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Review

The effects of vitamin D on skeletal muscle function and cellular signaling

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ABSTRACT

It is thought that every cell in the body expresses the vitamin D receptor, and therefore vitamin D may play a role in health and homeostasis of every organ system, including skeletal muscle. Human, animal, and cell culture studies have collectively shown that vitamin D affects muscle strength and function. Vitamin D functions in a plethora of cellular processes in skeletal muscle including calcium homeostasis, cell proliferation, cell differentiation, fiber size, prevention of fatty degeneration, protection against insulin resistance and arachidonic acid mobilization. These processes appear to be mediated by several signaling pathways affected by vitamin D. This review aims to explore the effects of vitamin D on skeletal muscle in each model system and to delineate potential cell signaling pathways affected by vitamin D.

Contents

1. Introduction

The role of vitamin D in health and disease has been a popular topic in the medical literature and popular news for two reasons. First, the prevalence of vitamin D deficiency and insufficiency is much more wide spread than once thought. Vitamin D insufficiency has been defined as 25(OH)D concentrations between 20 and 30 ng/ml, while concentrations below 20 ng/ml are considered deficient [1]. In the United States 70% of children, ranging in age from 1 to 21 years, were found to be vitamin D deficient or insufficient [2]. In adults the prevalence may be up to 73% and in the elderly nearly up to 78% [3]. Shown in the Third National Health and Nutrition Examination Survey 2001–2004, African American and Mexican American individuals had lower mean serum concentrations compared to white individuals [4]. Ethnic groups with darker skin require proportionally more sun exposure to synthesize equivalent amounts of vitamin D compared with people with lighter skin [5]. Obese individuals also have a

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higher risk of vitamin D insufficiency or deficiency compared to non-obese; likely due to decreased bioavailability of vitamin D caused by its sequestration in cutaneous fat depots [6]. Other contributing factors behind the wide spread vitamin D insufficiency or deficiency include inadequate vitamin D in the diet, lack of sun exposure, and genetic factors [7]. A recent study has identified common gene variants that increase the risk for vitamin D deficiency [7]. Hence, the majority of the U.S. population may have suboptimal concentrations of vitamin D that are potentially impacting health.

Secondly, it has come to light that vitamin D has much more of a global role in health and disease of numerous organ systems than once realized. Scientists and physicians are revealing that the physiological role of vitamin D is quite expansive beyond its classical role in calcium homeostasis and skeletal health. For example, vitamin D has been shown to reduce the risk of various cancers, hypertension, heart disease, infectious diseases, multiple sclerosis, rheumatoid arthritis, asthma, and depression [8–13]. Furthermore, low vitamin D concentrations have been implicated in the development of type I diabetes as well as insulin insensitivity and type II diabetes [14]. Vitamin D status may also be linked to body weight. It has been shown that insufficient vitamin D can stunt growth and increase body weight, body mass index, and abdominal fat during puberty [15]. Further, vitamin D has been associated with the aging process. It has been shown that premature aging occurs in vitamin D receptor mutant mice [16]. Women with higher concentrations of vitamin D had longer leukocyte telomeres, which is a sign of being biologically younger and healthier [17]. While much attention in the medical literature has been given to the effects of vitamin D on the skeletal system, cardiovascular system, and its role in preventing various cancers, much less attention has focused on the effects of vitamin D on skeletal muscle, which is the focus of this review. Current research has shown that vitamin D has beneficial actions that lead to enhanced muscle strength, function, and performance. The purpose of this review is to discuss the effects of vitamin D on skeletal muscle in human subjects, animal models, and cell culture models and to delineate potential signaling pathways affected by vitamin D.

2. Metabolism of vitamin D

Activation of vitamin D involves multiple organs (see Fig. 1). Vitamin D_2 or D_3, derived from plants or conversion of 7-dehydrocholesterol in the skin by ultraviolet B radiation of mammals respectively, is hydroxylated in the liver to 25-hydroxyvitamin D [25(OH)D] by vitamin D-25-hydroxylase. 25(OH)D is the major circulating form of vitamin D in the blood, although, this form is inactive and must be converted in the kidneys to the biologically active form, 1,25 hydroxyvitamin D [1,25-$(OH)_2D_3$] by 25-hydroxyvitamin D-1α-hydroxylase (1-OHase). This renal production of 1,25-$(OH)_2D_3$ regulates calcium and phosphorous metabolism [8]. Importantly, 25(OH)D can be converted to 1,25-$(OH)_2D_3$ locally in many tissues, which plays an autocrine or paracrine role in regulating a variety of cellular processes such as cell growth, differentiation and apoptosis [18]. Every tissue in the body expresses the vitamin D receptor (VDR), which is thought to regulate both genomic and non-genomic responses to 1,25-$(OH)_2D_3$ [19–22]. In skeletal muscle, VDR has been shown to be localized to the nucleus and sarcolemma associated with caveolae [20,23].

3. Human studies: the effects of vitamin D on skeletal muscle function and performance

Until recently, studies involving vitamin D and human subjects have focused on its role in bone health and its impact on calcium homeostasis. However, it has become clear that vitamin D affects muscle function through the binding of 1,25-$(OH)_2D_3$ to VDR, resulting in muscle growth as well as other adaptations [23,24]. The effect of vitamin D on skeletal muscle suggests that its relationship to muscle strength may influence the prevalence of falls in the elderly. Poor muscle strength and function have a detrimental effect on balance and consequently increase the risk of falling in vulnerable populations such as the elderly [25,26]. Low vitamin D concentrations are common in hip fracture patients [27–29] and, in particular, those with vitamin D concentrations in the severe deficiency range (<10 ng/ml) show the

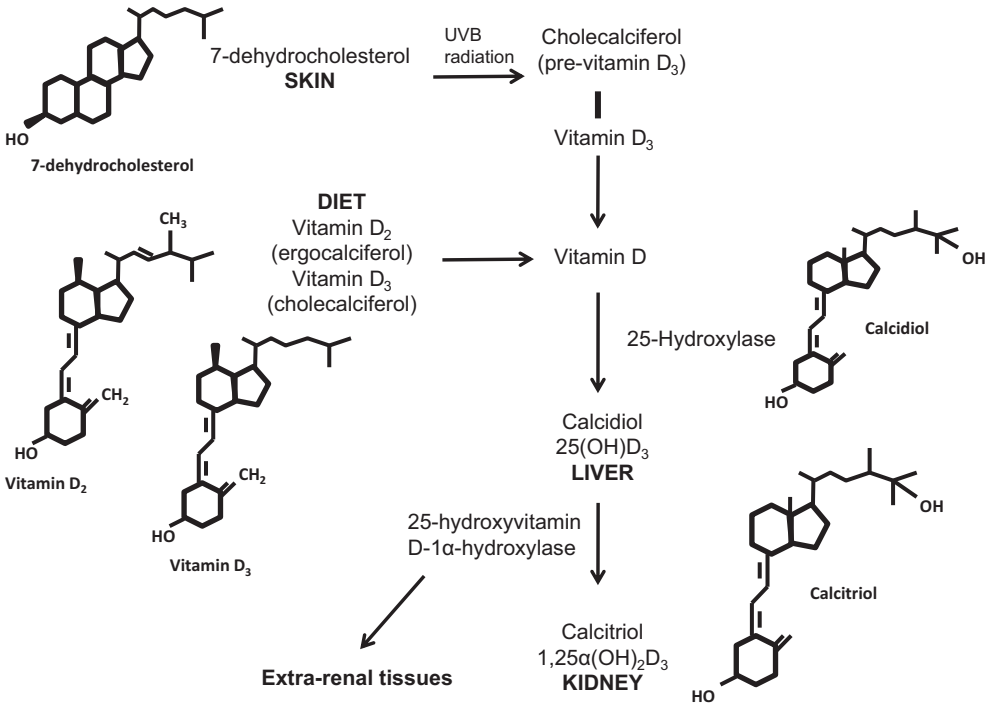

Fig. 1. Metabolism of vitamin D.

poorest performance on lower extremity tests and have higher fall rates [29].

The elderly are at risk for vitamin D insufficiency/deficiency for several reasons. Aging is associated with reduced concentrations of 7-dehydrocholestrol in the skin [18]. It has been shown that concentrations of 7-dehydrocholesterol in a 70 year old person are approximately 25% of a young adult [30]. The elderly also spend less time in the sun and consume less vitamin D in their diet [1,27,31]. Further, the effects of aging on liver and kidney function result in decreased activation of vitamin D [27,28,30]. Aging also leads to decreased expression of the VDR [23]. Not only are the elderly at risk for poor vitamin D status, several studies have shown a high prevalence of vitamin D deficiency among the young, the healthy, and in those living in regions where high sun exposure is prevalent [32–40].

3.1. Cross-sectional studies: vitamin D status and muscle strength

Several large cross-sectional studies have examined the relationship between vitamin D status and muscle strength. General muscle strength is often evaluated by handgrip strength and/or thigh muscle strength measured by a dynamometer. Assessment of physical performance may include, but not limited to, walking speed or gait, a sit-to-stand test, balance tests, and a timed up and go test (TUG) which consists of a timed test to rise from a chair, walk a given distance while avoiding obstacles, and then return to the chair.

Two large studies, the InCHIANTI and Malmo Osteoporosis Prospective Risk Assessment or OPRA, showed that individuals with insufficient and deficient vitamin D concentrations exhibited lower handgrip strength [41,42]. Results of physical performance markers in these two studies varied as the InCHIANTI study found vitamin D concentrations below 10 ng/ml to be correlated with lower scores on walking speed, ability to stand, and balance tests [41]. The OPRA study found that vitamin D concentrations below 30 ng/ml exhibited inferior gait speed ($r = 0.17$, $p < 0.001$), poorer balance ($r = 0.14$, $p < 0.001$), and lower thigh muscle strength ($r = 0.08$, $p = 0.02$) [42]. Additionally, women with concentrations below 30 ng/ml sustained at least one fracture during a 3-year follow-up [42].

A loss of handgrip strength associated with low vitamin D status was also seen in the Longitudinal Aging Study Amsterdam, a prospective study [43]. Furthermore, data from the Third National Health and Nutrition Examination Survey also support the notion that poor vitamin D status correlates with reduced muscle strength [4]. Bischoff-Ferrari et al. correlated vitamin D concentrations and markers of lower extremity function in over 4000 individuals aged 60 and older [44]. The lower extremity function tests included an 8-foot walk test and a sit-to-stand test. Both have been previously well described [45] but, briefly, the 8-foot walk test requires participants to walk eight feet at a normal pace. The test is performed twice and the faster trial is scored according to quartiles for the length of time required. In this study, a crude analysis of 25(OH)D concentrations compared to the 8-foot walk test and the sit-to-stand test both showed positive associations and reached similar significance ($p < 0.0001$). When subjects with vitamin D concentrations in the highest 25(OH)D quartile (34.4–160.1 ng/ml) were compared to subjects in the lowest quartile (3.4–17.4 ng/ml), an average improvement of 0.27 s (5.6%) in the 8-foot walk test was seen. Further, the highest quartile showed an average decrease of 0.67 s (3.9%) compared to the lowest quartile in the sit-to-stand test. The authors concluded that 25(OH)D concentrations between 16 and 38 ng/ml exhibited better musculoskeletal function regardless of activity level compared to those with vitamin D concentrations below 16 ng/ml [44].

In contrast to the above studies, data from the EPIDOS study involving 440 older women in France found no significant difference among measurements of muscle strength regardless of vitamin D status [46]. However, those subjects having the highest serum concentrations (>30 ng/ml) tended to exhibit greater handgrip strength than those with serum concentrations <15 ng/ml, although it did not reach statistical significance [46]. It should be noted that the number of subjects in the lowest vitamin D group was five times larger than the group with the highest vitamin D concentrations. Therefore, a lack of significance may be due to differences in subject numbers between the groups. Further, muscle strength is likely dependent on additional factors including level of physical fitness, genetics, and body size. As stated by the authors, these subjects were relatively healthy and might not be truly representative of community-dwelling older adults.

In summary, the majority of cross-sectional studies in elderly individuals support the relationship between poor vitamin D status and poor muscle function and strength. However, as these studies are cross-sectional in design, it does not support a clear causal relationship. Also, as the selected ranges of vitamin D are stratified differently from study to study, it is more challenging to compare results since the cutoff points between groups overlap and make interpretation of changes in muscle strength difficult. Furthermore, in studies looking at an elderly population, it is not clear if these changes are solely due to vitamin D deficiency, a result of aging, or a combination of the two.

3.2. Randomized control trials: vitamin D supplementation and falls

The prevalence of falls in individuals age 65 and older is common, with reports documenting at least one fall in every one in three individuals in this population [47]. Eight randomized controlled trials (RCTs) that have evaluated vitamin D supplementation or the combination of calcium and vitamin D supplementation on falls in the elderly will be reviewed (see Table 1). Supplementation periods vary ranging from eight weeks to three years. Further, most intervention groups received calcium (in the range of 500–1200 mg/day) and vitamin D (700–1000 IU/day) compared to the control group, which received calcium only. Finally, all the studies report baseline 25(OH)D serum concentrations which ranged from a defined severe deficiency of 10 ng/ml to a more moderate deficiency of 25–30 ng/ml. However, all the studies did not report 25(OH)D serum concentrations at the end of the supplementation period.

Two short-term calcium and vitamin D supplementation studies (8–12 weeks) in elderly women demonstrated a reduction in falls during a one year follow-up period [48] as well as during the treatment period [49]. In particular, recurrent fallers appear to respond best to the intervention. In both studies, 90% of the subjects qualified as vitamin D insufficient, with concentrations below 31 ng/ml. However, the response to supplementation differed. Baseline 25(OH)D concentrations in the study by Pfeifer et al. were 25 ng/ml in a population of healthy ambulatory seniors [48]. Serum concentrations rose to 40 ng/ml by the end of 8 weeks. This is in contrast to the study by Bischoff et al., who reported mean concentrations under 13 ng/ml with an average increase to 26 ng/ml after 3 months of supplementation in women living in geriatric units in Switzerland [49].

The results of two one-year supplementation trials have shown some variability in the percentage of subjects reporting falls. Prince et al. [50] supplemented older women with 1000 IU/day of vitamin D_3 and found that 53% of the vitamin D group reported at least one fall compared to 62.9% of the control group. Baseline 25(OH)D concentration was below 24 ng/ml for all subjects. At one year, the vitamin D_3 group had 25(OH)D concentrations that were 28% higher in winter/spring and 12.5% higher in summer/autumn compared to the control group. Interestingly, the

Table 1
Vitamin D supplementation and falls.

Reference	Primary endpoints	Subjects	Treatment groups and duration	Results
Pfeifer et al. [48]	Body sway, incidence of secondary hyperparathyroidism	148 females, mean age 74 ± 1 years (range 70–86 years), 25(OH)D level below 50 nmol/L	Intervention: 1200 mg/day elemental Ca and 800/day IU D_3 Control: 1200/day mg Ca 8 weeks	Decrease in body sway by 9% ($p = 0.0435$); mean number of falls 0.24 for Ca and Vit D, 0.45 for Ca alone ($p = 0.0346$); increased serum 25(OH)D of 72% ($p < 0.0001$) and decrease of PTH of 18% ($p = 0.0432$)
Bischoff et al. [49]	Falls	122 females, mean age 85.3 years (63–99 years)	Intervention: 1200 mg/day Ca carbonate and 800/day IU D_3 Control: 1200 mg/day Ca 12 weeks	Mean falls per person per week: 0.034 for Ca + D_3 and 0.076 for Ca. Ca + D_3 accounted for 49% reduction of falls (95% CI, 14–71%; $p < 0.01$)
Bischoff-Ferrari et al. [26,53]	Falls	199 males and 246 females 65+ years	Intervention: 500 mg/day Ca citrate malate and 700/day IU D_3 Control: placebo 3 years	Ca + D_3 reduced odds of falling in women (OR 0.54; 95% CI 0.50–0.97) but not in men (OR 0.93; 95% CI 0.15–0.81)
Prince et al. [50]	Falls	302 females, mean age 77 years (70–90 years)	Intervention: 1000 mg/day Ca citrate and 1000 IU/day D_2 Control: 1000 mg/day Ca citrate 1 year	Ca + D_2 had lowered risk of falling (OR 0.61; 95% CI 0.46–1.42); Ca + D_2 reduced first falls in winter/spring (OR 0.55; 95% CI; 0.32–0.96) but not summer/autumn (OR 0.81; 95% CI; 0.46–1.42)
Pfeifer et al. [51]	Falls	242 participants over the range of 70 (mean age76 years); 25% males	Intervention: 1000 mg/day elemental Ca and 800/day IU D_3 Control: 1000 mg/day elemental Ca 1 year	40% of Ca + D_3 experienced one fall vs. 63% in Ca alone ($p < 0.001$); mean number of falls in Ca 1.41 vs. 0.63 in Ca + D_3
Sato et al. [54]	Falls	96 females with poststroke hemiplegia, mean age 74 years	Intervention: 1000 IU/day D_2 Control: placebo 2 years	D_2 treatment decreased falls by 59% (estimate: −0.95; 95% CI 39–92%; $p = 0.0002$)
Broe et al. [55]	Falls	124 participants (73% female), mean age 89 years (68–104 years)	4 Intervention groups: 200 IU, 400 IU, 600 IU, or 800 IU/day D_2 Control: placebo 5 months	Number of falls over 5 months: 11/25 in placebo, 15/26 in 200 IU, 15/25 in 400 IU, 15/15 in 600 IU, 5/23 in 800 IU; 800IU group had 72% lower adjusted incidence rate ratio of falls (rate ratio = 0.28; 95% CI 0.11–0.75)
Flicker et al. [52]	Falls	625 participants (95% female), mean age 83.4 years	Intervention: 600 mg Ca carbonate and 10,000 IU/week D_2 (used initially) but changed to 1000 IU/day Control: 600 mg Ca carbonate 2 years	Incident rate for falling was lower in the D_2 group (0.63; 95% CI 0.48–0.82) compared to control (0.73; 95% CI 0.57–0.95). In compliant subjects, 65% of the control group fell compared to 56% in the D_2 group

control group did show increased 25(OH)D concentrations during the summer/autumn but not winter/spring seasons. In relation to falls, vitamin D supplementation reduced the risk of a first fall in winter and spring but not summer or autumn. Pfeifer et al. [51] documented only 40% of the vitamin D group reporting falls compared to 63% of the control. Their subjects also had low average 25(OH)D concentrations of 22 ng/ml. The vitamin D group improved their serum concentrations to ∼34 ng/ml at 12 months while the calcium control group did not change. It is interesting to note that Prince et al. reported an increase in 25(OH)D concentrations with calcium alone [50]. Both studies recruited community-dwelling participants with an average age of 77 years and used similar supplementation protocols. Similarly, both studies reported a 25(OH)D threshold for which vitamin D supplementation may be beneficial in reducing the risk of falls.

Two and three year supplementation studies appear to result in similar conclusions as one year supplementation trials. Flicker et al. studied predominantly older females residing in residential care [52]. When subjects with greater than 50% compliance were evaluated, 65% of the control group reported a fall compared to 56% of the vitamin D group. Only baseline 25(OH)D concentrations were reported and therefore changes in serum concentrations with supplementation cannot be discussed. Bischoff-Ferrari et al. supplemented older men and women with 700 IU of vitamin D and 500 mg calcium citrate malate for three years [53]. Over the course of the study, 55% of women and 45% of men reported at least one fall; however the data supported a reduction of falling only in women, not men, at the conclusion of the study. Furthermore, the least active women were most responsive to supplementation, while physical activity levels were not a factor for the men. The difference in gender response has not been noted previously, as most investigations focus on women exclusively or they represent at least 75% of the subjects. Bischoff-Ferrari et al. did report an increase in serum 25(OH)D concentrations as baseline values for females averaged 25 and 28 ng/ml for the placebo and vitamin D group, respectively, and increased to 27 and 41.6 ng/ml [53]. Baseline 25(OH)D concentrations for the males was 33 ng/ml across both groups and increased to 44 ng/ml with vitamin D supplementation and fell to 30.6 ng/ml in the placebo group. Sato et al. looked at fall reduction in elderly women with poststroke hemiplegia [54]. Hemiplegia is total paralysis on only one side of the body. They reported a 59% reduction in falls with 1000 IU/day of vitamin D_2 at the end of two years. These subjects were severely deficient in vitamin D as serum baseline concentrations were 9.8 ng/ml which increased to 33.4 ng/ml by the end of two years.

Finally, a dose response to vitamin D supplementation was performed by Broe et al. [55]. Four groups received 200, 400, 600, or 800 IU of vitamin D/day for 5 months. No dose response trend was seen, and a reduction in falls was observed in the 800 IU group alone; however, the risk of falling was not reduced. Also, the 800 IU group increased serum 25(OH)D concentrations from 21.4 to 29.8 ng/ml, however samples were only collected from 73% of the subjects. This study did suffer from some limitations, including a lack of control for use of multivitamins, which was inconsistent among participants. In summary, short-term and long-term studies collectively demonstrate a relationship between vitamin D

status and fall prevention independent of calcium status; however, additional research is needed in order to make strong conclusive statements.

3.3. Randomized control trials: vitamin D supplementation and muscle strength

In addition to falls prevention, several studies have included measures of muscle strength. A recent RCT treated subjects for twelve months with vitamin D_3 and followed them for an additional eight months under a blinded observation period [51]. Improvements in quadriceps strength and body sway and a decreased time in the TUG test were seen in the vitamin D_3 group [51]. In contrast, some studies have shown no improvement with vitamin D supplementation. A single dose of 300,000 IU of vitamin D followed by 10 weeks of high intensity home-based quadriceps resistance exercise did not improve rehabilitation outcomes in frail older adults [56]. A second trial supplemented 65 healthy older men with 1000 IU/day of vitamin D_3 over a six month period. These individuals were not vitamin D insufficient or deficient. They showed no differences in strength, power, or physical performance following supplementation at the end of six months [57].

A recent meta-analysis on the effects of low serum vitamin D and vitamin D supplementation on muscle strength, balance, and gait performance in subjects 65 years and older showed mixed results as well [46]. Slightly more than half of the studies showed a positive association, illustrating that the effect of vitamin D on muscle strength and performance is not clear at this time. Therefore, while research in the area of vitamin D supplementation and muscle strength is promising, it is not conclusive. Many trials utilizing a combination of vitamin D and calcium appear most successful, as opposed to vitamin D or calcium alone. Further, vitamin D deficient individuals appear to be the most responsive to vitamin D treatment compared to apparently healthy, vitamin D sufficient individuals. It has been shown that vitamin D deficient adults exhibit atrophy of type II skeletal muscle fibers [58]. This is important to note because type II muscle fibers are the first to be recruited when preventing a fall [59].

3.4. Vitamin D and adolescents

Research on the effects of vitamin D has primarily focused on elderly individuals who are most prone to sarcopenia. However, recently research has expanded to include adolescents and younger adults. Several studies have shown insufficient and/or deficient 25(OH)D concentrations in these populations [2,34,36]. It was shown that six months of vitamin D_3 supplementation improved skeletal muscle strength and physical performance in vitamin D deficient Asian Indians aged 20–40 years with normal BMI [60]. Subjects were given 60,000 IU/week of vitamin D_3 for 8 weeks followed by 60,000 IU/month for 4 months. Strength was assessed by a dynamometer for handgrip and the gastro-soleus muscle, while physical performance was assessed by a six-minute walk test.

Studies have shown correlations between vitamin D status and muscle strength and fitness in adolescents [34]. Data from 99 post-menarchal girls (12–14 years) in the UK demonstrated a positive relationship between 25(OH)D concentrations and jumping mechanics by measuring power ($p = 0.003$), force ($p = 0.05$), velocity ($p = 0.002$), and jump height ($p = 0.005$) [34]. These data suggested that female adolescents with low vitamin D status generate less power and, therefore, less height and velocity with jumps. A study of 559 US adolescents (age range 14–18 years) residing in the southeastern portion of the country reported vitamin D insufficiency (<30 ng/ml) in 56.4% and vitamin D deficiency ($\leq$20 ng/ml) in 28.8% [36]. Further, this cross-sectional study showed a higher prevalence of vitamin D insufficiency and deficiency in black girls and boys compared to white girls and boys. Positive correlations between serum 25(OH)D levels and physical activity and cardiovascular fitness were found. However, this was not seen in 179 girls (aged 10–17 years) in Lebanon [61]. Subjects were stratified into three groups: a placebo group, a low dose (200 IU/day) and a high dose (2000 IU/day) vitamin D supplement group for one year. While vitamin D concentrations increased significantly in the supplementation groups, resulting in increased hip bone mineral content, no change in grip strength was seen. Further, vitamin D supplementation had the greatest impact on the pre-menarchal girls, a population that was not studied by the other groups. In conclusion, there appears to be a trend linking vitamin D with improved muscle strength and fitness in adolescents; however, this may relate to specific periods of growth during adolescence. Much more research on the effects of vitamin D in adolescents is needed.

3.5. Vitamin D and fatty degeneration in muscle

Vitamin D may impact fatty degeneration in muscle. Oh et al. found that low serum concentrations of vitamin D correlated with fatty degeneration of the muscles in the rotator cuff as seen by MRI [62]. A relationship between fatty degeneration and poor vitamin D status was also seen in the thigh muscle [63]. A limitation of these studies is the inability to identify this fat as intracellular. These studies can only show muscular atrophy with fatty substitutions of the particular muscle studied. Furthermore, the mechanism explaining the link between vitamin D insufficiency/deficiency and fatty degeneration is unclear. It has been suggested that atrophy of skeletal muscle and infiltration of fat results in impaired muscular performance in older individuals with vitamin D deficiency [62].

To summarize the data from studies involving human subjects, vitamin D insufficiency/deficiency appears to be associated with a loss in muscle strength and function and an increase in falls. These findings are not limited to the elderly and may extend to young, apparently healthy individuals. Correlative studies have established a relationship between muscle strength and vitamin D concentrations while more tightly controlled randomized trials have predominantly shown a positive impact of vitamin D supplementation, with or without calcium, on muscle strength and function tests. However, there is still discrepancy in the literature, and more well controlled trials are needed before strong conclusions can be drawn.

4. Animal studies: the effects of vitamin D on skeletal muscle

Animal studies exploring the role of vitamin D on skeletal muscle have predominantly used the VDR knockout mouse known as the VDR null mutant mouse. The VDR null mutant mouse is characterized by hair loss [64], a reduction in body weight, and a reduction in body size [64–66]. Loss of the VDR also causes a shorter gait and impaired motor coordination [64–66]. Furthermore, these animals show abnormal swimming ability as illustrated by vertical swimming and sinking which suggests muscular and motor impairment [65,66].

The VDR null mouse has also been used to study skeletal muscle development. The VDR mouse appears to grow normally until weaning [19,67]. After weaning, the VDR mouse develops abnormalities consistent with rickets. This has been noted by several researchers [67–69]. It is suggested that a lack of critical nutrients from breast milk may be a primary factor in the development of bone and metabolic abnormalities post-weaning [19]. At 4 weeks, the VDR-null mutant mouse shows reduced serum levels of calcium and phosphate with elevated levels of serum alkaline phosphatase [68]. To tease out the direct effect of vitamin D on muscle develop-

ment, rather than secondary metabolic abnormalities, three-week pre-weaned VDR −/− mice were studied. The mice still exhibited a 20% reduction in skeletal muscle cell diameter, which became more prominent as the animal aged, suggesting a progressive negative effect of the loss of the vitamin D receptor. These muscle abnormalities affected both type I and II fibers. Additionally, the mice exhibited a high expression of the myogenic differentiation factors, including myf5, myogenin, and E2A as well as increased expression of the early myosin heavy chain isoforms. These transcription factors play a key role in muscle cell differentiation [19]. These genes are normally down-regulated during the stages in which VDR is expressed, leading these authors to conclude that the VDR is involved in transcriptional down-regulation of these genes during muscle differentiation, as shown in their cell culture myoblast studies [19]. In summary, this study suggests that vitamin D directly affects skeletal muscle development.

While vitamin D may affect skeletal muscle development, vitamin D deficiency induced through dietary restriction may not be the mechanism behind muscle weakness. A recent study evaluated the impact of vitamin D and phosphate deficiency on muscle strength in male Wistar rats [70]. The soleus and epitrochlearis muscles were selected to evaluate muscle strength. In the rachitic rodents, vitamin D treatment significantly improved soleus strength and phosphorus concentrations. When phosphorus was then provided, greater strength production occurred. Further, the improved soleus strength correlated well with phosphorus levels. The epitrochlearis, a predominantly fast twitch muscle, was not significantly affected by deficiency of either nutrient. Finally, repletion of phosphate alone resulted in the quick restoration of muscle strength suggesting that correction of hypophosphatemia corrects muscle weakness while vitamin D does not.

Although limited, animal studies suggest that the VDR is involved in normal skeletal development, and altered expression of its gene results in abnormal muscle differentiation characterized by a changed expression of the myogenic differentiation factors and muscle cell size. Furthermore, these animals exhibit changes in muscle and motor development relative to functional tests. Understanding the mechanisms of vitamin D in muscle development and function may be clinically relevant to recommendations of its use in prevention or attenuation of muscle aging and/or various muscle diseases/disorders. Cell culture studies have begun to elucidate mechanisms of the effects vitamin D on skeletal muscle.

5. Cell culture studies: cellular effects of vitamin D on muscle cells

Early on it was shown that both myoblasts and myotubes express 1,25-$(OH)_2D_3$ receptor(s) and showed no differences in the quantity or characteristics of the receptor(s) between the two cells [71]. Specifically, it was shown that cloned human myoblasts and fused myotubes exhibited similar specific binding data between 1,25-$(OH)_2D_3$ and its receptor(s); both cells responded with a dose-dependent increase in 25-hydroxyvitamin D_3-24-hydroxylase enzyme activity after treatment with 1,25-$(OH)_2D_3$ [71]. Hence, in culture it is evident that myoblasts and differentiated myotubes are both susceptible to 1,25-$(OH)_2D_3$-induced cellular responses and adaptations.

1,25-$(OH)_2D_3$-induced cellular responses and adaptations are mediated via both genomic and non-genomic mechanisms in skeletal muscle cells [72]. Most cell culture studies using myoblasts and myotubes address the rapid non-genomic response to 1,25-$(OH)_2D_3$. It has been shown that these rapid responses to 1,25-$(OH)_2D_3$ involve activation of a multitude of cell signaling pathways, including cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA), protein kinase C (PKC),

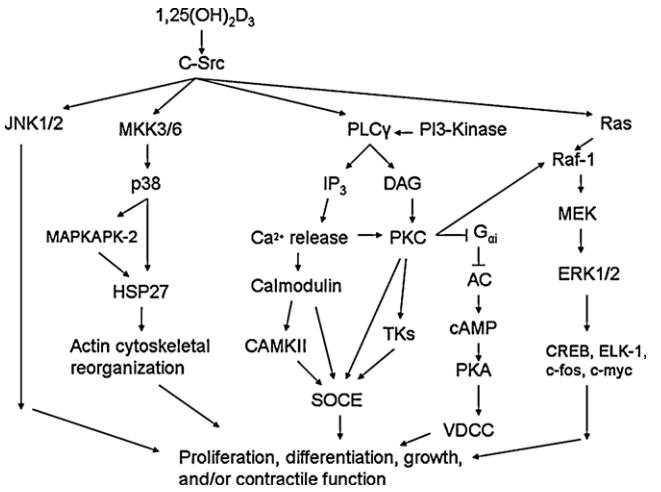

Fig. 2. Potential non-genomic vitamin D-induced cellular signaling in skeletal muscle. Arrows indicate direct or indirect activation or increased expression. Blunt lines indicate inhibition.

calmodulin/calmodulin-dependent kinase (CaM-kinase), protein kinase B (PKB/Akt), and multiple mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), p38, and c-Jun NH_2-terminal 1 and 2 (JNK1/2) (see Fig. 2). Activation of these pathways has been shown to participate in various cellular processes such as rapid Ca^{2+} influx, proliferation, differentiation, protection against insulin resistance, and/or stimulation of arachidonic acid (AA) mobilization. The effects of 1,25-$(OH)_2D_3$ on each of these cellular processes that have been studied in myoblast and/or myotube cell culture will be reviewed with discussion of the potential cellular signaling involved.

The rapid non-genomic actions of 1,25-$(OH)_2D_3$ are thought to be mediated by a plasma membrane-associated VDR. Upon myoblast treatment with 1,25-$(OH)_2D_3$, there is rapid translocation of the VDR to the plasma membrane [20,73]. It was shown that translocation of VDR is dependent upon microtubular transport and activity of tyrosine kinase(s) [73] and later shown to also be dependent on the presence of caveolae, lipid rafts in the plasma membrane that serve as platforms for signaling molecules [20]. Confocal microscopy imaged the abolished 1,25-$(OH)_2D_3$-induced VDR translocation to the plasma membrane in C_2C_{12} myoblasts pretreated with methyl beta cyclodextrin (MβCD), a caveolae disrupting agent [20]. Hence, the first step in 1,25-$(OH)_2D_3$-induced non-genomic responses is likely translocation of VDR to the plasma membrane.

5.1. Calcium homeostasis

1,25-$(OH)_2D_3$ regulates calcium homeostasis via VDR genomic and non-genomic mechanisms. The non-genomic 1,25-$(OH)_2D_3$-induced calcium influx involves rapid mobilization from the sarcoplasmic reticulum (SR) followed by influx from the extracellular milieu via activation of the store-operated calcium entry (SOCE) pathway and L-type voltage-dependent calcium channels (VDCC) [74–79]. First, 1,25-$(OH)_2D_3$ -induced mobilization of calcium from the SR has been shown to be regulated by activation of phospholipase C gamma (PLCγ) and production of inositol triphosphate (IP_3) [22,79]. c-Src and phosphoinositide 3-kinase (PI-3 kinase) are responsible for activating PLCγ [22]. Secondly, extracellular calcium influx via SOCE results from the release of calcium from the SR and the concurrent production of diacylglycerol (DAG) [74]. Calcium mobilization from the SR leads to activation of calmodulin and calmodulin-dependent protein kinase II (CAMKII) and also leads to the activation of PKC; PKC activation requires both

calcium mobilization from the SR and production of DAG. Calmodulin, CAMKII, PKC, and other tyrosine kinases activated by PKC all have been shown to participate in SOCE (see Fig. 2) [74]. Importantly, although 1,25-$(OH)_2D_3$ does activate the cAMP/PKA pathway in skeletal muscle cells, it was shown not to be involved in SOCE [74]. Later, it was shown that the molecular identity of the channels involved in myocyte SOCE may be the transient receptor potential channel (TRPC)-like proteins [77]. TRPC-like proteins co-immunoprecipitated with VDR after 1,25-$(OH)_2D_3$ treatment, suggesting that VDR may play a direct role in regulating channels involved in SOCE [77]. Lastly, 1,25-$(OH)_2D_3$-induced extracellular calcium entry via VDCC is mediated via the cAMP/PKA and PLC/PKC pathways (see Fig. 2) [78]. Evidence supports that adenylyl cyclase may be activated via 1,25-$(OH)_2D_3$-induced phosphorylation and inhibition of $G_{\alpha i}$, rather than activation of G_s [78]. It has been hypothesized that PKC may be responsible for $G_{\alpha i}$ phosphorylation [78]. Indeed, it was shown that VDCC calcium influx required activation of PKC, which in turn cross-talks with cAMP/PKA pathway [75,80]. Hence, VDCC calcium influx involves both PKA and PKC. In summary, 1,25-$(OH)_2D_3$-induced calcium fluxes in cultured skeletal muscle cells involve rapid mobilization from the SR via PLC/IP3 followed by extracellular calcium influx via SOCE and VDCC. SOCE activation involves calmodulin, CAMKII, and PKC, while extracellular calcium influx via VDCC involves cAMP/PKA and PLC/PKC pathways. In addition, activation of the cAMP/PKA pathway also leads to expression of calmodulin, which likely contributes to 1,25-$(OH)_2D_3$-induced calcium signaling [81]. The 1,25-$(OH)_2D_3$-induced calcium fluxes and alterations in calcium signaling may play a role in regulating muscle contractile force in differentiated muscle fibers and play a role in proliferation and differentiation of myoblasts, among a plethora of other cellular processes that may be affected by 1,25-$(OH)_2D_3$.

5.2. Proliferation and differentiation

1,25-$(OH)_2D_3$ stimulates proliferation and differentiation of myoblasts. It is well known that MAPK pathways are involved in proliferation and differentiation. Six subgroups of the MAPK family have been described and include ERKs, p38, and JNK, among others. It has been shown that 1,25-$(OH)_2D_3$ induces the activation of ERK1/2, p38, and JNK1/2 MAPKs, and they may all be involved in proliferation and differentiation of myoblasts to some degree (see Fig. 2) [21,75,82,83]. The role of the ERK1/2 signaling pathway has been the most studied in regards to 1,25-$(OH)_2D_3$-induced myoblast proliferation. ERK1/2 activation is mediated via MEK, and MEK is activated via Raf-1. Raf-1 is activated by Ras and PKCα together [83]. Therefore, once ERK1/2 is activated via a Ras/PKCα, Raf-1, or MEK pathway, ERK1/2 then phosphorylates a host of proteins and transcription factors, two of which are the cAMP response element binding protein (CREB) and ElK-1 [84]. It has also been shown that 1,25-$(OH)_2D_3$-induced ERK1/2 activation leads to expression of c-fos and c-myc [84,85]. In addition to ERK1/2 activation, p38 is another MAPK that is activated in cultured myoblasts in response to 1,25-$(OH)_2D_3$ treatment [21]. 1,25-$(OH)_2D_3$-induced activation of p38 involves phosphorylation of the MAPK kinases 3 and 6 (MKK3/6) [21]. It has been shown that p38 then phosphorylates MAPK activating protein kinase-2 (MAPKAPK-2) and heat shock protein 27 (HSP27). p38 and MAPKAPK-2 may both play a role in the phosphorylation and activation of HSP27 [21]. HSP27 may be playing a role in proliferation and differentiation via actin cytoskeleton reorganization [21]. The last MAPK known to be activated via 1,25-$(OH)_2D_3$ treatment in myoblasts is JNK1/2; however, details of this signaling pathway have not been studied [21].

One of the earliest steps in 1,25-$(OH)_2D_3$-induced activation of these MAPKs, at least shown for ERK1/2 and p38, is the activation of c-Src [20,21]. Under basal conditions, c-Src is held in an inactive state by caveolin-1 (cav-1), a component of caveolae. Upon stimulation with 1,25-$(OH)_2D_3$, the VDR translocates to the membrane and disrupts this interaction, thereby leading to the activation of c-Src [20]. Disruption of caveolae or silencing of cav-1 abolished the activation of c-Src, ERK1/2, and p38 in response to 1,25-$(OH)_2D_3$ treatment [20]. JNK1/2 was not assessed.

1,25-$(OH)_2D_3$ has also been shown to play a role in muscle differentiation via down-regulation of myogenic transcription factors myf5, myogenin, and E2A [19]. The mechanisms leading to 1,25-$(OH)_2D_3$-induced down-regulation of the myogenic transcription factors were not studied. Potentially these mechanisms could include VDR-mediated genomic and/or non-genomic mechanisms.

In summary, activation of MAPKs and PKC have been shown to be involved in cultured myoblast proliferation and differentiation, but the relative contributions are not clear, and the vast majority of the downstream targets of these signaling pathways remain to be elucidated.

5.3. Protection against insulin resistance

In addition to playing a role in calcium homeostasis and myoblast proliferation and differentiation, 1,25-$(OH)_2D_3$ also protects skeletal muscle from insulin resistance [86]. Vitamin D deficiency has been linked to type II diabetes mellitus and metabolic syndrome [87,88]. In culture, it was shown that treatment of myotubes with free fatty acids (FFA) induced insulin resistance and atrophy [86]. Co-administration of 1,25-$(OH)_2D_3$ with the FFA improved insulin-stimulated glucose uptake in a dose- and time-dependent manner and completely prevented atrophy [86]. FFA-induced insulin resistance involves increased serine phosphorylation and depressed tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) as well as depressed phosphorylation of Akt [86]. Furthermore, FFA caused increased JNK phosphorylation [86]. The 1,25-$(OH)_2D_3$-mediated protection against insulin resistance involves improved insulin signaling, specifically improved tyrosine phosphorylation and decreased serine phosphorylation of IRS-1 and increased phosphorylation of Akt. 1,25-$(OH)_2D_3$ also inhibited JNK phosphorylation induced by FFA [86]. Thus, 1,25-$(OH)_2D_3$ protects against insulin resistance via modulation of insulin signaling pathways.

5.4. Arachidonic acid mobilization

Lastly, 1,25-$(OH)_2D_3$ induces the release of arachidonic acid (AA) [89]. It was shown that 1,25-$(OH)_2D_3$ promotes mobilization of AA in myoblasts, which is dependent on influx of extracellular calcium and activation of phospholipase A_2 (PLA_2) and is enhanced by activation of PKC [89]. It was suggested that 1,25-$(OH)_2D_3$-induced PLA_2 activity and mobilization of AA may alter membrane fluidity and permeability, thereby affecting muscle cell membrane function [89]. This may play a role in the effects of vitamin D on insulin sensitivity. It has been shown that the arachidonic acid phospholipid content of skeletal muscle is inversely correlated with the fasting serum insulin concentration (a measure of insulin resistance) [90]. It is postulated that arachidonic acid and other long-chain polyunsaturated fatty acids may modulate the function of membrane insulin receptors and/or glucose transporters or, alternatively, they might influence the action of insulin by acting as precursors for the generation of second messengers such as eicosanoids or diacylglycerols [90]. AA plays a role in a variety of cellular processes. For example, it is well known that metabolism of AA plays a central role in the process of inflammation, producing pro-inflammatory as well as anti-inflammatory metabolites [91]. However, very little is known about vitamin D-induced AA mobilization and its downstream metabolism so it difficult to speculate the role AA

may be playing in regards to the effects of vitamin D on skeletal muscle.

In summary, both myoblasts and myotubes are responsive to 1,25-$(OH)_2D_3$ via genomic and non-genomic mechanisms. The non-genomic mechanism involves translocation of the VDR receptor to the plasma membrane and activation of a plethora of signaling pathways resulting in calcium influx, proliferation and differentiation, protection against insulin resistance, and/or arachidonic acid mobilization. It is likely that numerous other cellular processes are affected by 1,25-$(OH)_2D_3$ in skeletal muscle cells, but have not yet been researched.

6. Conclusion

The health benefits of vitamin D are widespread, and it is becoming evident that they may involve most, if not all, of the organ systems. Vitamin D insufficiency and/or deficiency are prevalent and reach individuals of all ages. Recently discovered, various gene variants may contribute to the vitamin D insufficiency and/or deficiency that seem to be a pandemic and therefore, may be contributing to various disease states. In skeletal muscle, it has become clear that vitamin D is important for optimal strength and functioning. Vitamin D deficiency and/or insufficiency in humans have shown deficits in strength and balance and have also been shown to result in atrophy and fatty degeneration of muscle fibers. Animal studies have also shown that vitamin D is important for skeletal muscle growth and homeostasis. VDR knockout mice have shown atrophy of muscle fibers compared to wild-type mice and also have demonstrated altered expression levels of muscle differentiation factors. In culture, vitamin D has been shown to affect both myoblasts and differentiated myotubes. Vitamin D plays a role in a plethora of cellular process such as calcium homeostasis, proliferation, differentiation, protection of skeletal muscle cells from insulin resistance, and arachidonic acid mobilization. These processes are mediated through genomic and non-genomic mechanisms via the VDR with the non-genomic mechanisms involving one or more of the several pathways currently known to be activated by vitamin D, which include cAMP/PKA, PKC, calmodulin/CaM-kinase, PKB/Akt, and multiple MAPKs such as ERK1/2, p38, and JNK1/2. Although it is currently known that vitamin D affects several processes in skeletal muscle, it is likely that future research will elucidate even more processes affected by this dynamic hormone.

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