Calcified Tissue International (2020) 106:47–57
https://doi.org/10.1007/s00223-019-00583-4

REVIEW

Vitamin D and Skeletal Muscle: Emerging Roles in Development, Anabolism and Repair

Christian M. Girgis[1,2,3]

Received: 8 December 2018 / Accepted: 29 April 2019 / Published online: 16 July 2019

Abstract

This special issue article will focus on morphologic and functional roles of vitamin D in muscle, from strength to contraction to development and ageing and will characterise the controversy of VDR's expression in skeletal muscle, central to our understanding of vitamin D's effects on this tissue.

Keywords Muscle · Vitamin D · Vitamin D receptor · Sarcopenia · Development · Satellite cells

Introduction

Osteomalacia and rickets are classical disorders of bone and mineral homeostasis. Defective skeletal mineralisation due to deficiency of vitamin D and substrates necessary for hydroxyapatite deposition, namely calcium and phosphate, are predominant mechanisms. However, effects of vitamin D on muscle are less well recognised. In the clinic, muscle weakness, pain and hypotonia are observed in subjects with severe vitamin D deficiency. Under the microscope, changes in muscle fibre size with preferential atrophy of type 2 (i.e. fast twitch) muscle fibres are seen and on electromyogram (EMG), reduced motor unit action potentials have long been reported in patients with vitamin D deficiency [1, 2]. In fact, in his seminal report on rickets in 1645, Daniel Whistler gave equal credence to the muscle and bone defects seen in children with this condition (i.e. he described both their "flabby, toneless muscles and flexible, waxy bones") [3].

Vitamin D deficiency is generally defined as a 25-OH-vitamin D less than 50 nmol/l (~ 20 ng/ml) [4], but seasonal variations, ethno-specific differences, body composition and alterations in the vitamin D-binding globulin amongst individuals may impact on "normal" levels. Optimal levels of vitamin D for musculoskeletal function may be lower than this threshold, and levels > 20 nmol/l (~ 8 ng/ml) are needed for calcium homeostasis.

How does vitamin D exert such critical effects on skeletal muscle? Attempts to answer this seemingly straightforward question have met challenges and controversy over the decades. The answer begins with the vitamin D receptor (VDR), a cognate nuclear receptor to which the active hormone, $1,25(OH)_2$Vitamin D (calcitriol) binds to exert both genomic and non-genomic effects in cells. The VDR is the *modus operandi* by which vitamin D exerts its diverse effects in physiology, from a central role in calcium and mineral homeostasis to non-classical effects on cell division, tissue pleiotropy, fibrosis and immune modulation [5]. The VDR is widely expressed, both at classical sites of vitamin D activity including intestine, parathyroid and bone, and non-classical sites such as immune cells, the mammary gland and brain [6]. However, its presence in skeletal muscle has been hotly contested for years. This special issue article will focus on morphologic and functional roles of vitamin D in muscle, from strength to contraction to development and ageing and will characterise the controversy of VDR's expression in skeletal muscle, central to our understanding of vitamin D's effects on this tissue.

✉ Christian M. Girgis
christian.girgis@sydney.edu.au

[1] Department of Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

[2] Department of Diabetes and Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

[3] University of Sydney, Sydney, NSW, Australia

Vitamin D Receptor and Muscle

We have learnt a great deal about the VDR since its discovery in 1974 by Brumbaugh and Haussler [7]. Key insights in its biological activity, its protein and structural composition examined by xray crystallography and conformational changes induced by binding to its ligand have come to light [8]. It exerts its effects on the genome by binding to its ligand 1,25(OH)2D together with Retinoid X-receptor (RXR) to form a transcription factor complex (1,25D-VDR-RXR). However, VDR also functions as an orphan nuclear receptor, that is, by direct binding to DNA without its ligand. VDR's ligand-bound and orphan nuclear effects differ, the former possibly being responsible for its classical effects in skeletal and mineral homeostasis and the latter being associated with its less classical effects, such as the maintenance of healthy hair follicles. Hence, mice with whole-body VDR knockout display alopecia (i.e. hair loss), an effect that is not seen in subjects with ligand deficiency (i.e. low levels of vitamin D) [5]. Classical effects of ligand deficiency (i.e. vitamin D and $1,25(OH)_2D$ deficiency) include reduced intestinal absorption of calcium and phosphate, reduced substrate for hydroxyapatite formation and subsequent osteomalacia (impaired mineralization of bone).

VDR is widely expressed in nature, in vertebrates and invertebrates, fish, avian species and mammals, and its expression spans a wide range of tissues and organs. However, its presence in skeletal muscle has been unclear for some time. Confirmation of VDR's expression in skeletal muscle is essential in determining whether vitamin D's effects are direct or indirect effects in this tissue. A number of issues have confounded the clear demonstration of this nuclear receptor in muscle. These include the heterogeneous, multi-cellular nature of skeletal muscle which comprises muscle fibres (i.e. a multinucleated, contractile syncytia of mycoytes) in addition to extracellular matrix, fibroblasts and satellite cells; time-dependent changes in VDR expression during the process of muscle development and; technical, experimental factors, such as the low specificity of VDR antibodies [9].

Through a wide range of techniques, including scintillation autoradiography, binding studies [10, 11], Reverse transcription polymerase chain reaction (RT-PCR) [12] and immunohistochemistry [13], VDR has been demonstrated in cultured myocytes of avian, murine and human origin. Table 1 summarises studies examining VDR's presence in skeletal muscle. Thirty years ago, in one of the first descriptions of VDR in human muscle cells, Costa and colleagues reported specific binding of tritiated $1,25\text{-}(OH)_2D_3$ with a protein component, presumably the VDR, with dose-dependent increases in the expression of *CYP24A1,* the classic VDR target gene [11]. VDR mRNA has been detected in muscle, both in cultured muscle cells and whole muscle tissue, but at much lower (4000-fold difference) levels compared to classical sites of VDR expression such as the duodenum [14]. Because RT-PCR is an exquisitely sensitive method that uses amplification of mRNA levels to produce a signal, the relevance of such low transcript levels of VDR in muscle that are found by this method remains unclear.

Contradictory reports on the detection of VDR protein by western blot and immunohistochemistry have been published [15–17]. Using a specific VDR antibody (D6-Ab), VDR protein was either detected at low levels [14] or not at all [17, 18] in skeletal muscle from mice and adult humans. In contrast to RT-PCR which is exquisitely sensitive, protein detection methods may not be sufficiently sensitive to detect extremely low levels of VDR in muscle at baseline. However, priming VDR by treatment with its ligand may lift levels above a critical threshold of detection. To support this, VDR protein was detected in the quadriceps of older human subjects following a course of oral vitamin D supplementation [19, 20].

Taking a novel approach to the question of whether VDR is present in muscle, Lee and colleagues explored whole-body expression patterns of VDR in a mouse model in which native *VDR* was replaced with a *VDR* transgene expressing luciferase, using a bacterial artificial chromosome (BAC)) [21]. Luciferase refers to a group of enzymes that produce bioluminescence. The BAC-VDR protein was *not* detected in adult muscle using western blot, immunohistochemistry or luciferase studies, but by comparison *was* expressed at classical sites of vitamin D action. Reasons for this lack of VDR expression in muscle may be methodological. Owing to the relatively low baseline levels of VDR in muscle, a finite amount of BAC-VDR in this model may be preferentially expressed in classical VDR sites.

A number of in vitro studies have characterised the activity of VDR in cultured myoblasts. Rapid translocation of VDR from nucleus to the cytoplasm was demonstrated following treatment of myoblasts with the ligand $1,25(OH)_2D_3$ [16, 22], a process dependent on microtubular transport. VDR interacted with a range of intracellular signalling pathways, the tyrosine phosphorylation cascade, [23], and membrane-scaffolding proteins such as caveolin-1 [16]. Following prolonged treatment of myoblasts with $1,25(OH)_2D_3$, VDR translocated back to the nucleus to carry out its role in regulating transcription [24]. Hence, depending on its location within the cell and exposure times to its ligand, VDR participated in both rapid and genomic actions in these cultured myoblasts.

VDR levels within muscle may change over time, particularly across the various stages of muscle development. Studies employing tissue culture report substantially

Table 1 Studies on VDR Expression in Skeletal Muscle

Author	Species	Models	Methodology	Key findings
In vitro studies				
Simpson (1985) [10]	Mouse, rat	G8 and H9c2 cell lines; rat longissimus	Chromatography, equilibrium binding studies	VDR present in muscle cytosol (*high-salt buffer*); anti-proliferative and anti-differentiation effect of $1,25(OH)_2D$ on muscle cells
Costa (1986) [11]	Human (five subjects)	Cloned muscle cells	Receptor binding studies, DNA-cellulose chromatography, 24-hydroxylase assay, ^{3}H-Leucine, ^{3}H-Thymidine incorporation	VDR present, $1,25(OH)_2D$ inhibited protein and DNA synthesis in human myoblasts
Buitrago (2010) [16]	Mouse	C2C12 cells	Immunohistochemistry, Immunoprecipitation, transfection studies	VDR present, translocates to membrane after $1,25(OH)_2D$ treatment VDR Interacts with caveolin-1, membrane scaffolding
Boland et al. (2000–2002) [23, 36, 86]	Chick	Muscle cells	Immunoprecipitation, western blot, spectrofluorimetric analysis, transfection studies	VDR present Non-genomic VDR effects: tyrosine phosphorylation of signalling proteins c-myc, c-Src, MAPK; effects on calcium flux
Garcia (2011) [24]	Mouse	C2C12 cells	PCR, immunohistochemistry, Western blot (VDR-C20 antibody)	VDR present $1,25(OH)_2D$ increases levels of myonuclear VDR Anti-proliferative and pro-myogenic effects
Girgis (2013) [32]	Mouse	C2C12 cells	PCR, immunohistochemistry, Luciferase reporter studies, Western blot	VDR & CYP27B1 present 25(OH)D & $1,25(OH)_2D$ exerted anti-proliferative, anti-myogenic effects on C2C12 cells Anabolic effects on C2C12 myotube size
Rodent studies				
Sandgren (1991) [87]	Rat	Muscle extracts	Immunoradiometric assay	VDR absent
Endo (2003) [12]	Mouse	Muscle extracts (WT and VDRKO) C2C12 cells	Northern blot, polymerase chain reaction (PCR)	VDR mRNA in 3 week but not 8 week old mice Developmental defects in muscles of VDRKO mice
Srikuea (2012) [26]	Mouse	Muscle extracts; C2C12 cells	$BaCl_2$—induced muscle injury Immunohistochemistry, Western blot (VDR-H81 antibody)	VDR & CYP27B1 present and are increased in regenerating muscle fibres following injury
Girgis (2014) [14]	Mouse	Muscle extracts (WT and VDRKO); primary muscle fibres and cells	PCR, Immunohistochemistry, Western blot (VDR-D6 Antibody), Luciferase reporter studies, ^{3}H-25(OH)D muscle fibre uptake assay	VDR present VDR levels in muscle decline with age VDR has novel role in ligand-mediated 25(OH)D uptake in muscle fibres
Makanae (2015) [25]	Rats	Muscle extracts	Western blot (VDR-sc13133 Antibody), Endurance and resistance exercise	VDR protein increases acutely in muscle following resistance exercise

Table 1 (continued)

Author	Species	Models	Methodology	Key findings
Chen (2016) [41]	Mice	Muscle-specific VDRKO mice (MLC1f-Cre)	PCR, phenotypic characterization	Distinct phenotype in these mice supporting the presence and function of VDR in muscle: smaller muscle fibres, insulin resistance
Human studies				
Bischoff (2001) [15]	20 subjects	Muscle extracts (20 subjects)	Immunohistochemistry (VDR-9A7 Antibody)	VDR present, localised within nucleus VDR level declines with age No correlation with serum vitamin D
Ceglia (2010) [13]	Four subjects	Muscle extracts	Immunohistochemistry (VDR-NR1I1; D-6 and 333C6a Antibodies), Fibre-typing	VDR present Not specific to muscle fibre-type
Wang (2011) [17]	One human subject	Muscle extracts (1 human subject)	PCR, Immunohistochemistry, Western blot (VDR-D6 Antibody)	VDR mRNA present at very low levels in muscle VDR protein absent
Ceglia (2013) [19]	♀ ≥ 65 years	Muscle extracts (12 subjects)	Immunohistochemistry (VDR-NR1I1 Antibody)	Subjects randomized to vitD3 (4000 IU d) had increased myonuclear VDR and fibre size
Pojednic (2015) [20]	♀ ≥ 65 years	Muscle extracts Primary muscle cells	PCR, Western blot, Immunohistochemistry (VDR-NR1I1 Antibody)	VDR present Muscle VDR increases following oral vitamin D supplementation, correlates with serum 25(OH)D
Olsson (2016) [18]	9 subjects, 20–27 years	Muscle extracts Primary muscle cells	PCR, Western blot, Immunohistochemistry (VDR-D6 Antibody)	VDR present in primary muscle cells VDR undetectable in whole muscle extract
Ryan (2016) [76]		Muscle extracts	Immunohistochemistry, western blot	VDR present in muscle fibres, $1,25(OH)_2D$ increased mitochondrial oxygen consumption in muscle cells
Antinozzi (2017) [69]	Subjects with inflammatory myopathy	Muscle extracts	Immunofluorescence analysis	VDR present but at lower levels in subjects with muscle inflammation
Roh (2019) [88]	40 subjects with wrist fracture	Muscle extracts	PCR, Western blot (VDR-D6 antibody)	VDR present but at lower levels in patients with sarcopenia

greater levels of VDR in immature muscle cells, myoblasts and muscle precursor cells, as opposed to fully differentiated myotubes and whole muscle fibres [14, 18]. Newborn mice, whose muscles are actively involved in the process of secondary myogenesis (a process that occurs in utero in humans) express significantly higher levels of muscle VDR than 3-week old mice and adult mice [14]. However, following muscle injury in adult mice, the process of post-natal muscle repair is activated, essentially a recapitulation of embryonic myogenesis, and a significant increase in muscle VDR is seen [25, 26]. Therefore, higher VDR expression levels in primordial muscle cells and newborn mice, together with muscle fibres undergoing repair support a pleiotropic role of VDR in muscle and potential effects in muscle development and repair. It is conceivable that VDR in adult muscle may sequester within satellite cells and hence, escape detection on assays that examine whole muscle tissue.

In summary, the controversy of VDR's presence in skeletal muscle has stemmed from wide differences in muscle models used to answer this question, non-specificity of VDR antibodies, and protein detection methods that may be insufficiently sensitive to detect low levels of VDR protein in this tissue. Low baseline levels do not preclude a biological role for VDR in this tissue. Indeed, transcription factors are known to exert genomic effects even at low levels of expression, dependent on their binding affinity to DNA [27]. In addition, VDR may sequester within a specific cell population, such as satellite cells, and thus escape detection methods employing whole muscle (in which satellite cells comprise a minority, i.e. ~5% cells in adult muscle) [28]. To date, evidence indicates that VDR is indeed expressed in muscle but at very low baseline levels in adults. VDR in muscle predominates in precursor cells and in developing and regenerating muscle fibres, and hence its activity in this tissue appears related to muscle development and pleiotropy.

Vitamin D and Muscle Development

The VDR makes its first appearance early during embryonic life (i.e. day 13 in rats) and is initially expressed within the mesoderm, the embryonic tissue which gives rise to the musculoskeletal system [29]. Mesenchymal stem cells (MSCs) express VDR in addition to components of the vitamin D –endocrine system including *CYP27B1 (*1α-hydroxylase) and CYP24A1 (24-hydroxylase) and respond to treatment with the ligand $1,25(OH)_2D_3$, which induces myogenic and osteogenic programs [30].

Experiments on C2C12 cells, an immortalized murine muscle cell line, indicate that vitamin D alters myogenesis in its various stages, including myoblast proliferation, myocyte differentiation and fusion to form myotubes and the determination of myotube size [12, 24, 30–32]. $1,25(OH)_2D_3$ exerted effects on post-translational modification in C2C12 myoblasts by altering phosphorylation of Rb (Retinoblastoma protein), JNK (c-Jun N-terminal kinase), Raf-1 (retinoblastoma associated factor 1), cAMP response element binding protein (CREB) and ElK-1 signalling, resulting in an anti-proliferative effect in these cells [33–36]. Vitamin D modulated C2C12 myotube formation, a complex process in which myocytes fuse to form tubular, contractile syncytia, dependent on well characterised pathways involving IGF-1 and myogenin [12, 24, 32]. Key myogenic regulatory factors includng *myogenin, myf5, myoD* and *IGF* were all modulated by vitamin D treatment in these cells. Vitamin D exerted an anabolic effect in increasing C2C12 myotube size by downregulation of myostatin, a TGF-β which negatively regulates muscle mass [24, 32]. These effects of the ligand $1,25(OH)_2D_3$ were directly related to VDR activation and were negated by VDR knockdown [37].

The Vitamin D receptor knockout (VDRKO) mouse model has provided key insights in the biologic activity of the vitamin D and its role in muscle development [5]. In addition to wide-ranging defects including rickets, reduced calcium and phosphate levels, alopecia and immune dysfunction, these mice displayed lighter muscles, global reduction in muscle fibre size and increased myonuclei, changes that persisted despite normalisation of calcium and phosphate by the provision of a rescue diet [12, 38]. This muscle phenotype was seen early in VDRKO mice, initially at 3 weeks of age, preceding the development of phosphate and calcium abnormalities and systemic defects. Increased transcript levels of *myostatin* (>2-fold), alteration of myogenic regulatory factors *myf5* and *myogenin* and persistent expression of neonatal *myosin heavy chain (MHC) isoforms* were seen in the muscle of adult VDRKO mice [12, 38]. However, the VDRKO mouse model is subject to confounding, specifically whole-body changes in these mice may have independent effects on muscle morphology. In addition, attempts to identify vitamin D response elements (VDRE) in the promoter regions of myf5 and myogenin genes have been unsuccessful, suggesting VDR's effects on key myogenic regulatory factors may be indirect [39, 40].

To bypass potential confounding by systemic effects of the VDR, tissue-specific knockout mice have been generated [41]. The promoter gene used to ablate *VDR* in the muscle of these mice, *MLC1f*, is expressed in embryonic life, making this an appropriate model to examine effects on muscle differentiation. Reductions in type II muscle fibre diameter were demonstrated in these mice but the primary focus of this paper was to examine effects on insulin sensitivity and hence, further detail on the muscle morphology of these mice was not presented in detail [41].

Effects of maternal vitamin D on muscle development in offspring have been examined in several species. In humans, maternal vitamin D levels were associated with arm muscle

size in offspring as well as grip strength [42, 43]. In rodent studies, offspring of vitamin D-deficient dams demonstrated smaller muscle fibres with effects on protein catabolism and genes involved in muscle differentiation and the cytoskeleton [44, 45]. In pigs, vitamin D supplementation during pregnancy led to increased muscle fibre size and number in offspring, associated with higher transcript levels of *myoD, myogenin* and reduced *myostatin* transcript [46]. European sea bass treated with vitamin D after hatching demonstrated increases in muscle fibre size that were dose-dependent and associated with changes in myogenic genes [47]. Maternal vitamin D levels may possibly play an epigenetic role in foetal development, being associated with methylation at 4 sites of the RXR-A (retinoid X receptor alpha) in umbilical cord tissue [48].

In summary, in vitro studies in muscle cells suggest a role for VDR in muscle proliferation, differentiation and myotube development and size. In vivo studies in mice corroborate this effect by demonstrating reduced muscle mass and smaller fibres in mice with whole-body or muscle-specific VDR ablation. Alterations in myogenic regulatory gene and TGF-β expression have been demonstrated as potential mechanisms for these changes. To confirm direct genomic effects of VDR in muscle, chromatin immunoprecipitation (ChIP) studies are necessary to characterise the VDR cistrome in this tissue (i.e., DNA binding sites).

Vitamin D and Muscle Strength

People with vitamin D deficiency display muscle weakness and higher risk of falls, features that are reversible with vitamin D supplementation [49]. In addition to changes in muscle mass described in the previous section, VDRKO mice displayed reduced grip strength [38] and abnormal swimming with reduced buoyancy and greater fatigue [50]. In open field testing, VDRKO mice displayed shorter steps and abnormal gait and on rotarod testing, they displayed reduced balance with shorter retention times implying abnormal muscle coordination [50, 51]. These defects in muscle function progressed with ageing and a dose-dependent effect of VDR on grip strength was seen [38, 51]. However, no impairment in swimming was demonstrated in 1α-hydroxylase knockout mice [51]. The contrasting phenotypes of VDR and 1α-hydroxylase knockout mice suggest different activities of the vitamin D endocrine system on muscle function. Whilst VDR is important in muscle function, as suggested by the VDRKO model, the active hormone, 1,25(OH)2D is not prerequisite for VDRs actions in this tissue, as suggested by lack of muscle phenotype in 1α-hydroxylase knockout mice. Taken together, this indicates that VDR exerts ligand-independent functions in muscle.

On the other hand, reduced muscle strength has been demonstrated in animal models of vitamin D deficiency [38, 52, 53]. Significant reduction in muscle contraction and impaired recovery in vitamin D-deficient rats and chicks were demonstrated and reversed with vitamin D supplementation [53, 54]. Alterations in expression of components of the sarcomere, including actin and the troponin-tropomyosin complex, provide a mechanism for reduced strength in these studies [55–57]. Alternatively, vitamin D may exert its effects on muscle strength via intracellular calcium handling. In vitamin D deficient mice, reduced grip strength was associated with altered expression of mRNAa responsible for calcium-handling and sarco-endoplasmic reticulum calcium transport ATPase (Serca) channels [38]. Reduced calcium concentrations within muscle mitochondria and sarcoplasmic reticulum have been reported in vitamin D deficient animals [54, 58].

A study proposed a primary function for phosphate in vitamin D's effects on muscle. Rats deficient in vitamin D, phosphorus and calcium underwent muscle strength testing via force transduction on soleus muscle [52]. Phosphate levels displayed the strongest correlation with muscle dysfunction and phosphate repletion reversed defects independent of calcium and vitamin D levels. However, in another study muscle defects in vitamin D deficient mice persisted despite adjusting calcium and phosphate deficiency [38].

Vitamin D effects on the neuromuscular junction were examined in a study of vitamin D deficient rats. In association with defects in muscle balance and coordination, vitamin D deficient rats had increased muscle hypersensitivity and a higher number of nocioceptor axons [59]. In other studies, treatment of WT mice with eldecalcitol, a vitamin D analogue, improved coordination and locomotor performance, with increased expression of IGF1 and myelin in Schwann cells and increased AchR density in neuromuscular junction [60]. Therefore, vitamin D exerts neuronal effects that may further impact on muscle function.

Recent studies have reported a novel ex vivo effect of VDR on muscle function [14, 61]. Muscles of VDRKO were dissected and examined in a controlled environment, unperturbed by systemic changes, and in these studies, VDR modulated the uptake and retention of $25(OH)D_3$ within muscle fibres [14, 61]. Upon entry into muscle, $25(OH)D_3$ may be locally converted to $1,25(OH)2D_3$ and elicit rapid effects on calcium handling thereby altering contraction and muscle strength. Alternatively, muscle may be a storage depot for $25(OH)D_3$, in which the molecule may be bound to the D-binding protein (DBP), and as required, diffuse back into circulation upon degradation of DBP and its release from actin [61].

Rapid effects of $1,25(OH)_2D_3$ on intramuscular calcium handling have been elucidated by a range of in vitro studies [62–64]. $1,25(OH)_2D_3$ resulted in rapid calcium shifts from

the sarcoplasmic reticulum to the cytosol, through the action of signal transduction pathway including c-src, phospholipase C gamma (PLC-gamma) and inositol triphosphate (IP3) [65]. Prolonged exposure to $1,25(OH)_2D_3$ led to sustained calcium entry from the extracellular compartment via L-type voltage-dependent calcium-channel (VDCC) and store-operated calcium entry (SOCE) mechanisms. By modulating intracellular calcium, vitamin D may play an indirect role in muscle contraction, plasticity and metabolism, processes which are determined by calcium [66]. To confirm these effects, fura-2 studies in muscle would allow for real-time in vivo assessment of calcium flux in response to vitamin D, and provide a mechanistic basis for effects on contraction and strength.

In summary, muscle weakness and increased fatigue have been reported in vitamin D deficient humans and animals in addition to VDRKO mice. However, there are difficulties in differentiating direct effects of vitamin D deficiency in muscle dysfunction from those of associated phosphate, calcium and parathyroid hormone defects. Cell studies indicate that $1,25(OH)_2D$ regulates muscle cell calcium and phosphate handling in rapid and genomic fashions and uptake of $25(OH)D_3$ within muscle, positing mechanisms for effects on contraction and strength. Definitive studies are needed to study contractile physiology, endurance and muscle fatigue in mice with distinct alterations of vitamin D signalling, independent of mineral defects.

Vitamin D and Muscle Ageing

Serum vitamin D levels predict the rate of functional decline and age-related atrophy of skeletal muscle in older subjects [67]. Vitamin D deficiency is common in older subjects and levels of VDR decline in muscle with age [15]. Type 2 muscle fibres (i.e. fast twitch) undergo preferential atrophy, predisposing vitamin D deficient subjects to falls [2].

In ageing rodents with vitamin D deficiency, muscle atrophy pathways were upregulated with increased muscle protein catabolism via activation of ubiquitin ligases (MAFBx and MuRF1), TGF-β, FOXO and the ubiquitin-proteosome system were seen [38, 68]. These molecular changes were associated with significant muscle fibre atrophy that was only partly corrected by adjusting calcium levels [68]. *Myostatin* expression was greater in the muscles of vitamin D deficient rodents and expression of mygenic regulatory factors was also altered [38]. Studies on human myocytes corroborate effects of vitamin D and VDR agonists on pathways known to regulate muscle ageing including ubiquitin ligases, inflammatory markers TNF-alpha and IL6 and PI3K/AKT signalling [20, 69].

Vitamin D may exert indirect effects on muscle ageing by its interaction with other hormones. FGF23 and its co-factor klotho regulate phosphate, vitamin D synthesis and have novel effects on ageing. Mice lacking FGF23 display premature ageing, osteopenia and sarcopenia, and these features were completely reversed by concomitant ablation of *CYP27B1* [70]. This suggests that the ligand $1,25(OH)_2D$ may modulate age-related responses to FGF23, possibly through its action on the VDR. Klotho deficient mice display a muscle phenotype remarkably similar to VDRKO mice with weakness, impaired endurance and premature ageing associated with alterations in TGF-β and wnt signalling [71, 72]. It is possible that vitamin D and klotho share inter-connected effects on skeletal muscle morphology and function during ageing on the basis of these similarities.

Vitamin D deficiency results in mitochondrial dysfunction and oxidative stress in muscle cells with reduction in superoxide dismutase (SOD) [73]. Serum Vitamin D levels correlated with lactic acid, creatine kinase and total antioxidant activity (TAC) in elderly subjects following exercise and vitamin D supplementation improved oxidative phosphorylation [74, 75]. In vitro, $1,25(OH)_2D_3$ regulated transcripts of mitochondrial genes in muscle cells with increased mitochondrial volume and oxygen consumption rates [76]. Thus, vitamin D may reduce oxygen free radicals in muscle and alleviate effects of mitochondrial dysfunction, thereby counteracting sarcopenia.

In summary, vitamin D deficiency accelerates muscle ageing with atrophy of muscle fibres and subsequent sarcopenia with a risk of falls and functional decline. Mechanisms elucidated in animal studies include increased muscle protein turnover via activation of ubiquitin-proteosome and oxidative stress. Vitamin D supplementation may reverse these effects and further research is needed on the potential anti-ageing effects of vitamin D on skeletal muscle.

Vitamin D and Muscle Repair

Muscle repair is an intricate process in which satellite cells, unique muscle stem cells, are activated by mitogenic factors and differentiate into myocytes which fuse into muscle fibres, governed by the myogenic regulatory program. Vitamin D may also play a role in this process.

In vitro, vitamin D altered muscle cell response to mechanical injury with an increase in muscle cell migration, myotube fusion and expression of tissue regeneration and angiogenic markers [31, 77]. In vivo, muscle damage induced by via freeze-crush or $BaCl_2$-induced mechanisms resulted in significant activation of *VDR* and *CYP27B1* in rodents, specifically within regenerating muscle fibers [26, 78]. Resistance training, inducing lesser degrees of muscle damage, also induced *VDR* and *CYP27B1* in muscles of rodents [25, 79].

Muscle injury induced by freeze-crush injury or high-intensity exercise was ameliorated by vitamin D supplementation in rats, associated with attenuated increase in creatine kinase (CK) and lactate dehydrogenase (LDH) [78, 79]. Functionally, improved recovery in contractile force in the injured muscle was demonstrated. Mechanistically, reduced oxidative stress and inflammation, together with an effect on stress-related proteins (p38 MAPK, ERK1/2, IKK, IkappaB), may explain these beneficial effects [79, 80]. However, there may be a U-shaped effect as excessive doses of 1,25(OH)D_3 were not beneficial on muscle regeneration with deleterious effects on satellite cell activity and muscle fibre repair following injury [81].

In human clinical studies, baseline levels of vitamin D correlated inversely with muscle weakness following exercise [82]. Vitamin D altered cytokine levels following exercise, including IL-10, IL-13 and inflammatory mediators TNF-a and IFN-g, suggesting a modulatory effect on inflammation [82, 83]. Levels of VDR protein and *IL6* displayed a positive correlation in human muscle, suggesting effects of vitamin D on the inflammatory response to muscle damage [84]. Vitamin D supplementation had a beneficial effect on muscle recovery in adult males in whom muscle injury was induced by repetitive eccentric contractions, i.e. jumping [77, 85].

In summary, vitamin D's effects on muscle repair are suggested by increases in VDR expression in regenerating muscle tissue. Direct effects on oxidative stress, inflammatory cytokines and satellite cell activity in response to vitamin D have been demonstrated.

Conclusions

This special issue article summarises the current understanding of vitamin D's effects on skeletal muscle, specifically in development, strength, ageing and repair. While functional effects of vitamin D on muscle, particularly in relation to muscle strength and contraction, appear related to calcium and phosphate levels, pleiotropic effects on muscle development, ageing and repair may be related to direct actions of vitamin D signalling within muscle cells.

At a basic level, vitamin D modulates intramuscular calcium flux via the rapid activation of signalling cascades and second messenger systems [62–64]. Genomic responses to vitamin D involve myogenic regulatory factors, TGF-β signalling including myostatin and the ubiquitin-proteosome [24, 32]. Morphologically, muscle mass, fibre size, strength and the regenerative response to muscle damage are altered by vitamin D [26, 38]. Age-related changes in muscle function, protein turnover, oxidative stress and atrophy pathways are postulated mechanisms by which this occurs [38, 68].

The controversial question of VDR's expression in skeletal muscle has also been discussed. Technical factors giving rise to this controversy, in addition to the heterogeneous, multicellular nature of skeletal muscle, in which individual components may respond differently to vitamin D, have been mentioned. Current evidence indicates that VDR *is* indeed expressed in muscle, but at low levels that may elude detection. VDR predominates in immature forms of muscle, primordial muscle cells, such as satellite cells, and in developing and regenerating muscle fibres [14, 18]. VDR's predominant expression in these early muscle cells indicates a primarily pleiotropic role in this tissue. At a functional level, VDR knockout mice display a distinct muscle phenotype, also in support of its presence and activity at this site.

Uncertainties remain. Although gene targets of vitamin D signalling in muscle have been reported, vitamin D response elements (VDRE) within these genes have not been clearly demonstrated and hence, it is unclear if these are direct targets. Chromatin immunoprecipitation (ChIP) studies to characterise the muscle VDR cistrome are needed. Non-genomic effects of vitamin D on calcium flux have been reported in many in vitro studies but the translation of these findings to in vivo muscle physiology is not a foregone conclusion. For confirmation, fura-2 studies in muscle would allow for real-time in vivo assessment of calcium flux in response to vitamin D. A greater understanding of direct effects of VDR on muscle function will come to light with characterisation of tissue-specific models, circumventing systemic effects of vitamin D [41]. Finally, effects of vitamin D on muscle regeneration raise the intriguing possibility that vitamin D modulates satellite cells in their response to damage, and thereby enhances regeneration. Thus, vitamin D exerts diverse effects on skeletal muscle, with a broad functional repertoire in development, pleiotropy and ageing.

Compliance with Ethical Standards

Conflict of interest Christian M. Girgis declares that he has no conflict of interest to disclose.

References

1. Floyd M, Ayyar DR, Barwick DD, Hudgson P, Weightman D (1974) Myopathy in chronic renal failure. Q J Med 43(172):509–524
2. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE (2013) The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocr Rev 34(1):33–83
3. Whistler D (1645) De morbo puerili Anglorum quem patrio idiomate indigenae vocant. The Rickets. Londini, London
4. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al (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(1):53–58
5. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF et al (2008) Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 29(6):726–776
6. Cui X, Pelekanos M, Liu PY, Burne TH, McGrath JJ, Eyles DW (2013) The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. Neuroscience 236:77–87
7. Brumbaugh PF, Haussler MR (1974) 1 Alpha,25-dihydroxycholecalciferol receptors in intestine. I. Association of 1 alpha,25-dihydroxycholecalciferol with intestinal mucosa chromatin. J Biol Chem 249(4):1251–1257
8. Girgis CM (2018) Vitamin D and skeletal muscle. In: Feldman DJPW, Bouillon R, Giovanucci E, Goltzman D, Hewison M (eds) Vitamin D. Elsevier, Atlanta, pp 597–613
9. Wang Y, Becklund BR, DeLuca HF (2010) Identification of a highly specific and versatile vitamin D receptor antibody. Arch Biochem Biophys 494(2):166–177
10. Simpson RU, Thomas GA, Arnold AJ (1985) Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. J Biol Chem 260(15):8882–8891
11. Costa EM, Blau HM, Feldman D (1986) 1,25-Dihydroxyvitamin D3 receptors and hormonal responses in cloned human skeletal muscle cells. Endocrinology 119(5):2214–2220
12. Endo I, Inoue D, Mitsui T, Umaki Y, Akaike M, Yoshizawa T et al (2003) Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology 144(12):5138–5144
13. Ceglia L, da Silva Morais M, Park LK, Morris E, Harris SS, Bischoff-Ferrari HA et al (2010) Multi-step immunofluorescent analysis of vitamin D receptor loci and myosin heavy chain isoforms in human skeletal muscle. J Mol Histol 41(2–3):137–142
14. Girgis CM, Mokbel N, Minn Cha K, Houweling PJ, Abboud M, Fraser DR et al (2014) The vitamin D receptor (VDR) is expressed in skeletal muscle of male mice and modulates 25-hydroxyvitamin D (25OHD) uptake in myofibers. Endocrinology 155(9):3227–3237
15. Bischoff HA, Borchers M, Gudat F, Duermueller U, Theiler R, Stahelin HB et al (2001) In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. Histochem J. 33(1):19–24
16. Buitrago C, Boland R (2010) Caveolae and caveolin-1 are implicated in 1alpha,25(OH)2-vitamin D3-dependent modulation of Src, MAPK cascades and VDR localization in skeletal muscle cells. J Steroid Biochem Mol Biol 121(1–2):169–175
17. Wang Y, DeLuca HF (2011) Is the vitamin d receptor found in muscle? Endocrinology 152(2):354–363
18. Olsson K, Saini A, Stromberg A, Alam S, Lilja M, Rullman E et al (2016) Evidence for vitamin D receptor expression and direct effects of 1 alpha, 25(OH)2D3 in human skeletal muscle precursor cells. Endocrinology 157(1):98–111
19. Ceglia L, Niramitmahapanya S, Morais MD, Rivas DA, Harris SS, Bischoff-Ferrari H et al (2013) A randomized study on the effect of vitamin D3 supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. J Clin Endocrinol Metab 98(12):1927–1935
20. Pojednic RM, Ceglia L, Olsson K, Gustafsson T, Lichtenstein AH, Dawson-Hughes B et al (2015) Effects of 1,25-dihydroxyvitamin D3 and vitamin D3 on the expression of the vitamin d receptor in human skeletal muscle cells. Calcif Tissue Int 96(3):256–263
21. Lee SM, Bishop KA, Goellner JJ, O'Brien CA, Pike JW (2014) Mouse and human BAC transgenes recapitulate tissue-specific expression of the vitamin D receptor in mice and rescue the VDR-null phenotype. Endocrinology 155(6):2064–2076
22. Capiati D, Benassati S, Boland RL (2002) 1,25(OH)2-Vitamin D3 induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells. J Cell Biochem 86(1):128–135
23. Buitrago C, de Boland R, Boland AR (2001) The tyrosine kinase c-Src is required for 1,25(OH)2-vitamin D3 signalling to the nucleus in muscle cells. Biochimica et Biophysica Acta. 1541(3):179–187
24. Garcia LA, King KK, Ferrini MG, Norris KC, Artaza JN (2011) 1,25(OH)2vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. Endocrinology 152(8):2976–2986
25. Makanae Y, Ogasawara R, Sato K, Takamura Y, Matsutani K, Kido K et al (2015) Acute bout of resistance exercise increases vitamin D receptor protein expression in rat skeletal muscle. Exp Physiol 100(10):1168–1176
26. 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(4):C396–C405
27. Cheng C, Alexander R, Min R, Leng J, Yip KY, Rozowsky J et al (2012) Understanding transcriptional regulation by integrative analysis of transcription factor binding data. Genome Res 22(9):1658–1667
28. Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93(1):23–67
29. Johnson JA, Grande JP, Roche PC, Kumar R (1996) Ontogeny of the 1,25-dihydroxyvitamin D3 receptor in fetal rat bone. J Bone Miner Res 11(1):56–61
30. Artaza JN, Norris KC (2009) Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. J Endocrinol 200(2):207–221
31. Garcia LA, Ferrini MG, Norris KC, Artaza JN (2013) 1,25(OH)(2)Vitamin D(3) enhances myogenic differentiation by modulating the expression of key angiogenic growth factors and angiogenic inhibitors in C(2)C(12) skeletal muscle cells. J Steroid Biochem Mol Biol 133:1–11
32. Girgis CM, Clifton-Bligh RJ, Mokbel N, Cheng K, Gunton JE (2014) Vitamin D signaling regulates proliferation, differentiation, and myotube size in C2C12 skeletal muscle cells. Endocrinology 155(2):347–357
33. Morelli S, Buitrago C, Boland R, de Boland AR (2001) The stimulation of MAP kinase by 1,25(OH)(2)-vitamin D(3) in skeletal muscle cells is mediated by protein kinase C and calcium. Mol Cell Endocrinol 173(1–2):41–52
34. Buitrago CG, Pardo VG, de Boland AR, Boland R (2003) Activation of RAF-1 through Ras and protein kinase Calpha mediates 1alpha,25(OH)2-vitamin D3 regulation of the mitogen-activated protein kinase pathway in muscle cells. J Biol Chem 278(4):2199–2205
35. Ronda AC, Buitrago C, Colicheo A, de Boland AR, Roldan E, Boland R (2007) Activation of MAPKs by 1alpha,25(OH)2-Vitamin D3 and 17beta-estradiol in skeletal muscle cells leads to phosphorylation of Elk-1 and CREB transcription factors. J Steroid Biochem Mol Biol 103(3–5):462–466
36. Boland R, De Boland AR, Buitrago C, Morelli S, Santillan G, Vazquez G et al (2002) Non-genomic stimulation of tyrosine phosphorylation cascades by 1,25(OH)(2)D(3) by VDR-dependent and independent mechanisms in muscle cells. Steroids 67(6):477–482
37. Tanaka M, Kishimoto KN, Okuno H, Saito H, Itoi E (2014) Vitamin D receptor gene silencing effects on differentiation of myogenic cell lines. Muscle Nerve 49(5):700–708
38. Girgis CM, Cha KM, Houweling PJ, Rao R, Mokbel N, Lin M et al (2015) Vitamin D receptor ablation and vitamin D deficiency

result in reduced grip strength, altered muscle fibers, and increased myostatin in mice. Calcif Tissue Int 97(6):602–610
39. Seoane S, Alonso M, Segura C, Perez-Fernandez R (2002) Localization of a negative vitamin D response sequence in the human growth hormone gene. Biochem Biophys Res Commun 292(1):250–255
40. Sakoda K, Fujiwara M, Arai S, Suzuki A, Nishikawa J, Imagawa M et al (1996) Isolation of a genomic DNA fragment having negative vitamin D response element. Biochem Biophys Res Commun 219(1):31–35
41. Chen S, Villalta SA, Agrawal DK (2016) FOXO1 mediates vitamin D deficiency-induced insulin resistance in skeletal muscle. J Bone Miner Res 31(3):585–595
42. Krishnaveni GV, Veena SR, Winder NR, Hill JC, Noonan K, Boucher BJ et al (2011) Maternal vitamin D status during pregnancy and body composition and cardiovascular risk markers in Indian children: the Mysore Parthenon Study. Am J Clin Nutr 93(3):628–635
43. Harvey NC, Moon RJ, Sayer AA, Ntani G, Davies JH, Javaid MK et al (2014) Maternal antenatal vitamin D status and offspring muscle development: findings from the Southampton Women's Survey. J Clin Endocrinol Metab 99(1):330–337
44. Max D, Brandsch C, Schumann S, Kuhne H, Frommhagen M, Schutkowski A et al (2013) Maternal vitamin D deficiency causes smaller muscle fibers and altered transcript levels of genes involved in protein degradation, myogenesis, and cytoskeleton organization in the newborn rat. Mol Nutr Food Res 58:343–352
45. Oku Y, Tanabe R, Nakaoka K, Yamada A, Noda S, Hoshino A et al (2016) Influences of dietary vitamin D restriction on bone strength, body composition and muscle in rats fed a high-fat diet: involvement of mRNA expression of MyoD in skeletal muscle. J Nutr Biochem 32:85–90
46. Zhou H, Chen Y, Lv G, Zhuo Y, Lin Y, Feng B et al (2016) Improving maternal vitamin D status promotes prenatal and postnatal skeletal muscle development of pig offspring. Nutrition 32(10):1144–1152
47. Alami-Durante H, Cluzeaud M, Bazin D, Mazurais D, Zambonino-Infante JL (2011) Dietary cholecalciferol regulates the recruitment and growth of skeletal muscle fibers and the expressions of myogenic regulatory factors and the myosin heavy chain in European sea bass larvae. J Nutr 141(12):2146–2151
48. Harvey NC, Sheppard A, Godfrey KM, McLean C, Garratt E, Ntani G et al (2014) Childhood bone mineral content is associated with methylation status of the RXRA promoter at birth. J Bone Miner Res 29(3):600–607
49. Girgis CM, Clifton-Bligh RJ, Turner N, Lau SL, Gunton JE (2014) Effects of vitamin D in skeletal muscle: falls, strength, athletic performance and insulin sensitivity. Clin Endocrinol (Oxf). 80(2):169–181
50. Burne TH, Johnston AN, McGrath JJ, Mackay-Sim A (2006) Swimming behaviour and post-swimming activity in Vitamin D receptor knockout mice. Brain Res Bull 69(1):74–78
51. Minasyan A, Keisala T, Zou J, Zhang Y, Toppila E, Syvala H et al (2009) Vestibular dysfunction in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol 114(3–5):161–166
52. Schubert L, DeLuca HF (2010) Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch Biochem Biophys 500(2):157–161
53. Rodman JS, Baker T (1978) Changes in the kinetics of muscle contraction in vitamin D-depleted rats. Kidney Int 13(3):189–193
54. Pleasure D, Wyszynski B, Sumner A, Schotland D, Feldman B, Nugent N et al (1979) Skeletal muscle calcium metabolism and contractile force in vitamin D-deficient chicks. J Clin Investig 64(5):1157–1167
55. Pointon JJ, Francis MJ, Smith R (1979) Effect of vitamin D deficiency on sarcoplasmic reticulum function and troponin C concentration of rabbit skeletal muscle. Clin Sci (Lond). 57(3):257–263
56. Stroder J, Arensmeyer E (1965) Actomyosin content of the skeletal muscles in experimental rickets. Klin Wochenschr 43(22):1201–1202
57. de Boland AR, Albornoz LE, Boland R (1983) The effect of cholecalciferol in vivo on proteins and lipids of skeletal muscle from rachitic chicks. Calcif Tissue Int 35(6):798–805
58. Curry OB, Basten JF, Francis MJ, Smith R (1974) Calcium uptake by sarcoplasmic reticulum of muscle from vitamin D-deficient rabbits. Nature 249(452):83–84
59. 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(39):13728–13738
60. Sakai S, Suzuki M, Tashiro Y, Tanaka K, Takeda S, Aizawa K et al (2015) Vitamin D receptor signaling enhances locomotive ability in mice. J Bone Miner Res 30(1):128–136
61. Abboud M, Puglisi DA, Davies BN, Rybchyn M, Whitehead NP, Brock KE et al (2013) Evidence for a specific uptake and retention mechanism for 25-hydroxyvitamin D (25OHD) in skeletal muscle cells. Endocrinology 154(9):3022–3030
62. Vazquez G, Boland R, de Boland AR (1995) Modulation by 1,25(OH)2-vitamin D3 of the adenylyl cyclase/cyclic AMP pathway in rat and chick myoblasts. Biochim Biophys Acta 1269(1):91–97
63. Capiati DA, Vazquez G, Boland RL (2001) Protein kinase C alpha modulates the Ca^{2+} influx phase of the Ca^{2+} response to 1alpha,25-dihydroxy-vitamin-D3 in skeletal muscle cells. Horm Metab Res 33(4):201–206
64. Boland RL (2011) VDR activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol 347(1–2):11–16
65. Morelli S, de Boland AR, Boland RL (1993) Generation of inositol phosphates, diacylglycerol and calcium fluxes in myoblasts treated with 1,25-dihydroxyvitamin D3. Biochem J. 289(Pt 3):675–679
66. Berchtold MW, Brinkmeier H, Muntener M (2000) Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 80(3):1215–1265
67. Sohl E, van Schoor NM, de Jongh RT, Visser M, Deeg DJ, Lips P (2013) Vitamin d status is associated with functional limitations and functional decline in older individuals. J Clin Endocr Metab 98(9):E1483–E1490
68. Bhat M, Kalam R, Qadri SS, Madabushi S, Ismail A (2013) Vitamin D deficiency induced muscle wasting occurs through the ubiquitin proteasome pathway and is partially corrected by calcium in male rats. Endocrinology 154(11):4018–4029
69. Antinozzi C, Corinaldesi C, Giordano C, Pisano A, Cerbelli B, Migliaccio S et al (2017) Potential role for the VDR agonist elocalcitol in metabolic control: evidences in human skeletal muscle cells. J Steroid Biochem Mol Biol 167:169–181
70. Montero-Odasso M, Duque G (2005) Vitamin D in the aging musculoskeletal system: an authentic strength preserving hormone. Mol Aspects Med 26(3):203–219
71. Phelps M, Pettan-Brewer C, Ladiges W, Yablonka-Reuveni Z (2013) Decline in muscle strength and running endurance in klotho deficient C57BL/6 mice. Biogerontology 14(6):729–739
72. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C et al (2012) Relationship of low plasma klotho with poor grip strength in older community-dwelling adults: the InCHIANTI study. Eur J Appl Physiol 112(4):1215–1220
73. Bhat M, Ismail A (2015) Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. J Steroid Biochem Mol Biol 152:171–179
74. Sinha A, Hollingsworth KG, Ball S, Cheetham T (2013) Improving the vitamin D status of vitamin D deficient adults is associated

with improved mitochondrial oxidative function in skeletal muscle. J Clin Endocr Metab 98(3):E509–E513
75. Al-Eisa ES, Alghadir AH, Gabr SA (2016) Correlation between vitamin D levels and muscle fatigue risk factors based on physical activity in healthy older adults. Clin Interv Aging 11:513–522
76. Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS et al (2016) 1alpha,25-Dihydroxyvitamin D3 regulates mitochondrial oxygen consumption and dynamics in human skeletal muscle cells. J Biol Chem 291(3):1514–1528
77. Owens DJ, Sharples AP, Polydorou I, Alwan N, Donovan T, Tang J et al (2015) A systems-based investigation into vitamin D and skeletal muscle repair, regeneration, and hypertrophy. Am J Physiol Endocrinol Metab. 309(12):E1019–E1031
78. Stratos I, Li Z, Herlyn P, Rotter R, Behrendt AK, Mittlmeier T et al (2013) Vitamin D increases cellular turnover and functionally restores the skeletal muscle after crush injury in rats. Am J Pathol 182(3):895–904
79. Choi M, Park H, Cho S, Lee M (2013) Vitamin D3 supplementation modulates inflammatory responses from the muscle damage induced by high-intensity exercise in SD rats. Cytokine 63(1):27–35
80. Ke CY, Yang FL, Wu WT, Chung CH, Lee RP, Yang WT et al (2016) Vitamin D3 reduces tissue damage and oxidative stress caused by exhaustive exercise. Int J Med Sci 13(2):147–153
81. Srikuea R, Hirunsai M (1985) Effects of intramuscular administration of 1alpha, 25(OH)2D3 during skeletal muscle regeneration on regenerative capacity, muscular fibrosis, and angiogenesis. J Appl Physiol 120(12):1381–1393
82. Barker T, Henriksen VT, Martins TB, Hill HR, Kjeldsberg CR, Schneider ED et al (2013) Higher serum 25-hydroxyvitamin D concentrations associate with a faster recovery of skeletal muscle strength after muscular injury. Nutrients 5(4):1253–1275
83. Barker T, Martins TB, Hill HR, Kjeldsberg CR, Dixon BM, Schneider ED et al (2014) Vitamin D sufficiency associates with an increase in anti-inflammatory cytokines after intense exercise in humans. Cytokine 65(2):134–137
84. Pojednic RM, Ceglia L, Lichtenstein AH, Dawson-Hughes B, Fielding RA (2015) Vitamin D receptor protein is associated with interleukin-6 in human skeletal muscle. Endocrine 49(2):512–520
85. Barker T, Schneider ED, Dixon BM, Henriksen VT, Weaver LK (2013) Supplemental vitamin D enhances the recovery in peak isometric force shortly after intense exercise. Nutr Metab (Lond). 10(1):69
86. Buitrago C, Vazquez G, De Boland AR, Boland RL (2000) Activation of Src kinase in skeletal muscle cells by 1, 1,25-(OH(2))-vitamin D(3) correlates with tyrosine phosphorylation of the vitamin D receptor (VDR) and VDR-Src interaction. J Cell Biochem 79(2):274–281
87. Sandgren ME, Bronnegard M, DeLuca HF (1991) Tissue distribution of the 1,25-dihydroxyvitamin D3 receptor in the male rat. Biochem Biophys Res Commun 181(2):611–616
88. Roh YH, Hong SW, Chung SW, Lee YS (2019) Altered gene and protein expressions of vitamin D receptor in skeletal muscle in sarcopenic patients who sustained distal radius fractures. J Bone Miner Metab. https://doi.org/10.1007/s00774-019-00995-0