor to this finding. Via immunohistochemical analysis, Minasyan et al. [88] localized VDR-positive nuclei in epithelium of different structures in the vestibular system in wild-type mice and a significantly reduced expression of VDR in these same structures in the VDR null mutant animals. To further support the presence of a vestibular impairment in the VDR knockout mouse, measurement of postural control on balance tests, such as the accelerating rotarod and tilting platform, revealed significantly greater abnormal results in the VDR knockout than wild type mouse.

$1,25(OH)_2D$ and Genomic Effects

$1,25(OH)_2D$ via intranuclear VDR activates gene transcription which results in the synthesis of specific proteins believed to influence muscle calcium handling, phosphate transport across the cell membrane, and muscle cell differentiation and proliferation. In vitro and in vivo experiments in chick skeletal muscle have shown that $1,25(OH)_2D$ regulates muscle calcium uptake by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma. Modifications in intracellular calcium levels control contraction and relaxation of muscle [89, 90]. In vitro studies have suggested that $1,25(OH)_2D$ modulates intracellular calcium levels by stimulating the expression of a calcium-binding protein called calbindin-D9K. This finding was shown via different techniques including Western blot in chick embryo myoblasts [91, 92], Northern blot in chick muscle cells, and in combined reverse transcription and polymerase chain reaction in chicken muscle cells [93].

Prolonged treatment with $1,25(OH)_2D$ affects the synthesis of certain muscle cytoskeletal proteins which are important in controlling muscle cell surface properties [89]. One of these $1,25(OH)_2D$-dependent proteins has been described as a calmodulin-binding component of the myoblast cytoskeleton [94]. Calmodulin is a calcium-binding protein that regulates several cellular processes including muscle contraction. Experiments in mitotic myoblasts treated with $1,25(OH)_2D$ revealed increased synthesis of calmodulin [95].

$1,25(OH)_2D$ appears to play a role in the regulation of phosphate metabolism in myoblasts [89]. 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. The clinical manifestations of hypophosphatemia can include a proximal myopathy. Exposure to $1,25(OH)_2D$ stimulates accelerated phosphate uptake and accumulation in cells. As mentioned earlier, studies by Birge and Haddad [64] were the first to show that $25(OH)D_3$ increased the rate of accumulation of inorganic phosphate by diaphragmatic muscle tissue of vitamin D–deficient rats. In cultured chick embryonic muscle cells, preincubation with physiological levels of $1,25(OH)_2D_3$ resulted in a significant accumulation of phosphate by the cells [96]. These effects may occur through $1,25(OH)_2D$'s reported effects on the expression of fibroblast growth factor-23 (FGF-23), a key hormone regulating phosphate homeostasis and effects on phosphate transporters. These $1,25(OH)_2D$ effects in muscle are hypothesized to be mediated through the nuclear VDR given abnormalities in FGF-23 and phosphate transporters in VDR null mice [97].

Finally, via its genomic pathway, $1,25(OH)_2D$ 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 of treatment with $1,25(OH)_2D$ at physiological levels increased both cell density and fusion [98]. $1,25(OH)_2D$ was found to exert a biphasic effect on DNA synthesis [99]. Specifically, the hormone had a mitogenic effect in proliferating myoblasts followed by an inhibitory effect during the subsequent differentiation phase. Additionally, expression of cell cycle genes, such as *c-myc* and *c-fos*, and other skeletal muscle cell proteins were altered during $1,25(OH)_2D$'s stimulatory and inhibitory effects on proliferation. Overall, the regulation of gene products via the genomic pathway occurs over hours to days.

However, more recent studies in C2C12 skeletal muscle cells have demonstrated that $1,25(OH)_2D$ actually inhibits proliferation and modulates cell differentiation [67, 100]. The study by Garcia et al. [100] found that administration of $1,25(OH)_2D$ to C2C12 myoblasts up-regulated gene pathways responsible for myogenic differentiation including insulin growth factor 2 (IGF-2) expression. It down-regulated IGF-1 expression. Other myogenic differentiation genes affected by $1,25(OH)_2D$ included

down-regulated myostatin expression (a negative regulator of muscle mass), and up-regulated follistatin expression (an inhibitor of myostatin). Furthermore, $1,25(OH)_2D$ stimulated expression myogenic factors such as MyoD, myogenin, and desmin, in the C2C12 myoblasts at different stages of differentiation resulting in significantly increased mean diameter and size of the muscle fibers [100]. Overall, these findings indicated an up-regulation of myogenesis in C2C12 muscle cells exposed to $1,25(OH)_2D$.

$1,25(OH)_2D$ and Nongenomic Effects

Activation of rapid, nontranscriptional signal transduction pathways have been reported with administration of $1,25(OH)_2D$ [101]. These actions are probably occurring via nongenomic mechanisms as the timing of these events is within seconds to minutes and thus via a nonnuclear or membrane-associated VDR. Although its characterization remains incomplete, the nonnuclear VDR is thought to be associated with caveolae microdomains [77, 78]. Caveolin-associated proteins include the Src-tyrosine kinases which are stimulated by IGF-1 and result in activation of mitogen-activated protein kinase (MAPK) and phosphoinositol-3 kinase (PI3K)/Akt cascades [102]—pathways that regulate cell differentiation and growth. Caveolin holds these signal transducers in the sensitive or inactive conformation until activation by an appropriate stimulus, such as $1,25(OH)_2D$. Experiments in cell culture suggest that the nonnuclear VDR interacts with caveolins by affecting caveolin-associated signal transduction pathways. A study that used murine cell lines of myoblasts and myotubes found that administration of $1,25(OH)_2D$ rapidly (within 1 min) activates Src and the MAPK cascade [78]. Another study by the same authors found that $1,25(OH)_2D$ activated the PI3K/Akt pathway over a 60-min period and that these effects were mediated by caveolae [103]. Although the latter study did not address whether these effects occurred via a nonnuclear VDR, a study in osteoblasts found that PI3K/Akt signaling was indeed VDR-dependent [104].

Nongenomic mechanisms also mediate the actions of $1,25(OH)_2D$ on calcium influx and muscle contractility. In vitro studies in vitamin D-deficient chicks and rat soleus muscle showed that administration of $1,25(OH)_2D$ had a rapid (1–15 min) effect on calcium uptake [105, 106]. 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 $1,25(OH)_2D$ was acting at the membrane level affecting calcium entry into the cell. These nongenomic responses consist of G-protein-mediated activation of both phospholipase C (PLC) [107] and adenylyl cyclase, which generates diacylglycerol (DAG) and inositol 1,4,5-trisphosphatase (IP_3) and adenylyl cyclase with the simultaneous acute increase in cyclic AMP levels [108], leading to the activation of protein kinase A and C [109–111], release of calcium from intracellular stores [112], and activation of voltage-gated and store-operated calcium channels [113, 114].

VDR Polymorphisms

VDR polymorphisms, which are defined as subtle variations in DNA sequence of the VDR gene, have been associated with a wide range of biological characteristics including muscle strength and falls. One well-described single nucleotide polymorphism, *Fok*I, is a polymorphism involving the translation start site resulting in a VDR protein shortened by three amino acids and not linked to any of the other VDR polymorphisms [115, 116]. 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 [117]. In light of the clinical data reporting a positive association between vitamin D status and muscle strength, this would suggest that greater VDR activity could result in improved muscle strength. On the contrary, the C allele has been associated with reduced fat-free mass and quadriceps strength in healthy elderly men [118], healthy middle-aged women [119], and older individuals with chronic obstructive pulmonary disease [120].

There are several studies examining potential associations between restriction fragment length polymorphisms in VDR gene and muscle mass and strength. *Bsm*I is a restriction fragment length polymorphism in intron 8 at the 3′ end of the VDR gene, where B allele indicates absence and the b allele the presence of the restriction site. In nonobese 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 [121]. *Taq*I is another restriction fragment length polymorphism at the 3′ end of the VDR gene in a silent site in exon 9. In men, Bt/Bt homozygotes for the *Bsm*I–*Taq*I haplotype outperformed bT carriers in both isometric and concentric quadriceps torques at different contraction velocities [119].

There are two recent studies examining a relationship between VDR polymorphisms and the risk of falls in older individuals. A study in a small group ($n = 259$) of very elderly (over 80 years) women [122] found an association between *Bsm1* and falls within 90 days of assessment, with a reduction in falls for those carrying the bb genotype, while no association was found for *Fok1*. Another recent study in two separate population cohorts (the Aberdeen Prospective Osteoporosis Screening Study and the Osteoporosis and Ultrasound study) also found an association

between falls and polymorphisms in *Bsm1*. In this analysis, postmenopausal women carrying the B allele were at higher risk of falls in both cohorts [123] compared to those not carrying the B allele. The study also showed that the association was independent of serum 25(OH)D levels.

However, across these and several other [119, 120, 124, 125] observational studies on specific VDR polymorphisms and skeletal muscle outcomes, the findings are not consistent. Explanations for at least some of this variability may be found in a better understanding of how these polymorphisms are linked to and interact with other genetic variations and environmental factors.

C27B1 in Skeletal Muscle

The kidney has been described as the predominant site expressing *CYP27B1*, the gene encoding the mitochondrial enzyme 1α-hydroxylase that hydroxylates 25(OH)D to $1,25(OH)_2D$ [126]. Previous studies have been unsuccessful at isolating this gene in skeletal muscle [127, 128]; yet, the study by Srikuea et al. [67] has demonstrated *CYP27B1* expression in C2C12 myoblasts and myotubes and in regenerating mouse skeletal muscle in vivo. In this study, administration of vitamin D_3 and $25(OH)D_3$ led to an up-regulation in VDR protein expression and decrease in proliferation of the myoblasts. A knockdown of the *CYP27B1* resulted in no response in VDR expression or proliferation in response to $25(OH)D_3$ administration, suggesting that the muscle-specific *CYP27B1* may mediate the effects of $25(OH)D_3$ in muscle. Yet, the possibility of a direct interaction between $25(OH)D_3$ and the VDR has been proposed in CYP27B1$^{-/-}$ cells [129].

Conclusion

An association between vitamin D status and skeletal muscle health has been described in case reports of a reversible proximal myopathy, in many large population-based studies, and in several well-designed randomized clinical trials. Much of the published data on this association are in older individuals, who often have low vitamin D status, are at risk for sarcopenia, and have an increased risk of falls and disability. Not all trials have shown significantly positive improvements in muscle performance or reductions in the risk of falls; however, in those individuals with low serum levels of 25(OH)D at baseline, supplementation with various forms of vitamin D has generally shown beneficial effects on muscle strength, tests of physical performance, and rate of falls. Thus, replenishing vitamin D stores in an aging population may be important for the preservation of physical function and the reduction in falls risk and its sequelae. Additional research is needed to determine optimal serum 25(OH)D concentration for muscle benefits.

Regarding research delving into the underlying actions of vitamin D in human skeletal muscle, some important advances have been made. A small body of evidence suggests that improvements in muscle strength and performance are mediated through an increase in muscle fibers (size and number). Underlying mechanisms by which vitamin D acts on skeletal muscle appear to be both indirect via alterations in calcium and phosphate balance and direct via the VDR. Several experimental studies in cell culture, animals, and humans in the last two decades have identified the VDR in skeletal muscle using different antibodies and techniques. Details on genomic effects of $1,25(OH)_2D$ via the nuclear VDR in muscle tissue need further elucidation, but thus far, they involve synthesis of proteins involved in muscle cell contractility, proliferation, and differentiation. Mainly in vitro experiments demonstrate nongenomic pathways of $1,25(OH)_2D$ activity in muscle cells via a nonnuclear VDR, and these rapid actions alter calcium handling and muscle cell development and growth.

However, there are still many gaps in knowledge that need further investigation. First, evidence is scarce on whether vitamin D supplementation affects muscle mass, muscle contraction, or both. Research is needed to fully characterize molecular mechanisms of vitamin D action on muscle cells downstream of the VDR. And finally, it is important to study whether the molecular and cellular effects seen at the level of the tissue translate into clinical improvements in muscle performance. Progress in these areas of investigation will provide important direction for future clinical research to improve functional outcomes in older adults.

Acknowledgments This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under agreement No. 58-1950-7-707. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

References

1. DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 80:1689S–1696S
2. Reichel H, Koeffler HP, Norman AW (1989) The role of the vitamin D endocrine system in health and disease. New Engl J Med 320:980–991
3. Walters MR (1992) Newly identified actions of the vitamin D endocrine system. Endocr Rev 13:719–764
4. Bikle DD (2010) Vitamin D: newly discovered actions require reconsideration of physiologic requirements. Trends Endocrinol Metab 21:375–384
5. Boland R (1986) Role of vitamin D in skeletal muscle function. Endocr Rev 7:434–448
6. Visser M, Deeg DJ, Lips P (2003) Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle

strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab 88:5766–5772
7. Snijder MB, van Schoor NM, Pluijm SM, van Dam RM, Visser M, Lips P (2006) Vitamin D status in relation to one-year risk of recurrent falling in older men and women. J Clin Endocrinol Metab 91:2980–2985
8. Schubert L, DeLuca HF (2010) Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch Biochem Biophys 500:157–161
9. Simpson RU, Thomas GA, Arnold AJ (1985) Identification of 1,25-dihydroxyvitamin D_3 receptors and activities in muscle. J Biol Chem 260:8882–8891
10. Bischoff HA, Borchers M, Gudat F, Duermueller U, Theiler R, Stahelin HB, Dick W (2001) In situ detection of 1,25-dihydroxyvitamin D_3 receptor in human skeletal muscle tissue. Histochem J 33:19–24
11. Dent CE, Richens A, Rowe DJ, Stamp TC (1970) Osteomalacia with long-term anticonvulsant therapy in epilepsy. Br Med J 4:69–72
12. Smith R, Stern G (1967) Myopathy, osteomalacia and hyperparathyroidism. Brain 90:593–602
13. Prineas JW, Mason AS, Henson RA (1965) Myopathy in metabolic bone disease. Br Med J 1:1034–1036
14. Marsden CD, Reynolds EH, Parsons V, Harris R, Duchen L (1973) Myopathy associated with anticonvulsant osteomalacia. Br Med J 4:526–527
15. Smith R, Stern G (1969) Muscular weakness in osteomalacia and hyperparathyroidism. J Neurol Sci 8:511–520
16. Wassner SJ, Li JB, Sperduto A, Norman ME (1983) Vitamin D deficiency, hypocalcemia, and increased skeletal muscle degradation in rats. J Clin Invest 72:102–112
17. Gloth FM 3rd, Tobin JD, Sherman SS, Hollis BW (1991) Is the recommended daily allowance for vitamin D too low for the homebound elderly? J Am Geriatr Soc 39:137–141
18. Schott GD, Wills MR (1976) Muscle weakness in osteomalacia. Lancet 1(7960):626–629
19. Skaria J, Katiyar BC, Srivastava TP, Dube B (1975) Myopathy and neuropathy associated with osteomalacia. Acta Neurol Scand 51:37–58
20. Yoshikawa S, Nakamura T, Tanabe H, Imamura T (1979) Osteomalacic myopathy. Endocrinol Jpn 26:65–72
21. Palmucci L, Bertolotto A, Doriguzzi C, Mongini T, Coda R (1982) Osteomalacic myopathy in a case of diffuse nodular lipomatosis of the small bowel. Acta Neurol Belgica 82:65–71
22. McComas A (1996) Skeletal muscle. Form and function. Human Kinetics Publishers, Champaign
23. Gilsanz V, Kremer A, Mo AO, Wren TA, Kremer R (2010) Vitamin D status and its relation to muscle mass and muscle fat in young women. J Clin Endocrinol Metab 95:1595–1601
24. Lazaro RP, Kirshner HS (1980) Proximal muscle weakness in uremia. Case reports and review of the literature. Arch Neurol 37:555–558
25. Floyd M, Ayyar DR, Barwick DD, Hudgson P, Weightman D (1974) Myopathy in chronic renal failure. Q J Med 43:509–524
26. Tague SE, Clarke GL, Winter MK, McCarson KE, Wright DE, Smith PG (2011) Vitamin D deficiency promotes skeletal muscle hypersensitivity and sensory hyperinnervation. J Neurosci 31:13728–13738
27. Sorensen OH, Lund B, Saltin B, Lund B, Andersen RB, Hjorth L, Melsen F, Mosekilde L (1979) Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. Clin Sci (Lond) 56:157–161
28. Sato Y, Iwamoto J, Kanoko T, Satoh K (2005) Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovasc Dis 20:187–192
29. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, Dawson-Hughes B (2004) Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or = 60 y. Am J Clin Nutr 80:752–758
30. Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P (2007) Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab 92:2058–2065
31. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P (2009) Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab 94:1244–1250
32. Houston DK, Tooze JA, Davis CC, Chaves PH, Hirsch CH, Robbins JA, Arnold AM, Newman AB, Kritchevsky SB (2011) Serum 25-hydroxyvitamin D and physical function in older adults: the Cardiovascular Health Study All Stars. J Am Geriatr Soc 59:1793–1801
33. Chan R, Chan D, Woo J, Ohlsson C, Mellstrom D, Kwok T, Leung PC (2012) Not all elderly people benefit from vitamin D supplementation with respect to physical function: results from the Osteoporotic Fractures in Men Study, Hong Kong. J Am Geriatr Soc 60:290–295
34. Ceglia L, Chiu GR, Harris SS, Araujo AB (2011) Serum 25-hydroxyvitamin D concentration and physical function in adult men. Clin Endocrinol (Oxf) 74:370–376
35. Ward KA, Das G, Berry JL, Roberts SA, Rawer R, Adams JE, Mughal Z (2009) Vitamin D status and muscle function in postmenarchal adolescent girls. J Clin Endocrinol Metab 94:559–563
36. Foo LH, Zhang Q, Zhu K, Ma G, Hu X, Greenfield H, Fraser DR (2009) Low vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. J Nutr 139:1002–1007
37. Allali F, El Aichaoui S, Khazani H, Benyahia B, Saoud B, El Kabbaj S, Bahiri R, Abouqal R, Hajjaj-Hassouni N (2009) High prevalence of hypovitaminosis D in Morocco: relationship to lifestyle, physical performance, bone markers, and bone mineral density. Semin Arthritis Rheum 38:444–451
38. Garnero P, Munoz F, Sornay-Rendu E, Delmas PD (2007) Associations of vitamin D status with bone mineral density, bone turnover, bone loss and fracture risk in healthy postmenopausal women. The OFELY study. Bone 40:716–722
39. Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H (2009) Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. Osteoporos Int 20:315–322
40. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C (2000) Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. J Bone Miner Res 15:1113–1118
41. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA, Conzelmann M (2003) Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. J Bone Miner Res 18:343–351
42. Dhesi JK, Jackson SH, Bearne LM, Moniz C, Hurley MV, Swift CG, Allain TJ (2004) Vitamin D supplementation improves neuromuscular function in older people who fall. Age Ageing 33:589–595
43. Kenny AM, Biskup B, Robbins B, Marcella G, Burleson JA (2003) Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men. J Am Geriatr Soc 51:1762–1767

44. Latham NK, Anderson CS, Lee A, Bennett DA, Moseley A, Cameron ID (2003) A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). J Am Geriatr Soc 51:291–299
45. Brunner RL, Cochrane B, Jackson RD, Larson J, Lewis C, Limacher M, Rosal M, Shumaker S, Wallace R (2008) Calcium, vitamin D supplementation, and physical function in the Women's Health Initiative. J Am Diet Assoc 108:1472–1479
46. Lips P, Binkley N, Pfeifer M, Recker R, Samanta S, Cohn DA, Chandler J, Rosenberg E, Papanicolaou DA (2010) Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. Am J Clin Nutr 91:985–991
47. Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL (2011) Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporos Int 22:859–871
48. Muir SW, Montero-Odasso M (2011) Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc 59:2291–2300
49. Faulkner KA, Cauley JA, Zmuda JM, Landsittel DP, Newman AB, Studenski SA, Redfern MS, Ensrud KE, Fink HA, Lane NE, Nevitt MC (2006) Higher 1,25-dihydroxyvitamin D_3 concentrations associated with lower fall rates in older community-dwelling women. Osteoporos Int 17:1318–1328
50. Suzuki T, Kwon J, Kim H, Shimada H, Yoshida Y, Iwasa H, Yoshida H (2008) Low serum 25-hydroxyvitamin D levels associated with falls among Japanese community-dwelling elderly. J Bone Miner Res 23:1309–1317
51. Flicker L, Mead K, MacInnis RJ, Nowson C, Scherer S, Stein MS, Thomasx J, Hopper JL, Wark JD (2003) Serum vitamin D and falls in older women in residential care in Australia. J Am Geriatr Soc 51:1533–1538
52. Stein MS, Wark JD, Scherer SC, Walton SL, Chick P, Di Carlantonio M, Zajac JD, Flicker L (1999) Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. J Am Geriatr Soc 47:1195–1201
53. Dhesi JK, Bearne LM, Moniz C, Hurley MV, Jackson SH, Swift CG, Allain TJ (2002) Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. J Bone Miner Res 17:891–897
54. Michael YL, Whitlock EP, Lin JS, Fu R, O'Connor EA, Gold R (2010) Primary care-relevant interventions to prevent falling in older adults: a systematic evidence review for the US Preventive Services Task Force. Ann Intern Med 153:815–825
55. Murad MH, Elamin KB, Abu Elnour NO, Elamin MB, Alkatib AA, Fatourechi MM, Almandoz JP, Mullan RJ, Lane MA, Liu H, Erwin PJ, Hensrud DD, Montori VM (2011) Clinical review. The effect of vitamin D on falls: a systematic review and meta-analysis. J Clin Endocrinol Metab 96:2997–3006
56. 56. Gillespie LD, Robertson MC, Gillespie WJ, Lamb SE, Gates S, Cumming RG, Rowe BH (2009) Interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev (2):CD007146
57. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 96:53–58
58. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J (2009) Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ (Clin Res Ed) 339:b3692
59. Bischoff-Ferrari HA, Dawson-Hughes B, Platz A, Orav EJ, Stahelin HB, Willett WC, Can U, Egli A, Mueller NJ, Looser S, Bretscher B, Minder E, Vergopoulos A, Theiler R (2010) Effect of high-dosage cholecalciferol and extended physiotherapy on complications after hip fracture: a randomized controlled trial. Arch Intern Med 170:813–820
60. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC (2010) Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 303:1815–1822
61. Rodman JS, Baker T (1978) Changes in the kinetics of muscle contraction in vitamin D–depleted rats. Kidney Int 13:189–193
62. Curry OB, Basten JF, Francis MJ, Smith R (1974) Calcium uptake by sarcoplasmic reticulum of muscle from vitamin D-deficient rabbits. Nature 249:83–84
63. Matthews C, Heimberg KW, Ritz E, Agostini B, Fritzsche J, Hasselbach W (1977) Effect of 1,25-dihydroxycholecalciferol on impaired calcium transport by the sarcoplasmic reticulum in experimental uremia. Kidney Int 11:227–235
64. Birge SJ, Haddad JG (1975) 25-Hydroxycholecalciferol stimulation of muscle metabolism. J Clin Invest 56:1100–1107
65. Costa EM, Blau HM, Feldman D (1986) 1,25-Dihydroxyvitamin D_3 receptors and hormonal responses in cloned human skeletal muscle cells. Endocrinology 119:2214–2220
66. Ceglia L, da Silva Morais M, Park LK, Morris E, Harris SS, Bischoff-Ferrari HA, Fielding RA, Dawson-Hughes B (2010) Multi-step immunofluorescent analysis of vitamin D receptor loci and myosin heavy chain isoforms in human skeletal muscle. J Mol Histol 41:137–142
67. Srikuea R, Zhang X, Park-Sarge OK, Esser KA (2012) VDR and CYP27B1 are expressed in c2c12 cells and regenerating skeletal muscle: potential role in suppression of myoblast proliferation. Am J Physiol Cell Physiol 303:C396–C405
68. Dusso AS, Brown AJ, Slatopolsky E (2005) Vitamin D. Am J Physiol Renal Physiol 289:F8–F28
69. Pike JW (1991) Vitamin D_3 receptors: structure and function in transcription. Annu Rev Nutr 11:189–216
70. Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW (1988) Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA 85:3294–3298
71. McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR, O'Malley BW (1987) Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science 235:1214–1217
72. DeLuca HF (1988) The vitamin D story: a collaborative effort of basic science and clinical medicine. FASEB J 2:224–236
73. Haussler MR, Mangelsdorf DJ, Komm BS, Terpening CM, Yamaoka K, Allegretto EA, Baker AR, Shine J, McDonnell DP, Hughes M et al (1988) Molecular biology of the vitamin D hormone. Recent Prog Horm Res 44:263–305
74. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM (1990) Nuclear receptor that identifies a novel retinoic acid response pathway. Nature 345:224–229
75. Nemere I, Farach-Carson MC (1998) Membrane receptors for steroid hormones: a case for specific cell surface binding sites for vitamin D metabolites and estrogens. Biochem Biophys Res Commun 248:443–449
76. Baran DT, Quail JM, Ray R, Leszyk J, Honeyman T (2000) Annexin II is the membrane receptor that mediates the rapid actions of 1alpha,25-dihydroxyvitamin D(3). J Cell Biochem 78:34–46
77. Capiati D, Benassati S, Boland RL (2002) 1,25(OH)2-Vitamin D_3 induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells. J Cell Biochem 86:128–135

78. Buitrago C, Boland R (2010) Caveolae and caveolin-1 are implicated in 1alpha,25(OH)(2)-vitamin D(3)-dependent modulation of Src, MAPK cascades and VDR localization in skeletal muscle cells. J Steroid Biochem Mol Biol 121:169–175
79. Simons K, Toomre D (2000) Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1:31–39
80. Galbiati F, Razani B, Lisanti MP (2001) Emerging themes in lipid rafts and caveolae. Cell 106:403–411
81. Boland R, Norman A, Ritz E, Hasselbach W (1985) Presence of a 1,25-dihydroxy-vitamin D_3 receptor in chick skeletal muscle myoblasts. Biochem Biophys Res Commun 128:305–311
82. Wang Y, DeLuca HF (2011) Is the vitamin D receptor found in muscle? Endocrinology 152:354–363
83. Ceglia LMM, Park L, Harris S, Bischoff-Ferrari H, Fielding RA, Dawson-Hughes B (2008) Immunofluorescent analysis of vitamin D receptor loci and myosin heavy chain isoforms in human skeletal muscle fibers. Osteoporos Int 19(suppl 2):S418
84. Endo I, Inoue D, Mitsui T, Umaki Y, Akaike M, Yoshizawa T, Kato S, Matsumoto T (2003) Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology 144:5138–5144
85. Burne TH, McGrath JJ, Eyles DW, Mackay-Sim A (2005) Behavioural characterization of vitamin D receptor knockout mice. Behav Brain Res 157:299–308
86. Song Y, Kato S, Fleet JC (2003) Vitamin D receptor (VDR) knockout mice reveal VDR-independent regulation of intestinal calcium absorption and $ECaC_2$ and calbindin D9k mRNA. J Nutr 133:374–380
87. Kalueff AV, Lou YR, Laaksi I, Tuohimaa P (2004) Impaired motor performance in mice lacking neurosteroid vitamin D receptors. Brain Res Bull 64:25–29
88. Minasyan A, Keisala T, Zou J, Zhang Y, Toppila E, Syvala H, Lou YR, Kalueff AV, Pyykko I, Tuohimaa P (2009) Vestibular dysfunction in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol 114:161–166
89. Boland R, de Boland AR, Marinissen MJ, Santillan G, Vazquez G, Zanello S (1995) Avian muscle cells as targets for the secosteroid hormone 1,25-dihydroxy-vitamin D_3. Mol Cell Endocrinol 114:1–8
90. Ebashi S, Endo M (1968) Calcium ion and muscle contraction. Prog Biophys Mol Biol 18:123–183
91. Lila D, Susana Z, Ricardo B (1994) Induction of a calbindin-D9K-like protein in avian muscle cells by 1,25-dihydroxy-vitamin D_3. Biochem Mol Biol Int 32:859–867
92. Drittanti LN, Boland RL, de Boland AR (1989) Induction of specific proteins in cultured skeletal muscle cells by 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1012:16–23
93. Zanello SB, Boland RL, Norman AW (1995) cDNA sequence identity of a vitamin D-dependent calcium-binding protein in the chick to calbindin D-9K. Endocrinology 136:2784–2787
94. Brunner A, de Boland AR (1990) 1,25-Dihydroxyvitamin D_3 affects the synthesis, phosphorylation and in vitro calmodulin binding of myoblast cytoskeletal proteins. Z Naturforsch C 45:1156–1160
95. Drittanti L, de Boland AR, Boland R (1990) Stimulation of calmodulin synthesis in proliferating myoblasts by 1,25-dihydroxy-vitamin D_3. Mol Cell Endocrinol 74:143–153
96. Bellido T, Boland R (1991) Effects of 1,25-dihydroxy-vitamin D_3 on phosphate accumulation by myoblasts. Horm Metab Res 23:113–116
97. Inoue Y, Segawa H, Kaneko I, Yamanaka S, Kusano K, Kawakami E, Furutani J, Ito M, Kuwahata M, Saito H, Fukushima N, Kato S, Kanayama HO, Miyamoto K (2005) Role of the vitamin D receptor in FGF23 action on phosphate metabolism. Biochem J 390:325–331
98. Giuliani DL, Boland RL (1984) Effects of vitamin D_3 metabolites on calcium fluxes in intact chicken skeletal muscle and myoblasts cultured in vitro. Calcif Tissue Int 36:200–205
99. Drittanti L, de Boland AR, Boland R (1989) Modulation of DNA synthesis in cultured muscle cells by 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1014:112–119
100. Garcia LA, King KK, Ferrini MG, Norris KC, Artaza JN (2011) 1,25(OH)2 Vitamin D_3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. Endocrinology 152:2976–2986
101. Dirks-Naylor AJ, Lennon-Edwards S (2011) The effects of vitamin D on skeletal muscle function and cellular signaling. J Steroid Biochem Mol Biol 125:159–168
102. Williams TM, Lisanti MP (2004) The Caveolin genes: from cell biology to medicine. Ann Med 36:584–595
103. Buitrago C, Arango N, Boland R (2012) 1alpha,25(OH)(2)D(3)-dependent modulation of Akt in proliferating and differentiating C2C12 skeletal muscle cells. J Cell Biochem 113:1170–1181
104. Zhang X, Zanello LP (2008) Vitamin D receptor-dependent 1 alpha,25(OH)2 vitamin D_3–induced anti-apoptotic PI3K/AKT signaling in osteoblasts. J Bone Miner Res 23:1238–1248
105. Selles J, Boland R (1991) Rapid stimulation of calcium uptake and protein phosphorylation in isolated cardiac muscle by 1,25-dihydroxyvitamin D_3. Mol Cell Endocrinol 77:67–73
106. de Boland AR, Boland RL (1987) Rapid changes in skeletal muscle calcium uptake induced in vitro by 1,25-dihydroxyvitamin D_3 are suppressed by calcium channel blockers. Endocrinology 120:1858–1864
107. Morelli S, Boland R, de Boland AR (1996) 1,25(OH)2-vitamin D_3 stimulation of phospholipases C and D in muscle cells involves extracellular calcium and a pertussis-sensitive G protein. Mol Cell Endocrinol 122:207–211
108. Vazquez G, Boland R, de Boland AR (1995) Modulation by 1,25(OH)2-vitamin D_3 of the adenylyl cyclase/cyclic AMP pathway in rat and chick myoblasts. Biochim Biophys Acta 1269:91–97
109. Capiati DA, Vazquez G, Tellez Inon MT, Boland RL (2000) Role of protein kinase C in 1,25(OH)(2)-vitamin D(3) modulation of intracellular calcium during development of skeletal muscle cells in culture. J Cell Biochem 77:200–212
110. Vazquez G, de Boland AR, Boland RL (1997) 1 alpha,25-(OH)2-Vitamin D_3 stimulates the adenylyl cyclase pathway in muscle cells by a GTP-dependent mechanism which presumably involves phosphorylation of G alpha i. Biochem Biophys Res Commun 234:125–128
111. Vazquez G, de Boland AR (1996) Involvement of protein kinase C in the modulation of 1alpha,25-dihydroxy-vitamin D_3-induced $^{45}Ca^{2+}$ uptake in rat and chick cultured myoblasts. Biochim Biophys Acta 1310:157–162
112. Vazquez G, de Boland AR, Boland R (1997) Stimulation of Ca^{2+} release-activated Ca^{2+} channels as a potential mechanism involved in non-genomic 1,25(OH)2-vitamin D_3-induced Ca^{2+} entry in skeletal muscle cells. Biochem Biophys Res Commun 239:562–565
113. Vazquez G, de Boland AR, Boland RL (1998) 1alpha,25-Dihydroxy-vitamin-D_3-induced store-operated Ca^{2+} influx in skeletal muscle cells. Modulation by phospholipase c, protein kinase c, and tyrosine kinases. J Biol Chem 273:33954–33960
114. Vazquez G, de Boland AR (1993) Stimulation of dihydropyridine-sensitive Ca^{2+} influx in cultured myoblasts by 1,25(OH)2-vitamin D_3. Biochem Mol Biol Int 31:677–684
115. Nejentsev S, Godfrey L, Snook H, Rance H, Nutland S, Walker NM, Lam AC, Guja C, Ionescu-Tirgoviste C, Undlien DE, Ronningen KS, Tuomilehto-Wolf E, Tuomilehto J, Newport MJ, Clayton DG, Todd JA (2004) Comparative high-resolution

analysis of linkage disequilibrium and tag single nucleotide polymorphisms between populations in the vitamin D receptor gene. Hum Mol Genet 13:1633–1639
116. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E (1997) A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 12:915–921
117. Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, Haussler CA, Galligan MA, Thatcher ML, Encinas Dominguez C, Haussler MR (2001) Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol 177:145–159
118. Roth SM, Zmuda JM, Cauley JA, Shea PR, Ferrell RE (2004) Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. J Gerontol 59:10–15
119. Windelinckx A, De Mars G, Beunen G, Aerssens J, Delecluse C, Lefevre J, Thomis MA (2007) Polymorphisms in the vitamin D receptor gene are associated with muscle strength in men and women. Osteoporos Int 18:1235–1242
120. Hopkinson NS, Li KW, Kehoe A, Humphries SE, Roughton M, Moxham J, Montgomery H, Polkey MI (2008) Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease. Am J Clin Nutr 87:385–390
121. Geusens P, Vandevyver C, Vanhoof J, Cassiman JJ, Boonen S, Raus J (1997) Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. J Bone Miner Res 12:2082–2088
122. Onder G, Capoluongo E, Danese P, Settanni S, Russo A, Concolino P, Bernabei R, Landi F (2008) Vitamin D receptor polymorphisms and falls among older adults living in the community: results from the ilSIRENTE study. J Bone Miner Res 23:1031–1036
123. Barr R, Macdonald H, Stewart A, McGuigan F, Rogers A, Eastell R, Felsenberg D, Gluer C, Roux C, Reid DM (2010) Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). Osteoporos Int 21:457–466
124. Grundberg E, Brandstrom H, Ribom EL, Ljunggren O, Mallmin H, Kindmark A (2004) Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. Eur J Endocrinol 150:323–328
125. Bahat G, Saka B, Erten N, Ozbek U, Coskunpinar E, Yildiz S, Sahinkaya T, Karan MA (2010) BsmI polymorphism in the vitamin D receptor gene is associated with leg extensor muscle strength in elderly men. Aging Clin Exp Res 22:198–205
126. Zehnder D, Bland R, Walker EA, Bradwell AR, Howie AJ, Hewison M, Stewart PM (1999) Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in the human kidney. J Am Soc Nephrol 10:2465–2473
127. Choudhary D, Jansson I, Schenkman JB, Sarfarazi M, Stoilov I (2003) Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. Arch Biochem Biophys 414:91–100
128. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M (2001) Extrarenal expression of 25-hydroxyvitamin D(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 86:888–894
129. Lou YR, Molnar F, Perakyla M, Qiao S, Kalueff AV, St-Arnaud R, Carlberg C, Tuohimaa P (2010) 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. J Steroid Biochem Mol Biol 118:162–170