Myogenic, genomic and non-genomic influences of the vitamin D axis in skeletal muscle

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Despite vitamin D-deficiency clinically presenting with myopathy, muscle weakness and atrophy, the mechanisms by which vitamin D exerts its homeostatic effects upon skeletal muscle remain to be fully established. Recent studies have shown that the receptor by which 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3) exerts its biological actions (ie, the vitamin D receptor, VDR) elicits both genomic and non-genomic effects upon skeletal muscle. The controversy surrounding skeletal muscle VDR mRNA/protein expression in post-natal muscle has been allayed by myriad recent studies, while dynamic expression of VDR throughout myogenesis, and association of higher VDR levels during muscle regeneration/immature muscle cells, suggests a role in myogenesis and perhaps an enrichment of VDR in satellite cells. Accordingly, in vitro studies have demonstrated 1,25(OH)2D3 is anti-proliferative in myoblasts, yet pro-differentiation in latter stages of myogenesis. These effects involve modulation of gene expression (VDR as a transcriptional co-activator controls ~3% of the genome) and post-genomic intracellular signalling for example, via c-Src and alterations to intramuscular calcium homeostasis and proteostasis. The aim of this review is to consider the biomolecular role for the vitamin D/VDR axis in myogenesis, while also exploring global evidence for genomic and non-genomic mechanisms of action for 1,25(OH)2D3/VDR.

KEYWORDS
differentiation, myogenesis, proliferation, skeletal muscle, vitamin D, vitamin D receptor

1 | INTRODUCTION

Vitamin D is a fat-soluble hormone that exists in two main forms. Cholecalciferol (vitamin D3), the primary source of vitamin D, is obtained predominantly via subcutaneous ultraviolet B (UVB; 290-320 nm) irradiation of 7-dehydrocholesterol (7-DHC) into vitamin D, although it can also be attained through dietary consumption of animal products such as oily fish, egg yolks and red meat.1 Comparatively, ergocalciferol (vitamin D2) is the product of UV irradiation of ergosterol, a yeast sterol present in some plants and fungi, and has commonly been prescribed as a supplement.2,3 Following UVB irradiation to vitamin D, the metabolite is subjected to two hydroxyl-ation steps: (a) In the liver, by CYP2R1 (25-hydroxylase), to form 25(OH)D3; and (b) In the kidney, by CYP27B1 (1α-hydroxylase), to form the biologically active hormone—1,25(OH)2D3.1 Serum levels of 25(OH)D3 are used in the clinic as a means of determining an individual's vitamin D status and, following NICE guidelines, serum 25(OH)D3 concentrations of less than 25 nmol/L and 25 to 50 nmol/L indicate a diagnosis of vitamin D deficiency and insufficiency, respectively.4

Approximately, 1 billion people worldwide are considered to be either vitamin D deficient or insufficient.1 This pandemic of hypovitaminosis D is

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regarded as an important global public health issue due to vitamin D’s role in human physiology, for example, the immune system, and its deficiency being implicated in a number of chronic diseases.5 Vitamin D-deficiency is classically recognized as being associated with bone diseases, such as rickets and osteoporosis.6 However, vitamin D has effects on tissues outside the skeleton that may be crucial for a wide range of other chronic health issues, with one such area being skeletal muscle. Expression of both the vitamin D receptor (VDR)7,8 and CYP27B1 has been observed in the skeletal muscle of humans and rodents.9,10 As a member of the nuclear receptor super-family, the VDR is localized to the nucleus where it binds DNA vitamin D response elements (VDREs), thereby acting as a transcription factor.11 Through observational studies, it is now evident that low vitamin D is associated with reduced muscle mass (sarcopenia) and increased risk of falls in the elderly.12 As individuals age, the expression of VDR decreases which, paired with a higher prevalence of deficiency within the older population, suggests involvement of the vitamin D system in musculoskeletal ageing.8 Indeed, vitamin D deficiency is associated with reduced muscle mass and weakness, myalgia and an increased risk of sarcopenia, regardless of activity levels.13,14 Moreover, low vitamin D has also been implicated in muscle dysfunction in chronic obstructive pulmonary disease (COPD), cancer and diabetes.15-17 Finally, numerous randomized controlled trials (RCTs) have suggested an important physiological role of the hormone within muscle, demonstrating increases in muscle function following vitamin D3 supplementation.18-20 While the mechanisms by which vitamin D and the VDR exert its actions upon skeletal muscle remain to be fully established, here we review the current state of this area.

2 | VDR EXPRESSION AND SKELETAL MUSCLE

Early studies reported a positive association between VDR content of classical tissue targets of vitamin D, for example, bone and intestine, and the level of biological activity as a response to treatment with vitamin D2.21 It has since been demonstrated, that upon treatment with 1,25(OH)2D3, VDR expression is also upregulated in skeletal muscle cells.16,22,23 However, the confirmed expression of VDR in skeletal muscle has been subject to controversy. Initial animal studies used immunohistochemistry as a means for detection, but selectivity of the widely used anti-VDR antibody 9A7 was questionable in cross-reacting with unrelated proteins on western blots.24 More recent data, confirming the presence of VDR in both human and murine skeletal muscle cells, have been generated through studies using multiple VDR antibodies.22,25,26 However, in fully differentiated murine and human muscle, VDR levels have been detected at extremely low levels or not at all, whereas VDR expression is higher in cell-cycling myoblasts than differentiated cells.22,27,28 One potential explanation for these observations is that VDR levels are dynamic in nature, being highest during immaturity followed by an age-related decline. This hypothesis is supported by the fact that VDR is displayed to be upregulated in regenerating muscle of mice that have undergone muscular injuries or exercise.10,29,30 This raises an important question—is VDR expression enriched in satellite cells? In postnatal muscle fibres, this population of muscle stem cells represents approximately 30% to 35% of the total muscle cell population, before declining to 5% in healthy adult muscle.31 As satellite cells normally exist in a quiescent state, unless stimulated to differentiate into myocytes by exercise or muscular damage, this could explain the difficulties of detecting VDR in adult muscle.32 Nonetheless, DNA-bound VDR may also represent a challenge for detection using standard extraction techniques. Next, we review in vitro and in vivo evidence linking vitamin D/VDR to muscle biology.

2.1 | Vitamin D and myogenesis

The majority of studies investigating the vitamin D axis and muscle cell function have adopted in vitro cultures of muscle cells. Myogenesis, the means by which skeletal muscle is generated, is a multi-step process and can be largely broken down into four stages: (a) Activation of satellite cells, (b) Proliferation of myoblasts, (c) Myoblast differentiation and (d) Formation of myotubes. This process is displayed in Figure 1. For further information, more in-depth reviews of myogenesis are available.33

Studies using the C2C12 murine skeletal muscle cell line have demonstrated 1,25(OH)2D3 as being anti-proliferative, but stimulatory of differentiation.23,34 Treatment of myoblasts with 100 nM 1,25(OH)2D3 for 1-week resulted in a 75% reduction in proliferating cell nuclear antigen (PCNA—a biomarker of cellular proliferation),35 while significant reductions in BrdU+ cells have also been observed following 2-day treatments at the same dosage.10,22 It is important to note that, while a dosage of 100 nM 1,25(OH)2D3 is commonly used for in vitro studies, it is highly unlikely to occur in vivo at this concentration. Future studies should aim to optimize dosages of in vitro 1,25(OH)2D3 treatments to better reflect that of physiologically relevant dosages. At the onset of differentiation, expression of the majority of cyclins is down-regulated, as this is the stage at which cells arrest in the G0/G1 phases of cell division. Expression of cyclin D3 is associated with permanent cell-cycle withdrawal and induction of early myogenesis, whereas cyclin D1 is seen to be highly expressed in proliferating myoblasts.36,37 Treatment of C2C12 myoblasts with 1 nM 1,25(OH)2D3 increases cyclin D3 expression, supporting observations that 1,25(OH)2D3 causes cellular arrest in G0/G1 phase.22,36,38 It appears that this effect occurs simultaneously with induction of the cyclin-dependent kinase inhibitors (CDKIs) p21 and p27,26 and data from studies using VDR knockdown (VDR-KD) cell lines indicate that these cell-cycle influences are VDR-dependent.37

Fusion of myoblasts into multinucleated myotubes is a strictly controlled process. The first wave occurs during embryogenesis and is responsible for generating the myotubes of the preliminary muscular system, whereas the secondary wave takes place later in a vertebrate’s lifespan. It is this second wave that is responsible for the addition of secondary myotubes/myofibres that increase muscle mass, so the impact of vitamin D on this process is of particular interest.39 Myotube formation can occur spontaneously in high serum media and is believed to rely primarily on myogenin. In culture, C2C12 myoblasts...
can be induced to exit the cell cycle and differentiate into myotubes through switching "growth culture" medium (10% foetal bovine serum, FBS) to "differentiation medium" (2% FBS). This method of differentiation induction via serum starvation is primarily driven by IGF-1 and has been used to examine the differences that occur when myoblasts are treated with vitamin D3 either at the point of differentiation initiation or when they are fully differentiated.40 Interestingly, 1,25(OH)2D3 appears to stimulate myotube formation when high-sera medium is used for both C2C12 and primary muscle cell cultures,35,41 yet it has the opposing effect in cases of differentiation via serum starvation.22,42 Thus, it is possible to suggest that vitamin D elicits differential effects depending on the model of myogenesis used. When C2C12 myoblasts are treated with 100 nM 1,25(OH)2D3 during the early differentiation phases (ie, day 0 and day 4), myogenin and neonatal myosin heavy chain (nMHC) isoforms were down-regulated, but this effect was not present in the fully differentiated group (day 8).36 In fact, treatment with 1,25(OH)2D3 at day 8 post-differentiation appeared to have an opposing effect as myosin heavy chain type IIA expression was upregulated with an additional protective role against cellular detachment.36 The inhibitory influence of 1,25(OH)2D3 upon myotube formation has been supported further as C2C12 myoblasts also treated at day 4 differentiation were only able to fuse partially, resulting in abnormally shaped myotubes possessing an increased number of myonuclei.16

The results from these aforementioned in vitro studies have been translated in vivo through the use of vitamin D deficient and/or VDR-null animals. These animals possess similar phenotypes, namely presenting with smaller muscle fibres, reduced muscle strength, higher fatigue, fibre hyper-nuclearity and abnormal gait.42-45 In rats, vitamin D deficiency causes cell-cycle arrest, which, while initially seeming undesirable for muscle function, it is possible that this action of 1,25(OH)2D3 promotes entry of myoblasts into a quiescent state.46 Notch signalling is considered a master regulator of myoblast quiescence and, while expression of full-length Notch is unaffected by deficiency, a decrease in the cleaved form of Notch is present, indicating reduced activation of the Notch pathway in vitamin D deficient rats.46,47 1,25(OH)2D3 has been demonstrated to influence the enzymes responsible for proteolytic processing of Notch, a γ-secretase complex and an ADAM protease, in neuroblastoma cells but further investigations are required to evaluate this hypothesis in skeletal muscle cells.46,48 As this pro-myogenic pathway is involved in the proliferation of satellite cells, and deficiency of vitamin D leads to a blunted Notch response
within skeletal muscle, it may be possible to place this relationship as a potential aggravator for age-related decline in myoregenerative capacities.

Phenotypic alterations of the VDR-KO mouse model are not limited to just the muscular system—these animals also display alterations, for example, to cardiovascular and gastrointestinal systems. Due to this confounding systemic impact caused by the absence of the VDR gene, it has become difficult to draw final conclusions from results produced from the total-body VDR-null mice. Tissue-specific VDR-KO animal models have been generated in an attempt to bypass this problem, and work using mice with a myocyte-specific VDR deletion (mVDR null) has demonstrated the importance of VDR in muscle function. Stark differences can be seen between these mice and the whole-body knockout animals, as the mVDR mice were of a normal body size yet their muscle fibres were marginally larger in diameter. Thus, efforts should be focused on clarifying conclusions drawn from whole-body models using this new tissue-specific model, while also yielding novel information regarding the biological activity of vitamin D.

2.2 | Genomic action of the vitamin D/VDR axis

Upon entry into the cell, 1,25(OH)2D3 binds to the VDR resulting in conformational changes in the receptor and subsequent nuclear translocation. In the nucleus the VDR associates with its heterodimeric partner, retinoid X receptor (RXR), forming a 1,25D-VDR-RXR complex that is able to modulate gene expression by binding vitamin D response elements (VDREs) in DNA. Identification of multiple genes that are under transcriptional regulation of the vitamin D system, roughly 3% of the human genome, exemplifies the sheer breadth of potential explanations for vitamin D3 mechanisms of action within normal biological function, for example, cellular adhesion, cell cycle and cytoskeletal maintenance. PCR array analysis studies using human muscle biopsies have identified a number of myogenic genes that correlate with 1,25(OH)2D3 while RNA sequencing has demonstrated alterations to expression levels of over 1900 mRNAs following treatment of human skeletal muscle cells with 1,25(OH)2D3.

With regards to myogenic-related genes, several have been found to possess VDREs. FOXO1, a transcription factor belonging to the forkhead/winged helix family, has a VDRE in its promoter and regulates muscle atrophy through its influences upon muscle protein synthesis (MPS) and cellular differentiation. VDR signalling has been shown to inhibit both expression and nuclear translocation of FOXO1, but upon knockdown of the VDR, this suppressive effect is abolished, suggesting FOXO1 as a chief target in the altered signalling demonstrated in VDR-null skeletal muscle. The insulin-like growth factor (IGF) system comprises multiple ligands (IGF-I, IGF-II and insulin), receptors and six binding proteins (IGFBPs), several of which possess VDREs within their genes. Within skeletal muscle, IGFBP-5 is the primary secreted IGFBP and, through its activation of the IGF-II auto-regulation loop, it promotes myogenic differentiation. IGFBP-5 interacts directly with the VDR and seemingly prevents heterodimerization with RXR, thereby impairing 1,25(OH)2D3 induced cell-cycle progression and gene transcription. Similarly, the gene encoding thioesterase super-family member 4 (THEM4; also known as carboxyl-terminal modulator protein, CTMP) also possesses a VDRE in its promoter region. THEM4 inhibits the phosphorylation and subsequent activation of Akt, resulting in muscle protein breakdown (MPB) due to cessation of Akt’s inhibition and activation of members of the FOXO family and mTOR, respectively. Treatment with 1,25(OH)2D3 caused upregulation of THEM4 which, paired with the fact that it has been found to be elevated in atrophic C2C12 cells, further suggests a...
role for the vitamin D system in the maintenance of muscle proteostasis.62,63 Despite being primarily implicated in skeletal health, recent studies have implicated the bone-derived hormone osteocalcin in the regulation of muscle function.64,65 The presence of a VDRE in its promoter indicates that 1,25(OH)2D3 is able to directly regulate this gene, an effect that has been demonstrated in vitro by upregulation following 1,25(OH)2D3 treatment.66 As osteocalcin has been shown to influence muscular mitochondrial function, myofibre uptake and catabolism of glucose/fatty acids, and insulin sensitivity,55,67,68 it is sensible to suggest this genomic relationship as one potential explanation for the aberrant muscle phenotype seen in VDR-KO mice. Similar to the VDR, expression levels of the osteocalcin gene have been shown to decline as individuals age.69 Lastly, expression of integrinβ3 is also recognized to be under the control of a VDRE, and deficiency of this integrin in myoblasts results in impaired myoblast migration and fusion.70 The integrins are a major family of trans-membrane receptors that act to provide connections between the extracellular matrix and the cytoskeleton.71 It seems that vitamin D increases expression of integrinβ3, thereby inducing the formation of focal adhesions that are crucial for cellular adhesion, while also potentially initiating the differentiation process in satellite cells.70,72 Currently, uncertainty exists with regards to the directly impacted target genes of 1,25(OH)2D3 in myogenic cells. The use of transcriptomics and RNA sequencing following treatment of myogenic cells, that is, satellite cells, myoblasts or myotubes, with 1,25(OH)2D3 would provide further data that may clarify the issue, while also elucidating any differences that occur at each stage of the myogenic pathway.

Polymorphisms of the VDR gene has been shown to alter the biological activity of the vitamin D-VDR complex. The rs2228570 single nucleotide polymorphism (SNP), more commonly known as FokI, is located at the 5’ end of the VDR gene, leading to an altered start codon, and a subsequently abnormally sized VDR protein.73 Comparatively, the rs1544410 (BsmI) and rs731236 (TaqI) SNPs are localized close to the 3’ un-translated region (3’-UTR) and, therefore, have no impact on the structural characteristics of the receptor.73 Thus, it is probable that not only vitamin D status, but also expression and structure of VDR, govern molecular actions, and can potentially modify the risk of myopathies.74-76 Unlike environmental factors (eg, sunlight exposure, season, dietary or supplementary intake) that are all highly variable, genetic variants are constant from conception and un-modifiable, thereby eliminating concern for reverse causality. While VDR polymorphisms have been linked to several disease outcomes via systematic reviews, including skeletal health,77,78 there is an evident lack of studies investigating associations between skeletal muscle health and variants of the VDR gene. Future VDR polymorphism studies are imperative as potentially key SNPs could be identified that may predispose an individual to vitamin D deficiency or certain musculoskeletal disorders, for example, sarcopenia.

Finally, it appears that these genomic influences of vitamin D permit a relationship between the vitamin D/VDR axis and mitochondrial metabolism. Recent supplementation studies have indicated that 1,25(OH)2D3 results in improved mitochondrial function within skeletal muscle,79 but the mechanism for this beneficial effect is yet to be fully elucidated. Since mitochondrial translocation of VDR was first described,80 two key questions remain unanswered: (a) What is the general role of vitamin D in mitochondria, and (b) how does this relationship specifically translate into skeletal muscle? Potential explanations may be that 1,25(OH)2D3 can directly influence energy production (eg, ATP synthesis) by eliciting its genomic effects on mechanisms such as oxidative phosphorylation or the tricarboxylic acid (TCA) cycle.81 This theory has been explored by several research groups, all of which have demonstrated upregulation of expression levels of genes associated with the TCA cycle and electron transport chain (ETC).53,82 In addition, studies using VDR-KD cell lines have shown that the rate of ATP synthesis derived from oxidative phosphorylation is reduced by 20% vs a shRNA scrambled control,83 and that this absence of VDR is also associated with increased production of reactive oxygen species (ROS).84 While evidence does exist to indicate a relationship between mitochondria and the vitamin D system it is clear that further investigations are required for concise conclusions to be made.

2.3 | Signal transduction pathway (non-genomic) regulation of muscle by the vitamin D/VDR axis

Muscle proteostasis is tightly regulated by an intrinsic balance between the rates of muscle protein synthesis (MPS; anabolism) and breakdown (MPB; catabolism). If the net rate of MPS exceeds that of MPB, then the net muscle protein balance (NPB) is considered to be positive, and growth of muscle fibres is achieved; alternatively, muscle atrophy occurs when the NPB is negative due to rate of MPB being higher than MPS.85,86 Of the various proteolytic systems existing in muscle, vitamin D has been mainly implicated in one in particular, the ubiquitin-proteasomal pathway (UPP).87,88 In rats, deficiency of vitamin D resulted in increased levels of MPB, significantly elevated expression of both the ubiquitin-conjugating enzyme, E2, and its ubiquitin conjugates, but no alterations to either calpain or lysosomal enzymes were observed.87 At the heart of the UPP lies the 26S proteasome, a large complex comprised of two smaller sub-complexes: the 20S core peptidase and the 19S regulatory unit.89 The proteasomal activity of the 20S subunit was seen to be upregulated in biopsies from the vitamin D-deficient mouse model, thereby indicating an increase in non-lysosomal proteolysis and a place for the vitamin D system in muscle protein degradation.87 Further to this, expression of both muscle ring finger protein 1 (MuRF1) and muscle atrophy F-box protein (MaFbx, also known as Atrogin-1) has been seen to be higher in both the vitamin-deficient and VDR-KO mice when compared with the control animals,26,87 a result similar to that observed in the sarcopenic muscle of aged rats.90

Conversely, hypertrophy (an increase in size of muscle cells) is largely governed via intracellular signalling cascades, and several attempts have been made in order to try and clarify the relationship between these myogenic processes and 1,25(OH)2D3 (Figure 3). Potential explanations have stemmed from studies that investigated
influences upon cellular signalling, particularly post-translational modifications (PTMs) as they can be imperative to normal signalling, as exemplified by the effects exerted by 1,25(OH)\textsubscript{2}D\textsubscript{3} upon altered phosphorylation of certain members of the mitogen-activated protein kinase (MAPK) pathway.\textsuperscript{91} In its phosphorylated state, Raf-1 (proto-oncogene serine/threonine-protein kinase) is able to initiate a protein kinase cascade that results in gene transcription being induced via MAPK. When C2C12 myoblasts are treated with 1,25(OH)\textsubscript{2}D\textsubscript{3}, a rapid rise in serine phosphorylation of Raf-1 is seen alongside a steep rise in dephosphorylation of the Ras-GTPase-activating protein.\textsuperscript{91} In line with these results, it has also been demonstrated that 1,25(OH)\textsubscript{2}D\textsubscript{3} can activate the cellular sarcoma kinase (c-Src), a major transcription factor that lies upstream of Raf-1, thereby also promoting gene transcription through downstream activation of MAPK.\textsuperscript{92} Studies using VDR-KD cell lines have confirmed that activation of MAPK signalling is dependent on VDR, as both c-Src and p38 MAPK were significantly down-regulated in its absence.\textsuperscript{92} Furthermore, reports have revealed that 1,25(OH)\textsubscript{2}D\textsubscript{3} treatment of rats undergoing high-intensity exercise reduces expression of key components of the MAPK signal cascade, namely extracellular signal-regulated kinases 1 and 2 (ERK1/2), in comparison to the placebo group.\textsuperscript{93} Due to previous demonstrations that inhibition of ERK1/2 in myocytes leads to the induction of muscle atrophy and upregulation of MuRF1 and MaFbx, the idea of 1,25(OH)\textsubscript{2}D\textsubscript{3} reducing MPS via diminishing ERK1/2 expression levels seems rather paradoxical.\textsuperscript{94} It is possible that this effect of vitamin D is dependent on what developmental stage the myogenic cell is in at the time of the study because, as discussed, its effects can be anti-proliferative. Under basal conditions, c-Src is co-localized with caveolin-1 in close proximity to the plasma membrane but, upon treatment of 1,25(OH)\textsubscript{2}D\textsubscript{3}, VDR translocation to the plasma membrane occurs followed by disruption of the co-localization.\textsuperscript{95,96} It seems that association with caveolin-1 suppresses kinase activity of c-Src,
meaning it is possible to hypothesize that 1,25(OH)₂D₃-induced translocation of VDR permits activation of c-Src signalling, therefore suggesting that c-Src acts as a gateway to cell signalling for vitamin D.

As c-Src is under positive regulation by vitamin D₃ and is also known to be an upstream regulator of Akt, it is, therefore, possible that the hormone is able to have some element of control over mechanistic target of rapamycin (mTOR) and p70S6 kinase (p70S6K) signalling. The Akt/mTOR signalling pathway is deemed to be one of the principal regulators of muscle mass in skeletal muscle, and myogenesis is seen to be impaired when mTOR is inhibited following muscle injury.97 Akt is able to positively control MPS via its relationship with mTOR, while also repressing MPB through its inhibitive phosphorylation of members of the FOXO family of transcription factors.98 Vitamin D deficiency is associated with down-regulation of mTOR and subsequent suppression of its phosphorylation of eIF4E-binding protein 1 (4EBP1) and p70-S6K, implying an overall decrease in muscle protein synthesis (MPS).99 This was supported by the demonstration of sensitization of the mTOR/Akt pathway by 1,25(OH)₂D₃ in C2C12 myoblasts to the effects of insulin and leucine through upregulation of phosphorylation, and consequent inhibition, of glycogen synthase kinase 3β (GSK3β) by Akt.100

Upstream of Akt lies myostatin, a negative regulator of muscle mass whose absence in both KO animal models, and human loss-of-function mutations leads to muscular hypertrophy and hyperplasia (an increased number of muscle cells).101,102 In vitro studies using the C2C12 cell line have revealed that 1,25(OH)₂D₃ treatment at the myoblast stage induces a transition from cell proliferation to differentiation by reducing the expression of myostatin and up-regulating its antagonist, Follistatin.35 Paradoxically, these results confute previous work reporting a decrease in expression levels of pro-myogenic factors upon myoblast treatment with 1,25(OH)₂D₃ compared to muscle obtained from VDR-null mice.42 When taking the experimental protocols used into consideration, it is possible that these contradictions may be due to time-dependent and/or dosage effects. A single dose of 1,25(OH)₂D₃ treatment lasting 48 to 96 hours,42 may have elicited an acute response resulting in diminished myogenesis, whereas prolonged exposure (ie, seven daily doses) could be considered as chronic and brings about pro-myogenic influences.35

Recent data have suggested that it may be the relationship between vitamin D₃ and intracellular calcium (Ca²⁺) homeostasis that drives certain observations. The response of protein kinase C (PKC) to 1,25(OH)₂D₃ treatment was investigated in vitro using chick myoblasts and myotubes.103 Vitamin D-induced translocation of PKC from the cytosol to the cell membrane was markedly higher in myotubes, as was the rise in Ca²⁺ concentration.103 This intracellular influx of Ca²⁺ is notably reduced in antisense PKC knockdown models,104 but it appears to be VDR independent as no change is seen to the increase in PKC translocation following 1,25(OH)₂D₃ treatment in VDR-KO models.105 PKC modulates Ca²⁺ homeostasis via its activation of L-type voltage-dependent calcium channels (VDCCs) and store-operated calcium entry (SOCE).106 Hence it is possible to propose that the vitamin D-induced Ca²⁺ influx is mediated by these actions of PKC. Structurally, SOC channels are constituted of various members of the TRP-C (transient receptor potential-canonical) protein family, of which TRPC3 has been identified as a primary mediator of SOCE induced by 1,25(OH)₂D₃.107 In addition, TRPC1 is required for fusion of myoblasts into myotubes,108 which may partly explain conclusions made by Zhang et al regarding the positive influence of 1,25(OH)₂D₃ upon the influx of calcium ions in myotubes, but not myoblasts, when in a calcium sufficient environment.109 It is well recognized that calcium is required for fusion of myoblasts during their differentiation process,110,111 thus it is sensible to theorize that the suppressive influences upon myoblast-to-myotube formation may be partly due to a reduction in cytosolic calcium. Finally, it has been documented that the perturbed Ca²⁺ uptake that accompanies vitamin D deficiency alters the kinetics of muscle contraction, thereby extending the relaxation phase of contraction.112,113 Under basal conditions, mitochondrial uptake of Ca²⁺ occurs during the twitch and tetanic responses of muscle contraction,114 but when the cell is in a vitamin D deficient state it seems that oxidative phosphorylation is impaired, and mitochondria are unable to retain Ca²⁺.115 If contraction kinetics are altered by this perturbed Ca²⁺ then it may be possible that this is the underlying mechanism for the muscle weakness that is a classic clinical manifestation of vitamin D deficiency.

When this evidence is considered together, it is evident that the non-genomic pathway of 1,25(OH)₂D₃ simultaneously activates numerous rapid myogenic-related signalling pathways, however, their relative involvement to myogenesis is yet to be fully established. It is clear that 1,25(OH)₂D₃ modulates key signalling cascades that have the end-result of promoting VDR nuclear translocation and activation of gene transcription, hence it is probable that these processes are directly linked to one another. However, it is not clear whether the relationships exist in a time-ordered fashion, or whether they are coordinated, thus further highlighting the gaps within the field.

### 2.4 Key future considerations in skeletal muscle vitamin D/VDR research

Although the main aim of this review was to provide a state-of-the-art overview of the molecular and cellular mechanisms that underpin the proposed actions of vitamin D on muscle physiology and disease, it is important to recognize the difficulties in extrapolating between in vitro and in vivo studies of the vitamin D system and muscle. In common with many other studies of vitamin D and human health, the link between vitamin D and muscle function in vivo has focused primarily on the relationship between serum levels of 25(OH)D₃ and diverse muscle parameters.12 Overall, vitamin D-deficiency—low serum levels of 25(OH)D₃—has been shown to be associated with muscle pain and muscle weakness.116 However, the changes in serum inactive 25(OH)D₃ do not necessarily correspond to the changes in active 1,25(OH)₂D₃. The latter is rarely measured in human studies of muscle function and sarcopenia, although when this is carried out it is clear that serum 1,25(OH)₂D₃ is a better correlate of muscle function than serum 25(OH)D₃.32 Thus, in future studies, to investigate the potential benefits of vitamin D supplementation for muscle health, it
may be more meaningful to analyse the circulating levels of 1,25(OH)2D3, as well as the more conventionally assayed 25(OH)D3. In addition, it is probable that other metabolites of vitamin D could provide further information regarding an individual’s vitamin D status, and in-depth reviews are available that address this topic. It is also important to recognize that studies of vitamin D supplementation may lead to increased serum 25(OH)D3 that, despite being an inactive form of vitamin D, is still able to influence vitamin D function in target tissues. Specifically, it is recognized that many extra-skeletal actions of vitamin D are due to target tissue uptake of 25(OH)D3 and localized CYP27B1 conversion to 1,25(OH)2D3, which can then act on endogenous VDR. This intracrine mode of action may be strongly influenced by substrate 25(OH)D3 availability—in other words, by the vitamin D status of any given individual—and may therefore be the pivotal mechanism linking vitamin D-deficiency or—supplementation—with in vivo health parameters. It is unclear whether this mode of action is functional in muscle. Differentiated C2C12 cells and primary murine myoblasts have been reported to express both VDR and CYP27B1, with uptake of 25(OH)D3 being stimulated by 1,25(OH)2D3. Thus, it is possible that muscle cells operate an intracrine mode of vitamin D metabolism similar to that observed in many other cells/tissues, for example, monocytes of the immune system, with this being a 1,25(OH)2D3-enhanced process. Other studies, using C2C12 cells and primary myoblasts, have shown that uptake of 25(OH)D3 is also stimulated by parathyroid hormone (PTH), suggesting that multiple components of the vitamin D system overlap to promote vitamin D function in muscle. It is still unclear how muscle cells acquire 25(OH)D3. In many extra-renal and extra-skeletal tissues, it appears that uptake of 25(OH)D3 is independent of its serum carrier vitamin D binding protein (DBP). However, it has been reported that muscle cells utilize the same megalin-mediated receptor-dependent uptake of 25(OH)D3, which is characteristic of cells from the proximal tubules of the kidney. It is, therefore, possible that serum DBP plays a much more active role than previously thought in the effects of vitamin D on muscle. Specifically, disease or genetically determined changes in DBP concentrations may profoundly influence muscle cell uptake of 25(OH)D3, local levels of 1,25(OH)2D3 and subsequent muscle responses.

3 | CONCLUSIONS

Despite the clinical consequences of its deficiency, there still remains to be a clear lack of mechanistic understanding behind the biological functioning of 1,25(OH)2D3 upon skeletal muscle. Many supplementation studies, particularly in the elderly, have provided data implicating its beneficial effects in counteracting musculoskeletal disorders, such as sarcopenia, yet confliction within evidence obtained from both in vitro and animal models suggests that further clarification is required before definite conclusions can be made. Future research should seek to establish the mechanisms, both genomic and non-genomic, that underlie the actions of 1,25(OH)2D3 upon skeletal muscle, while also clarifying an optimal physiologically relevant dosage for obtaining its beneficial effects in vitro. VDR polymorphism studies would be useful in identifying potential SNPs that may predispose an individual to vitamin D deficiency or certain musculoskeletal disorders, for example, sarcopenia. Moving forward, great emphasis should be placed on generating concrete evidence for the role of vitamin D3 in the different cellular development stages, or sub-populations, while also focusing on clarifying the dynamics between mitochondrial function and the vitamin D system, particularly in skeletal muscle. This work would be of great physiological importance as it possesses the potential to finally unlock the full therapeutic potential of the “sunshine” vitamin, thereby permitting clinical translation.

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REFERENCES

1. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-281.
2. Nair R, Maseeh A. Vitamin D: the sunshine vitamin. J Pharmacoal Pharmacother. 2012;3:118-126. https://doi.org/10.4103/0976-500X.95506.
3. Mostafa WZ, Hegazy RA. Vitamin D and the skin: focus on a complex relationship: a review. J Adv Res. 2015;6:793-804. https://doi.org/10.1016/j.jare.2014.01.011.
4. NICE. Vitamin D deficiency in adults - treatment and prevention: how should I diagnose vitamin D deficiency in adults? 2018. Available: https://cks.nice.org.uk/topics/vitamin-d-deficiency-in-adults-treatment-prevention/diagnosis/diagnosis/
5. Priett B, Treiber G, Pieber T, Amrein K. Vitamin D and immune function. Nutrients. 2013;5:2502-2521. https://doi.org/10.3390/nu5072502.
6. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116:2062-2072.
7. Girgis CM, Mokbel N, Cha KM, et al. The Vitamin D receptor (VDR) is expressed in skeletal muscle of male mice and modulates 25-hydroxyvitamin D (25OHD) uptake in myofibers. Endocrinology. 2014;155:3227-3237. https://doi.org/10.1210/en.2014-1016.
8. Bischoff-Ferrari HA, Borcher M, Gudat F, Dürmüller U, Stähelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res. 2004;19:265-269. https://doi.org/10.1359/jbmr.2004.19.2.265.
9. Kim K, Gong HS, Lim JY, Kim JH, Baek GH. The vitamin D receptor expression in skeletal muscle of women with distal radius fracture. Arch Osteoporos. 2018;13:4-9. https://doi.org/10.1007/s11657-018-0442-8.
10. Srikueba R, Zhang X, Park-Sarge O-K, Esser KA. VDR and CYP27B1 are expressed in C2C12 cells and regenerating skeletal muscle: potential role in suppression of myoblast proliferation. Am J Physiol Cell Physiol. 2012;303:C396–C405. https://doi.org/10.1152/ajpcell.00014.2012.
11. Haussler MR, Whitfield GK, Haussler CA, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res. 1998;13:325-349. https://doi.org/10.1002/jbmr.1998.13.3.325.
12. Wicherts IS, Van Schoor NM, Boeke AJP, et al. Vitamin D status predicts physical performance and its decline in older persons. J Clin...
van der Meijden K, Bravenboer N, Dirks NF, et al. Effects of 1,25(OH)2D3 during skeletal muscle regeneration on regenerative capacity, muscular fibrosis, and angiogenesis. J Appl Physiol. 2016;120:1381-1393. https://doi.org/10.1152/japplphysiol.00180.2015.

Allbrook DB, Han MF, Hellmuth AE. Population of muscle satellite cells in relation to age and mitotic activity. Pathology. 1971;3:233-243. https://doi.org/10.1080/003302719073739.

Fu X, Wang H, Hu P. Stem cell activation in skeletal muscle regeneration. Cell Mol Life Sci. 2015;72:1663-1677. https://doi.org/10.1007/s00018-014-1819-5.

Chal J, Pourquèi O. Making muscle: skeletal myogenesis in vivo and in vitro. Development. 2017;144:2104-2122.

García LA, Ferrini MG, Norris KC, Artaza JN. 1,25(OH)2 vitamin D3 enhances myogenic differentiation by modulating the expression of key angiogenic growth factors and angiogenic inhibitors in C2C12 skeletal muscle cells. J Steroid Biochem Mol Biol. 2013;133:1-11. https://doi.org/10.1016/j.jid.2014.371.

García LA, King KK, Ferrini MG, Norris KC, Artaza JN. 1,25(OH)2 vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. Endocrinology. 2011;152:2976-2986. https://doi.org/10.1210/en.2011-0159.

Okuno H, Kishimoto KN, Hatori M, Itoi E. 1α,25-dihydroxyvitamin D3 enhances fast-myosin heavy chain expression in differentiated C2C12 myoblasts. Cell Biol Int. 2012;36:441-447. https://doi.org/10.1042/cbi20100782.

Irazoqui AP, Boland RL, Buitrago CG. Actions of 1,25(OH)2-vitamin D3 on the cellular cycle depend on VDR and p38 MAPK in skeletal muscle cells. J Mol Endocrinol. 2014;53:331-343. https://doi.org/10.1530/jme-14-0102.

Rohan JNP, Weigel NL. 1α,25-dihydroxyvitamin D3 reduces c-Myc expression, inhibiting proliferation and causing G1 accumulation in C4-2 prostate cancer cells. Endocrinology. 2009;150:2046-2054. https://doi.org/10.1210/en.2008-1395.

André LM, Ausems CRM, Wansink DG, Wieringa B. Abnormalities in skeletal muscle myogenesis, growth, and regeneration in myotonic dystrophy. Front Neurol. 2018;9:1-24. https://doi.org/10.3389/fneur.2018.00368.

Yoshiko Y, Hirao K, Maeda N. Differentiation in C2C12 myoblasts depends on the expression of endogenous IGFs and not serum depletion. Am J Physiol Cell Physiol. 2002;283:C1278–C1286. https://doi.org/10.1152/ajpcell.00168.2002.

Braga M, Simmons Z, Ferrini MG, Norris KC, Artaza JN. Vitamin D induces myogenic differentiation in skeletal muscle derived stem cells. Endocr Connect. 2017;6:139-150. https://doi.org/10.1530/ec-17-0008.

Endo I, Inoue D, Mitsui T, et al. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology. 2003;144:5138-5144. https://doi.org/10.1210/en.2003-1102.

Yoshiko Y, Hirao K, Maeda N. Differentiation in C2C12 myoblasts depends on the expression of endogenous IGFs and not serum depletion. Am J Physiol Cell Physiol. 2002;283:C1278–C1286. https://doi.org/10.1152/ajpcell.00168.2002.

Minasyan A, Keisala T, Zou J, et al. Vestibular dysfunction in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol. 2009;114:161-166. https://doi.org/10.1016/j.jsbmb.2009.01.020.

Burne THJ, Johnston ANB, McGrath JJ, Mackay-Sim A. Swimming behaviour and post-swimming activity in vitamin D receptor knockout mice. Brain Res Bull. 2006;69:74-78. https://doi.org/10.1016/j.brainresbull.2005.10.014.

Girgis CM, Cha KM, Houweling PJ, et al. Vitamin D receptor ablation in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology. 2003;144:5138-5144. https://doi.org/10.1210/en.2003-1102.

Srikuea R, Hirunsai M. Effects of intramuscular administration of 1α,25(OH)2D3 on muscle strength and motor function. J Steroid Biochem Mol Biol. 2010;114:5138-5144. https://doi.org/10.1016/j.jsbmb.2009.11.029.

Girgis CM, Cha KM, So B, et al. Mice with myocyte deletion of vitamin D receptor have sarcopenia and impaired muscle function. J Cachexia Sarcopenia Muscle. 2019;10:1228-1240. https://doi.org/10.1002/jcsm.12460.

Wang Y, DeLuca HF. Is the vitamin D receptor found in muscle? Endocrinology. 2011;152:354-363. https://doi.org/10.1210/en.2010-1109.

Sandgren ME, Brönnegärd M, DeLuca HF. Tissue distribution of the 1,25-dihydroxyvitamin D3 receptor in the male rat. Biochem Biophys Res Commun. 1991;181:611-616. https://doi.org/10.1016/0006-291X(91)91234-4.

Makanae Y, Ogasawara R, Sato K, et al. Acute bout of resistance exercise increases vitamin D receptor protein expression in rat skeletal muscle. Exp Physiol. 2015;100:1168-1176. https://doi.org/10.1113/EP085207.
atrophy in old Wistar rats. Nutr Metab. 2014;11:1-13. https://doi.org/10.1186/1743-7075-11-47.

47. Bjornson CRR, Cheung TH, Liu L, Tripathi PV, Steeper KM, Rando TA. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells. 2012;30:232-242. https://doi.org/10.1002/stem.773.

48. Grimm M, Thiel A, Lauer A, et al. Vitamin D and its analogues decrease amyloid-β (Aβ) formation and increase Aβ-degradation. Int J Mol Sci. 2017;18:2764. https://doi.org/10.3390/ijms18122764.

49. Bouillon R, Bischoff-Ferrari H, Willett W. Vitamin D and health: perspectives from mice and man. J Bone Miner Res. 2008;23:974-979. https://doi.org/10.1359/Jbmr.080420.

50. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. Endocrinol Metab Clin N Am. 2010;39:243-253. https://doi.org/10.1016/j.ecl.2010.02.002.

51. Wang T-T, Tavera-Mendoza LE, Lapierriere D, et al. Large-scale in-silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol. 2005;19:2685-2695.

52. Hassan-Smith ZK, Jenkinson C, Smith DJ, et al. 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 exert distinct effects on human skeletal muscle function and gene expression. PLoS One. 2017;12:1-20. https://doi.org/10.1371/journal.pone.0170665.

53. Ryan ZC, Craig TA, Holmes CD, et al. 1α,25-dihydroxyvitamin D3 regulates mitochondrial oxygen consumption and dynamics in human skeletal muscle cells. J Biol Chem. 2016;291:1514-1528.

54. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech. 2013;6:25-39.

55. Chen S, Villalta SA, Agrawal DK. FOXO1 mediates vitamin D metabolism. Endocrinol Metab Clin N Am. 2010;39:243-253. https://doi.org/10.1016/j.ecl.2010.02.002.

56. Schedlich LJ, Muthukaruppan A, O’Han MK, Baxter RC. Insulin-like growth factor binding protein-5 interacts with the vitamin D receptor to regulate Akt signaling during skeletal muscle atrophy in vitro and α V β 3 expression. Stem Cells Int. 2018;2018:1-9. https://doi.org/10.1155/2018/6958713.

57. Uitterlinden AG, Fang Y, van Meurs JBJ, Pols HAP, van Leeuwen JPTM. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004;338:143-156. https://doi.org/10.1016/j.gene.2004.05.014.

58. Sicmea M, Centofanti F, Celi M, et al. Vitamin D receptor in muscle atrophy of elderly patients: a key element of osteoporosis-sarcopenia connection. Aging Dis. 2018;9:952-964. https://doi.org/10.14336/AD.2018.0215.

59. Roth SM, Zmuda JM, Cauley JA, Shea PR, Ferrell RE. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. J Gerontol Ser A Biol Sci Med Sci. 2004;59:B10-B15. https://doi.org/10.1093/gerona/59.1.B10.

60. Walsh S, Ludlow AT, Metter EJ, Ferrucci L, Roth SM. Replication study of the vitamin D receptor (VDR) genotype association with skeletal muscle traits and sarcopenia. Aging Clin Exp Res. 2016;28:435-442. https://doi.org/10.1007/s00520-015-0447-8.

61. Zhang L, Yin X, Wang J, et al. Associations between VDR gene polymorphisms and osteoporosis risk and bone mineral density in postmenopausal women: a systematic review and meta-analysis. Sci Rep. 2018;8:981. https://doi.org/10.1038/s41598-017-18670-7.

62. Liu Y, Li C, Chen P, et al. Polymorphisms in the vitamin D receptor (VDR) and the risk of ovarian cancer: a meta-analysis. Miao X-P, editor. PLoS One. 2013;8:e66716. https://doi.org/10.1371/journal.pone.0066716.

63. Sinha A, Hollingsworth KG, Ball S, Cheetham T. Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. J Clin Endocrinol Metab. 2013;98:E509-E513. https://doi.org/10.1210/jc.2012-3592.

64. Silvagno F, Consiglio M, Foglizzo V, Destefanis M, Pescarmona G. Mitochondrial translocation of vitamin D receptor is mediated by the permeability transition pore in human keratinocyte cell line. Makishima M, editor. PLoS One. 2013;8:e54716. https://doi.org/10.1371/journal.pone.0054716.

65. Silvagno F, Pescarmona G. Spotlight on vitamin D receptor, lipid metabolism and mitochondria: some preliminary emerging issues. Mol Cell Endocrinol. 2017;450:24-31. https://doi.org/10.1016/j.mce.2017.04.013.

66. Muñoz García A, Eijssen LM, Kutmon M, et al. A bioinformatics workflow to decipher transcriptomic data from vitamin D studies. J Steroid Biochem Mol Biol. 2019;189:28-35. https://doi.org/10.1016/j.jsbmb.2019.01.003.

67. Clemens TL, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. J Bone Miner Res. 2011;26:677-680.

68. Sims NA, White CP, Sunn KL, et al. Human and murine osteocalcin gene expression: conserved tissue restricted expression and divergent responses to 1,25-dihydroxyvitamin D3 in vivo. Mol Endocrinol. 1997;11:1695-1708.

69. Oury F, Khirimian L, Denny CA, et al. Maternal and offspring pools of osteocalcin influence brain development and functions. Cell. 2013;155:226-241.

70. Liu H, Niu A, Chen S-E, Li Y-P. Beta3-integrin mediates satellite cell differentiation in regenerating muscle. FASEB J. 2011;25:1914-1921.

71. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. New York, NY: Garland Science; 2008.

72. Posa F, Di Benedetto A, Cavalcanti-Adam EA, et al. Vitamin D promotes MSC osteogenic differentiation stimulating cell adhesion and α V β 3 expression. Stem Cells. 2018;2018:1-9. https://doi.org/10.1158/2018/6958713.
84. Ricca C, Allion A, Bergandi L, Alotto D, Castagnoli C, Silvagno F. Vitamin D receptor is necessary for mitochondrial function and cell health. Int J Mol Sci. 2018;19:1672. https://doi.org/10.3390/ijms19061672.

85. Breen L, Phillips SM. Skeletal muscle protein metabolism in the elderly: interventions to counteract the "anabolic resistance" of ageing. Nutr Metab (Lond). 2011;8:68. https://doi.org/10.1186/1743-7075-8-68.

86. Tipton KD, Hamilton DL, Gallagher JI. Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans. Sports Med. 2018;48:53-64. https://doi.org/10.1007/s40279-017-0845-5.

87. Bhat M, Kalam R, Syh Qadri S, Madabushi S, Ismail A. Vitamin D deficiency-induced muscle wasting occurs through the ubiquitin proteasome pathway and is partially corrected by calcium in male rats. Endocrinology. 2013;154:4018-4029. https://doi.org/10.1210/ en.2013-1369.

88. Bhat M, Ismail A. Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. J Steroid Biochem Mol Biol. 2015;152:171-179. https://doi.org/10.1016/j.jsbmb.2015.05.012.

89. Bard JAM, Goodall EA, Greene ER, Jonsson E, Dong KC, Martin A. Vitamin D receptor is necessary for mitochondrial function and cell health. J Steroid Biochem Mol Biol. 2000;77:200-212. https://doi.org/10.1002/sici.1097-4644(20000501)77:2<200::aid-jcb4>3.0.co;2-5.

90. Capiati DA, Vazquez G, Boland RL. Protein kinase Cα regulates 1,25(OH)2-vitamin D3-induced store-operated Ca2+ influx in skeletal muscle cells. J Biol Chem. 2003;278:2199-2205. https://doi.org/10.1074/jbc.M205732200.

91. Capiati DA, Pardo V, Boland RL. Role of VDR in 1α,25-dihydroxyvitamin D3-dependent non-genomic activation of MAPKs, Src and Akt in skeletal muscle cells. J Steroid Biochem Mol Biol. 2013;136:125-130. https://doi.org/10.1016/j.jsbmb.2013.02.012.

92. Choi M, Park H, Cho S, Lee M. Vitamin D3 supplementation modulates inflammatory responses from the muscle damage induced by high-intensity exercise in SD rats. Cytokine. 2013;63:27-35. https://doi.org/10.1016/j.cyto.2013.03.018.

93. Shi H, Scheffler JM, Zeng C, et al. Mitogen-activated protein kinase signaling is necessary for the maintenance of skeletal muscle mass. Am J Physiol Cell Physiol. 2009;296:C1040–C1048. https://doi.org/10.1152/ajpcell.00475.2008.

94. Buitrago CG, Boland RL. Caveolae and caveolin-1 are implicated in 1,25(OH)2-vitamin D3-dependent modulation of Src, MAPK cascades and VDR localization in skeletal muscle cells. J Steroid Biochem Mol Biol. 2010;121:169-175. https://doi.org/10.1016/j.jsbmb.2010.03.002.

95. Capiati D, Benassati S, Boland RL. 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. 2002;86:128-135. https://doi.org/10.1002/jcb.10191.

96. Ge Y, Wu A-L, Warnes C, et al. mTOR regulates skeletal muscle regeneration in vivo through kinase-dependent and kinase-independent mechanisms. Am J Physiol Cell Physiol. 2009;297:C1434-C1444.

97. Sandri M, Sandri C, Gilbert A, et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell. 2004;117:399-412. https://doi.org/10.1016/s0092-8674(04)00400-3.
116. Dawson-Hughes B. Serum 25-hydroxyvitamin D and muscle atrophy in the elderly. *Proc Nutr Soc*. 2012;71:46-49. https://doi.org/10.1017/S0029665111003260.

117. Dirks N, Ackermans M, Lips P, et al. The when, what & how of measuring vitamin D metabolism in clinical medicine. *Nutrients*. 2018;10:482. https://doi.org/10.3390/nu10040482.

118. Adams JS, Hewison M. Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. *Arch Biochem Biophys*. 2012;523:95-102. https://doi.org/10.1016/j.abb.2012.02.016.

119. Hewison M. Vitamin D and immune function: autocrine, paracrine or endocrine? *Scand J Clin Lab Invest Suppl*. 2012;243:92-102. https://doi.org/10.3109/00365513.2012.682862.

120. Abboud M, Rybchyin MS, Liu J, et al. The effect of parathyroid hormone on the uptake and retention of 25-hydroxyvitamin D in skeletal muscle cells. *J Steroid Biochem Mol Biol*. 2017;173:173-179. https://doi.org/10.1016/j.jsbmb.2017.01.001.

121. Chun RF, Lauridsen AL, Suon L, et al. Vitamin D-binding protein directs monocyte responses to 25-hydroxy and 1,25-dihydroxyvitamin D. *J Clin Endocrinol Metab*. 2010;95:3368-3376. https://doi.org/10.1210/jc.2010-0195.

122. Abboud M, Puglisi DA, Davies BN, et al. Evidence for a specific uptake and retention mechanism for 25-hydroxyvitamin D (25OHD) in skeletal muscle cells. *Endocrinology*. 2013;154:3022-3030. https://doi.org/10.1210/en.2012-2245.

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