

Received Date : 25-Oct-2013

Returned for Revision: 08-Nov-2013

Finally Revised : 13-Nov-2013

Accepted Date : 15-Nov-2013

Article type : 6 Requested Review

Effects of Vitamin D in Skeletal Muscle: Falls, Strength, Athletic Performance and Insulin Sensitivity

Christian M. Girgis ^{1,2}, Roderick J. Clifton-Bligh ²⁻⁴, Nigel Turner ⁵, Sue Lynn Lau ^{1,2,6}, Jenny E. Gunton ^{1, 2,6,7}

¹ Diabetes and Transcription Factors Group, Garvan Institute of Medical Research (GIMR), Sydney, NSW, Australia

²Faculty of Medicine, University of Sydney, Sydney, NSW, Australia

³Department of Diabetes and Endocrinology, Royal North Shore Hospital, Sydney, Australia.

⁴Kolling Institute, University of Sydney, Sydney, Australia.

⁵ Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia

⁶Department of Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

⁷ St. Vincent's Clinical School, University of New South Wales, Sydney, NSW, Australia

Corresponding authors:

Christian Girgis and Jenny E. Gunton

Diabetes and Transcription Factors Group

Garvan Institute of Medical Research

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cen.12368

This article is protected by copyright. All rights reserved.

384 Victoria St, Darlinghurst

Sydney NSW 2010

Ph: +612 9295 8474

Email: c.girgis@garvan.org.au, j.gunton@garvan.org.au

ABSTRACT

Accompanying the high rates of vitamin D deficiency observed in many countries, there is increasing interest in the physiological functions of vitamin D. Vitamin D is recognised to exert extra-skeletal actions in addition to its classic roles in bone and mineral homeostasis. Here we review the evidence for vitamin D's actions in muscle on the basis of observational studies, clinical trials and basic research. Numerous observational studies link vitamin D deficiency with muscle weakness and sarcopaenia. Randomised trials predominantly support an effect of vitamin D supplementation and the prevention of falls in older or institutionalised patients. Studies have also examined the effect of vitamin D in athletic performance, both *inferentially* by UV radiation and *directly* by vitamin D supplementation. Effects of vitamin D in muscle metabolic function, specifically insulin sensitivity, are also addressed in this review. At a mechanistic level, animal studies have evaluated the roles of vitamin D and associated minerals, calcium and phosphate, in muscle function. *In vitro* studies have identified molecular pathways by which vitamin D regulates muscle cell signalling and gene expression. This review evaluates evidence for the various roles of vitamin D in skeletal muscle and discusses controversies that have made this a dynamic field of research.

Introduction

Long before the discovery of vitamin D, the sun was revered as a source of physical strength and vitality. In ancient Greece, heliotherapy (sun exposure) was prescribed as a cure for 'weak and flabby muscles' ¹. Sun exposure was considered a performance-enhancer; ancient Olympians were instructed to train in sunlight to increase their muscle size ¹.

It was in 1922 that the American physician, Alfred Hess, realised that children with rickets had profound muscle weakness, and that direct sunlight exposure improved their 'general vigour and nutrition' ². Adolf Windhaus was awarded the 1928 Nobel prize for his work on sterols, including the discovery of the chemical structure of vitamins D₂, D₃ and 7-dehydro-cholesterol ³. The name 'vitamin D' is a historical misnomer. The active form, calcitriol, is a

hormone; it is synthesised in humans, undergoes autocrine regulation and it interacts with a nuclear receptor ⁴. Following the photochemical conversion of 7-dehydrocholesterol to vitamin D under the influence of UV-radiation, this molecule circulates bound to vitamin D-binding protein (DBP) and undergoes two hydroxylation steps, firstly in the liver to form 25(OH)Vitamin D (25OHD) and subsequently in the kidney to form 1,25(OH)₂Vitamin D (1,25(OH)₂D) by the 1-alpha-hydroxylase enzyme (encoded by CYP27B1). 1,25(OH)₂D is the biologically active form that binds to the vitamin D receptor (VDR) to regulate gene expression, with major effects on calcium and mineral homeostasis ⁴. 1,25(OH)₂D regulates its own catabolism *via* upregulation of the expression of CYP24A1 gene (which encodes 24-hydroxylase). Although VDR is present in cell-types involved in mineral homeostasis, it is present in other cell types, suggesting that there may be non-traditional roles of vitamin D.

Vitamin D deficiency is now recognised to be common. In Australia, 31% of participants in a study of 11,247 adults were deficient (25OHD < 50 nmol/l) ⁵ and an incidence of 41% was recently reported in the US ⁶. Other parts of the world, such as Asia, India and the Middle East, have also reported high rates of vitamin D deficiency⁴. With increasing recognition of this pandemic, a plethora of observational studies have associated deficiency with several conditions including autoimmune diseases, diabetes, cancer and muscle weakness ⁷.

A direct link between vitamin D deficiency and muscle weakness was first described in the 1960s. In adults with osteomalacia, muscle strength improved following supplementation ⁸. Whether this improvement represented a direct effect on muscle or an indirect result of vitamin D-mediated changes in calcium homeostasis was unclear. In the years since this paper, clinical studies examining the effects of vitamin D on muscle performance, mass and the risk of falls in various populations have emerged ^{9,10}. There are also a variety of studies that have examined mechanisms responsible for vitamin D's effects in muscle. However, controversy remains regarding the significance of this research and whether the vitamin D receptor (VDR) is expressed in adult muscle ¹¹⁻¹³. In this review, we will cover the clinical evidence for effects of vitamin D in skeletal muscle function and potential mechanisms underlying a role for vitamin D at this site.

Vitamin D and Muscle Function in Older Populations

Low muscle mass (sarcopaenia) is characteristic of the ageing process and is associated with a greater risk of disability, falls and mortality in older people ¹⁴.

Vitamin D deficiency is common in the elderly and institutionalised and may contribute to sarcopaenia¹⁵. Reduced capacity for UV-mediated vitamin D synthesis in older skin may be partly responsible. Additionally, VDR expression in human muscle declines with age¹¹. This may render the muscles of elderly individuals more vulnerable to low vitamin D levels. Higher serum levels may be required to achieve comparable physiological effects. In this section, we discuss observational studies linking vitamin D deficiency and muscle dysfunction in elderly subjects and interventional studies examining potential effects of vitamin D supplementation in the risk of falls and muscle weakness.

Observational Studies

Several authors report an association between baseline 25OHD and subsequent falls risk in elderly people. In a prospective, longitudinal study of 1600 elderly women living in assisted care, Flicker and colleagues found a 20% reduction in falls risk over \approx 150 days with a doubling of 25OHD levels¹⁶. Similarly, amongst 1231 people over age 65, those with 25OHD levels <25 nmol/L were more likely to have recurrent falls in the subsequent year (OR 1.78 for ≥ 2 falls)¹⁷.

Prospective studies have also reported an association between baseline 25OHD levels and declining muscle function. In a Dutch study of 1000 subjects over age 65, baseline 25OHD level <50 nmol/l correlated with a greater 3-year decline in physical performance (sum score of walking test, chair stands, and tandem stand (*i.e.* ability to stand with feet in tandem position with eyes closed for 10 sec)¹⁸. The 3-year risk of sarcopaenia in older subjects was 2 times greater with baseline 25OHD levels < 25 nmol/l¹⁵.

However, not all studies confirm these associations¹⁹ and despite adjustments, potential confounding factors exist. Low baseline 25OHD levels may be a marker of pre-existing frailty, as the frail elderly are less likely to spend time outdoors, exposed to UV radiation. Malnutrition is common in the elderly; 40% of those living in institutions are at risk²⁰. Dietary deficiencies may contribute simultaneously to low vitamin D and to frailty. Factors not considered in adjustment models may confound data interpretation, making interventional studies important.

Intervention Studies

Many studies have examined the effect of exposure to UV radiation or vitamin D supplementation in the prevention of falls and muscle strength in older individuals.

Amongst 602 residents of aged-care facilities living in Sydney, Australia, those who increased sunlight exposure had fewer falls than those randomised to the control group over 12 months (Incidence Rate Ratio 0.52, $p=0.01$)²¹. However, compliance with sunlight exposure was poor (26%) and no effect was observed on intention-to-treat analysis.

Several studies reported beneficial effects of vitamin D on falls. Amongst 625 older residents of assisted-living facilities, those who received calcium (600 mg daily) and ergocalciferol (initially 10,000 IU weekly, then 1,000 IU daily) for 2 years were less likely to ever fall than those receiving calcium alone²². Similarly, in 122 older women in geriatric care, calcium (1200 mg/d) and cholecalciferol (800 IU/d) caused a 49% reduction in falls during the 12-week treatment versus the preceding 6-weeks, versus calcium alone²³. Those who fell most had the greatest benefit. Other studies have also suggested benefits in at-risk individuals such as less active older women²⁴ and those suffering stroke²⁵.

Not all studies, however, are concordant^{26,27}. A randomised study of 5292 subjects ≥ 70 years showed no benefit with cholecalciferol (800 IU daily) in the reduction of falls over 26-62 months²⁷. However in both negative studies^{26,27}, patients had recently sustained minimal-trauma fracture or were at risk of fracture, suggesting impaired mobility and falls were not the primary study endpoint.

One study suggested that ‘mega-doses’ *increased* falls risk. An annual oral dose of 500,000 IU of cholecalciferol increased falls in 2256 community dwelling older women, particularly in the 3 months following the dose (incidence rate ratio 1.31 compared to placebo)²⁸. The mechanism was unclear as serum levels were not toxic. As the vitamin D system is autoregulated by induction of 24-hydroxylase which inactivates 25OHD and 1,25OHD, we speculate that large single doses of cholecalciferol may result in excess 24-hydroxylase induction, rapidly degrading the administered dose and potentially contributing to subsequent deficiency.

Regarding muscle performance, studies show improved lower limb strength following vitamin D supplementation in institutionalised²⁹ and community-dwelling older individuals³⁰. Those with baseline 25-OHD levels <50 nmol/L had the greatest benefits. However, a study of single mega-dose therapy (300,000IU) in 243 frail, older patients did not improve

Accepted Article

performance even amongst those with baseline levels <30 nmol/L³¹. For a detailed list of studies examining vitamin D supplementation and falls, we refer readers to tables 4 and 5 in a recent review⁴.

Meta-analyses

In the widely cited meta-analysis by Bischoff-Ferrari, a dose-dependent effect of vitamin D supplementation in the reduction of falls was found when examining 8 trials and 2426 elderly individuals^{32,33}. Compared with placebo, higher doses of vitamin D (700 – 1000 IU) reduced falls risk by 19% but lower doses had no effect. Likewise, people who achieved serum 25OHD concentrations ≥ 60 nmol/l reduced falls by 23% but those who achieved concentrations ≤ 60 nmol/l had no effect.

The recent Institute of Medicine report criticised this meta-analysis for inconsistencies in study inclusion, perceived errors in statistical analysis and reliance on 2 studies to produce the positive effect, one of which was reportedly not well-powered³³. On re-analysis with two statistical alterations, the positive effect was not found.

The most recent and largest meta-analysis of 45,782 people, mainly elderly females, found a significant reduction in falls risk amongst those randomised to vitamin D supplementation (OR 0.86 for ≥ 1 fall)³⁴. There was no difference between higher (>800 IU) versus lower doses. Vitamin D was effective in both community-dwelling and institutionalised people with cholecalciferol or ergocalciferol. Reduction in falls was most prominent in patients who were deficient at baseline and with calcium co-administration. In studies included in this meta-analysis, the calcium doses ranged from 500 to 1200 mg daily and elemental calcium and calcium carbonate were used.

Meta-analyses dealing with vitamin D and muscle strength are limited by substantial study heterogeneity in design, including in the parameters of muscle function³¹. A recent meta-analysis that assessed 17 RCTs involving 5,072 participants reported no effect on grip strength or proximal lower limb strength in adults with 25OHD levels > 25 nmol/l¹⁰. However, on pooling data from two studies on vitamin D deficient adults (*i.e.* 25OHD < 25 nmol), a large effect on hip muscle strength was found although the studies used different measures.

Summary

Although observational studies link falls, muscle weakness and sarcopaenia with vitamin D deficiency, randomised interventional trials have yielded conflicting results. This may be explained by the heterogeneity in study design, variability in treatment dose, duration and the different analyses of falls and muscle function. Nevertheless, the data generally support a benefit of vitamin D supplementation in reducing falls amongst at-risk populations, specifically those with vitamin D deficiency and a history of falls³⁴. On this basis, we suggest the use of vitamin D in combination with calcium at a daily dose of at least 500 mg for falls prevention in older people with baseline vitamin D deficiency. The dose of vitamin D supplementation and target 25OHD levels are controversial. Whilst the Institute of Medicine recommends 25OHD target levels of 50 nmol/l and daily vitamin D doses of 800 IU d in older adults (>70yrs)³³, the US Endocrine society advocates higher serum target level of 75 nmol/l and daily doses of at least 1500-2000 IU in this age-group³⁵. Those targets are based on skeletal and biochemical outcomes and the evidence for muscle outcomes does not particularly favour one target over the other. It appears that single-dose megatherapy is not beneficial for muscle function and we recommend daily therapy in favour of intermittent mega-dosing.

Vitamin D and Muscle Function in the Young

Children with rickets, a condition characterised by chronic vitamin D deficiency, display profound muscle weakness and hypotonia. Similarly, those affected with the rare condition type II vitamin D dependent rickets display muscle weakness in association with mutations in the vitamin D receptor³⁶. These observations suggest that together with a critical role in bone mineralisation and metaphyseal fusion, vitamin D is important in muscle development.

Beyond the developmental stage, adults with osteomalacia display proximal myopathy characterised by proximal weakness, pain, a ‘waddling gait’ and difficulty rising from a seated or squat position^{37, 38}.

Here we review evidence for a role of vitamin D in muscle function and mass in those not yet affected by age-related muscle loss.

Physical Performance

There is extensive literature dating back to the 1930s reporting significant improvements in

physical performance following exposure to UV radiation. Although these studies do not directly refer to vitamin D, UV-mediated changes in vitamin D status may have played a role in muscle function amongst study participants. In 1938, a Russian group reported significant improvements in 100-metre sprint times amongst students receiving UV radiation (7.4% vs. 1.7% improvement in controls)³⁹. In 1944, a German group found that medical students receiving UV radiation over 6 weeks had a 13% improvement in performance on a bicycle ergometer⁴⁰. In a study from USA, 11 male students experienced a 19% increase in cardiovascular fitness following a course of UV radiation⁴¹.

Contemporary studies have reported surprisingly high rates of vitamin D deficiency amongst athletes, including Middle-eastern sportsmen (58% deficient)⁴², professional British athletes (57%)⁴³ and Australian gymnasts (33%)⁴⁴. However, studies examining vitamin D supplementation in athletes are sparse. In a study of thirty UK athletes randomised to cholecalciferol 20000 IU, 40000 IU or placebo for 12 weeks, despite significant increases in 25OHD levels at 6 and 12 weeks in the active arms, there was no significant improvement in muscle performance⁴³. In a larger study including 61 male athletes and 30 healthy male non-athletes in the UK, cholecalciferol (5000 IU per day) led to significant improvements in 10-metre sprint times and vertical jump over the 8-week period, with no improvement in the placebo group⁴⁵. Baseline 25OHD levels were lower in this study than in the previous one (mean ~ 40 vs. ~ 50 nmol/l) and higher 25OHD levels were achieved (mean 103 vs. ~ 85 – 91 nmol/l), possibly explaining the discrepancy. With the potential involvement of vitamin D in metabolic pathways⁴⁶, athletes may require a higher intake to ensure adequate availability for muscle metabolism and physical performance. Once again, there are suggestions that high-dose supplemental vitamin D does not improve muscle function compared to daily lower-doses^{43,45}.

A study of 14 recreationally active subjects found an association between baseline vitamin D levels and muscle recovery, assessed by leg isometric force, following a bout of intense exercise⁴⁷. Pre-exercise 25OHD levels predicted immediate and persistent muscle weakness (*i.e.* 48 and 72 hrs post-exercise) but there was no correlation at 24-hours. Interestingly, 25OHD levels initially increased (by ~ 5 nmol/l) and then decreased following exercise and the authors hypothesised this was due to exercise-related shifts in cytokine and protein levels.

In non-athletes, supplementation studies have yielded mixed results. Among 69 adolescent females, those randomised to receive 150,000 IU ergocalciferol orally every 3 months for 1

year demonstrated significant improvements in movement efficiency, a composite of jump height and velocity measured by mechanography, compared to baseline ⁴⁸. Additionally, higher baseline 25OHD correlated with greater jumping velocity.

In another study of 179 vitamin D deficient adolescent females in Lebanon, those randomised to receive cholecalciferol (doses 1,400 IU or 14,000 IU weekly) did not improve grip strength ⁴⁹. Adequate 25OHD levels were achieved in the high-dose group but not in the low dose-group ($\approx 95, 40$ nmol/l respectively, placebo 40 nmol/l), implying that increases in lean mass and bone mineral content seen in both groups versus placebo at 1 yr were not directly due to serum 25OHD levels.

In a case-control study, 55 veiled Arabic women with severe vitamin D deficiency (mean 25OHD 7nmol/L) were weaker on all tested parameters of muscle function than 22 Danish women with higher levels (47 nmol/L) ⁵⁰. Following vitamin D repletion (IM ergocalciferol: 100,000 IU per week for 1 month then monthly for 5 months and 400-600 IU orally daily), the Arabic women had significant improvements in muscle function and pain at 3 and 6 months. This may have been due to improvement in related biochemical defects and does not prove a *direct* role for vitamin D. In any case, mega-dose supplementation was helpful in this group.

Therefore, vitamin D deficiency is common in young populations and vitamin D supplementation may improve physical performance in athletes depending on the dose ^{43, 45}. Such an effect is less clear in non-athletic populations ^{48,49}.

Myalgia

Studies examining a putative association between vitamin D deficiency and myalgia (*i.e.* muscle pain) have generally been small, observational and potentially confounded.

Vitamin D deficiency was reported in 93% of individuals with persistent, nonspecific musculoskeletal pain ⁵¹. Significantly more frequent occurrence of 25OHD level <20 nmol/L was found in 40 premenopausal women with fibromyalgia compared to 37 age-matched controls ⁵². These studies were observational and potentially confounded by factors such as physical activity, smoking and BMI.

A single randomised trial of ergocalciferol supplementation (50,000 IU weekly for 3 months) in 50 subjects with 25OHD<50 nmol/L, failed to demonstrate improvement in pain scores compared to baseline or placebo ⁵³. As the study was adequately powered to detect a significant change in pain, results would suggest that high dose supplementation has no benefit in the management of generalised pain in the absence of osteomalacia. Therefore despite an association between vitamin D deficiency and myalgia, the single RCT addressing this question shows no effect.

VDR (Vitamin D Receptor) Polymorphisms

Vitamin D receptor (VDR) is the nuclear receptor to which 1,25(OH)₂D binds in order to regulate gene expression. Several VDR polymorphisms exist that are associated with a range of biological effects, including bone mineral density and muscle function ⁵⁴. The FokI polymorphism, found in ~ 35% of Caucasians ⁵⁴, is a T/C transition in exon 2 that results in a shorter protein with enhanced transactivation capacity. Although this greater activity might suggest improved muscle strength, the FokI polymorphism is in fact associated with reduced muscle strength. In 107 patients with Chronic Obstructive Pulmonary Disease (COPD), homozygosity was associated with reduced quadriceps strength compared to heterozygosity⁵⁵. In another study, the Fok I genotype associated with reduced muscle mass and sarcopaenia in 302 caucasian men aged 58-93 yrs ⁵⁶.

The BsmI polymorphism affects the 3' region which is important in the regulation of gene expression. It is reported in ~ 40% of Caucasians ⁵⁴. In studies of older individuals, the bb genotype was associated with greater strength and reduced risk of falls. Amongst 121 healthy women >70 years, the bb genotype was linked to higher quadriceps and wrist strength compared to the BB genotype ⁵⁷. In two population-based studies from Italy and the UK, the bb genotype of the BsmI polymorphism appeared to protect against falls ⁵⁸.

However, in younger people, studies link the bb genotype to *reduced* strength. Amongst 175 women (20–39 years), bb genotype associated with lower hamstring strength and lower fat-free mass compared to the BB genotype ⁵⁹. In a Chinese study of 109 female university students, bb genotype was associated with significantly lower peak torque in concentric knee flexors ⁶⁰.

While these polymorphisms are interesting, the associations are not consistent, and lack mechanistic explanation. Larger studies to assess the associations are needed. Importantly, polymorphisms of other components of the vitamin D system may also be relevant: DBP polymorphisms have been associated with different responses to vitamin D₃ supplementation⁶¹ and CYP27B1 polymorphisms have been linked to a greater risk of osteoporotic fracture⁶².

Mechanisms Linking Vitamin D and Muscle Function

A variety of mechanisms by which vitamin D impacts on muscle cells and fibres have been elucidated. This research includes the description of muscle morphology in vitamin D deficient subjects, signalling pathways by which vitamin D effects muscle cells *in vitro*, and *in vivo* studies that have sought to delineate effects and mechanisms of aberrant vitamin D signalling on muscle function. These have been summarised in **Figure 1**.

Changes in Muscle Morphology

Several reports dating back to the 1970s described striking morphological changes in the skeletal muscle of subjects with osteomalacia, likely related to severe vitamin D deficiency⁶³. The main change described is atrophy of the type 2 (*i.e.* fast twitch) muscle fibres with some reports of scattered necrosis and derangement of the inter-myofibrillar network⁶⁴.

These changes appear to be reversible. Eleven patients with a condition described as ‘bone loss of ageing’ displayed significant increases in the proportion and cross-sectional size of fast-twitch type IIa fibres of the vastus lateralis following treatment with the vitamin D analogue, 1 α -OHD₃, and calcium for 3 to 6 months⁶⁵. Interestingly, vitamin D supplementation resulted in changes to the oxidative capacity of muscle – succinate dehydrogenase and total phosphorylase activity were low at baseline and increased with treatment whilst lactate dehydrogenase activity, a measure of anaerobic metabolism, did not change⁶⁵.

At a functional level, Sato and colleagues suggested that the reduction in falls in a group of 48 women with post-stroke hemiplegia who received vitamin D₂ (1000 IU daily) supplementation could be explained by the increase in the proportion and diameter of type II muscle fibres at 1 year²⁵. A recent study also implicates improved mitochondrial function as a possible mechanism in the prevention of falls by vitamin D⁴⁶. In twelve individuals with severe vitamin D deficiency (<15 nmol/L), 10-12 weeks of treatment with cholecalciferol 20

000 IU on alternate days resulted in improved muscle mitochondrial function, as assessed by NMR spectroscopy.

There is an association between fatty infiltration of skeletal muscle and vitamin D deficiency. In 90 post-pubertal females in California, the proportion of quadriceps muscle fat, assessed by CT (computed tomography), was strongly inversely correlated with serum 25OHD levels, independent of body mass or subcutaneous and visceral fat ⁶⁶. Similar findings were reported in 20 older subjects who received MRI (magnetic resonance imaging) of the thigh ⁶⁷. Interestingly, selective and near total fatty degeneration of at least one muscle was observed amongst 55% of patients with 25OHD <50 nmol/L ⁶⁷.

These studies raise the interesting possibility that vitamin D deficiency may increase the deposition of intra-myocellular lipids, either directly or potentially as a consequence of the reduced physical activity or *via* reduced muscle mitochondrial capacity. Since defects in mitochondrial fuel metabolism and increases in intra-muscular lipids are thought to play a role in the pathogenesis of skeletal muscle insulin resistance ⁶⁸, these findings may potentially explain the association between vitamin D deficiency and insulin resistance.

VDR and Muscle

The presence of the VDR in avian, murine and human muscle cells is described by immunohistochemistry, Western blot and detection of VDR mRNA by RT-PCR ⁶⁹⁻⁷¹. However, a recent paper suggested that the VDR is not expressed in skeletal, cardiac or smooth muscle ¹². Differences in experimental conditions and the possibility of tight protein binding of VDR to DNA may have accounted for this finding. Another possible explanation is that VDR is expressed at low levels in resting muscle but is activated by particular conditions. In support of this, a recent paper reported that VDR expression increases following muscle injury in mice ¹³. Interestingly, this paper also reported the presence of 1-alpha-hydroxylase (CYP27B1) in muscle and that it *too* is activated during muscle injury. These findings support the intriguing possibility that VDR and CYP27B1 play roles in muscle regeneration and that muscle possesses the capacity for local production of 1,25(OH)₂D.

More recently, a pilot study linked vitamin D supplementation (4000 IU/d for 4 months) with an increase in muscle VDR expression and fibre size in older, mobility-limited women ⁷². Although this was a small study and the specificity of the particular antibody used is unclear (VDR-NR1H1), this study provides tantalizing evidence that vitamin D may influence muscle

fibre size in humans by up-regulating local VDR expression.

VDR defects also influence muscle function. Mice with congenital absence of VDR display a number of deficits in muscle function. On forced swim tests, they display more sinking episodes and fatigue compared to wild-type mice ⁷³. These changes are probably largely related to impaired muscle development with generalised atrophy of muscle fibres in this mouse model ⁷⁴. One important caveat is that these mouse models of VDR defects also display secondary biochemical abnormalities in calcium, phosphate and parathyroid hormone that could affect muscle function.

One study examined the precise aetiology of vitamin D deficiency myopathy by rendering rats vitamin D, phosphorus, and calcium deficient by dietary methods. Phosphorus levels correlated independently with the reduction in soleus muscle force in these rats and phosphorus repletion in the presence of persistent vitamin D deficiency resulted in complete restoration in slow-twitch muscle force ⁷⁵. Another study reported that calcium was predominantly important in the type 2 muscle fibre atrophy and the muscle protein degradation seen in vitamin D deficient rats ⁷⁶. Although calcium repletion mostly corrected these abnormalities, persistent vitamin D deficiency was itself associated with a degree of muscle fibre atrophy.

It therefore appears that muscle defects are due, in part, to vitamin D deficiency but predominantly to the associated hypocalcaemia and hypophosphataemia. Repletion with vitamin D remains the most effective treatment as it corrects all of those defects ^{50,77}.

Vitamin D and Intracellular Pathways in Muscle

A variety of molecular pathways by which vitamin D may affect muscle cells have been elucidated. These effects may be rapid, occurring within seconds to minutes of vitamin D treatment and include the release of calcium from intracellular stores and its subsequent entry *via* voltage-gated membrane channels ⁴. This suggests a role for vitamin D in the calcium-mediated functions of muscle, namely contraction, plasticity, mitochondrial function, insulin signalling and fuel handling. While evidence for a link between vitamin D and mitochondrial function is only recent ⁴⁶, potential alterations in muscle substrate metabolism may help explain the occurrence of insulin resistance ⁷⁸, intramuscular fatty deposition ⁶⁶ and muscle weakness ⁵⁰ in vitamin D deficient subjects. Serum 25OHD levels correlate with recovery

Accepted Article

rates of phospho-creatinine muscle stores after exercise, suggesting a broader link with ATP production and oxidative function ⁴⁶.

Vitamin D could lead to delayed effects on muscle *via* gene expression. This involves binding of the 1,25OHD-VDR-RXR (retinoid X-receptor) complex to vitamin D response elements of DNA and includes effects in the expression of contractile proteins and myogenic transcription factors which influence muscle development ⁷⁴.

More recently, it has emerged that skeletal muscle serves a storage site for 25OHD ⁷⁹. Skeletal muscle was found to express megalin and cubulin, proteins necessary for the endocytic internalisation of DBP-bound 25OHD and muscle fibres were noted to retain substantially higher proportions of tritium-labeled 25OHD than bone cells.

Therefore, evolving research points to both rapid and genomic effects of vitamin D in skeletal muscle and its storage at this site that may have a range of effects that remain unknown at this time.

Vitamin D and Insulin Sensitivity

Apart from the generation of force, skeletal muscle is a highly metabolic tissue that responds to a range of hormones including growth hormone, IGF-1, corticosteroids and insulin. Under normal physiological conditions, skeletal muscle is responsible for ~85% of whole-body insulin-mediated glucose uptake ⁸⁰ and is therefore of primary importance to insulin resistance. In this section, we discuss the evidence linking vitamin D deficiency with insulin resistance and the broader implications for the pathogenesis of type 2 diabetes.

Observational Studies (summarised in Table 1)

Amongst 808 non-diabetic participants of the Framingham Offspring Study, plasma 25OHD was inversely associated with fasting insulin concentrations and HOMA-IR (*i.e.* Homeostasis Model Assessment of Insulin Resistance) after adjustment for age, sex and BMI ⁸¹. A similar association between 25OHD and HOMA-IR was found in 712 subjects at risk of diabetes ⁸². HOMA is a mathematical model, based on data from physiological studies, which is used to estimate insulin resistance (HOMA-IR) and insulin sensitivity (HOMA-S). In a group of 126 healthy young adults, there was a significant association between 25OHD and insulin sensitivity assessed by hyperglycaemic clamps, after adjustment for a range of factors including BMI ⁸³.

Prospective studies show an association between baseline 25OHD levels and the long-term risk of developing insulin resistance (**Table 1**). In a study of 5,200 participants, a 25 nmol/L increment in baseline serum 25OHD levels was associated with a 24% reduced 5-year risk of diabetes ⁷⁸. A positive and independent association with HOMA-S at 5 years was also reported ($r = 0.16, p < 0.001$).

These observational studies suggest, but do not prove, a causal or directional relationship between vitamin D status and insulin resistance. Multiple possible confounders exist. In particular, adiposity has an independent inverse association with 25OHD, in many (though not all) studies ⁸⁴. This may relate to storage of fat-soluble compounds, the avoidance of sunlight exposure and outdoor activity amongst potentially self-conscious obese individuals. Adiposity is a key factor in insulin resistance. Other potential confounders include physical activity, parathyroid hormone levels, serum calcium, and dietary intake, all of which may influence or reflect 25OHD levels and also independently influence insulin sensitivity.

*Interventional Studies (summarised in **Table 2**)*

In people without diabetes or underlying insulin resistance, there is no clear evidence that vitamin D supplementation results in improved insulin sensitivity. However, limited evidence suggests that subjects at risk of diabetes may benefit. At-risk subjects randomised to receive various regimens of cholecalciferol and calcium over 6 weeks to 3 years, displayed significant improvements in insulin sensitivity, secretion and/or the disposition index (an integrated measure of insulin secretion and action)⁸⁵⁻⁸⁷.

The evidence is mixed for subjects with established diabetes. In a recent trial of 90 diabetic subjects, those randomised to receive daily vitamin D (1000 IU) in fortified yoghurt demonstrated improved glycaemic control and insulin resistance (HOMA-IR) versus those receiving plain yoghurt ⁸⁸. Importantly, an inverse correlation was observed between changes in serum 25OHD and HOMA-IR in this study.

As summarised in **Table 2**, the large number of small interventional studies in different populations with baseline vitamin D status, using different modes of supplementation, creates difficulty in drawing definite conclusions. Methods of assessing insulin sensitivity range from measuring fasting insulin to hyperinsulinaemic-euglycaemic clamps. Whilst insulin resistance is a factor in the genesis of type 2 diabetes, there is a complex interaction between glucose

and insulin levels, with glycaemic outcomes reflecting the relationship between both insulin resistance and insulin secretory capacity.

Thus, the question of whether vitamin D supplementation is effective in the amelioration of insulin resistance, and with particular respect to a role in muscle, remains unanswered. A number of large clinical trials are currently underway, which may definitively answer this question (e.g. NCT00736632, NCT01354964, NCT01315366).

Potential mechanisms

Mouse models of type 2 diabetes show improvements in insulin sensitivity following treatment with $1\alpha\text{OHD}_3$ ⁴. *In vitro* studies help to explain this link. $1,25\text{D}$ leads to increased expression of insulin receptors and amelioration of insulin resistance *via* effects on Akt and insulin receptor phosphorylation⁸⁹. Non-genomic effects of vitamin D may also be important. In addition to intracellular calcium regulation, vitamin D leads to the release of arachidonic acid, a polyunsaturated fatty acid, from the cell membrane and into the cytoplasm of muscle cells⁹⁰. This further links vitamin D with insulin sensitivity as does the possibility that vitamin D may influence caveolin-I⁹¹, a scaffolding protein within the membrane that plays roles in metabolism.

Conclusions (summarised in *Table 3*)

Our understanding of the role of vitamin D in skeletal muscle results from the combined efforts of basic scientists and clinical researchers. Reports of profound muscle weakness in children with rickets more than 80 years ago and more recent descriptions of muscle weakness and myalgia in adults with severe vitamin D deficiency have provided *prima facie* evidence of a role for vitamin D in muscle. Although historical literature reports substantial improvements in physical performance amongst athletes exposed to UV irradiation, a direct association with vitamin D status was not established in these early studies and remains to be substantiated by contemporary research.

Amongst older individuals, studies examining the effect of vitamin D on strength and falls have had mixed results. The general consensus is that correction of vitamin D deficiency is beneficial in preventing falls, in combination with calcium supplementation and with the recommended aim of achieving serum 25OHD levels of > 50 nmol/l according to the IOM or > 75 nmol/l according to the US Endocrine society.

Animal studies have demonstrated that congenital lack of the VDR and models of vitamin D deficiency are associated with defects in muscle function. From these studies, it has been difficult to dissect the individual contribution of vitamin D signalling to muscle function, distinct from its role in mineral homeostasis. At a molecular level, muscle cells show both rapid and delayed responses to the active form of vitamin D, 1,25OH₂D, that provides mechanistic support for these clinical and animal studies. Recent research showing the activation of VDR in regenerating muscle and the storage of 25OHD in skeletal muscle raise intriguing questions regarding its unknown roles at this site. In conclusion, vitamin D supplementation prevents falls in susceptible populations, vitamin D pathways regulate muscle development in animal models and vitamin D signalling alters various molecular pathways in cultured muscle cells. Further clinical and translational studies are needed, and will be of particular interest in muscle training, insulin resistance and muscle regeneration.

Figure 1: Potential Effects of Vitamin D on Muscle Cells

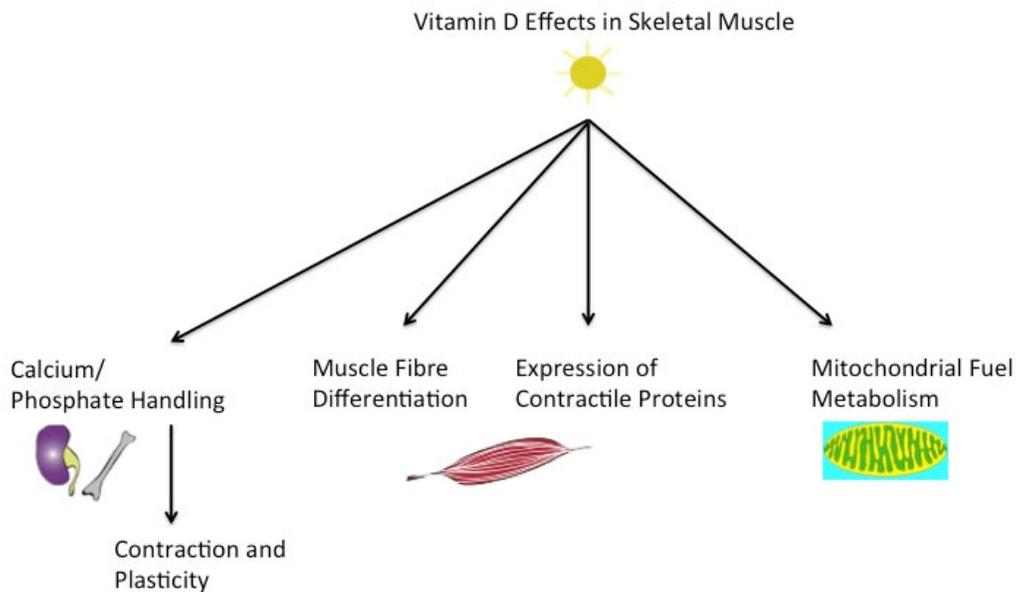


Table 1: Cross-sectional Studies Assessing the Correlation Between Serum Vitamin D Levels and Parameters of Insulin Sensitivity and Glucose Homeostasis

Study (ref)	Positive or negative	No.	Age, Sex	Other	Vitamin D nmol/l (ng/ml)	Tests	Findings	Risk or correlation	Adjustments
Kositsawat ⁹²	Positive, subgroup	9773	>18, M,F	NHANES		HbA1c	Inverse association 35-74 years	PE=-0.0035	Age, race, sex, DM, activity, supplements, PTH
						HbA1c	Inverse association non DM	PE=-0.0014	
Scragg ⁹³	Negative	6228	>20 M,F	All	M 78 (31), F 73 (29)	Log (HOMA-IR)	No association		Age, sex, BMI, activity, season
	Positive, subgroup	2766	>20 M,F	Caucasian	80 (32)	Log (HOMA-IR)	Positive association	beta = -0.009	Age, sex, BMI, activity, season
	Negative subgroup	1736	>20 M,F	African Americans	49 (19)	Log (HOMA-IR)	No association	beta= 0.001	Age, sex, BMI, activity, season
	Positive, subgroup	1726	>20 M,F	Mexican Americans	66 (26)	Log (HOMA-IR)	Positive association	beta= -0.016	Age, sex, BMI, activity, season
Cheng ⁹⁴	Negative	3890	40, M,F		92 (37)	Insulins, HOMA-IR	No association		Adipose tissue, season, WC, activity, D intake
Ford ⁹⁵	Positive, subgroup	1941	12-17 M,F		>75 (30) vs. <50 (20)	Fasting insulin, Hba1c	Association with insulin in males	24% lower insulin	Age, sex, BMI, HDL, ethnicity, activity, time, supplements, cholesterol

Kayani ⁸²	Positive	960	50, M,F	Caucasian	56	HOMA-IR, ISI on OGTT	Both markers associated	r= -0.29	SES, activity, supplements, PTH, BMI
Liu ⁸¹	Positive, subgroup	808	60 M, F		>54 (22) vs. <39 (16)	HOMA-IR, ISI, Insulins at OGTT	Improved fasting insulin & HOMA IR	(10% vs.13%)	Age, sex, BMI, waist circumference, smoking
Pinelli ⁹⁶	Positive, subgroup	542	38 M,F	46% Ins Res, 42% IGT	M 45 (18), F 35 (14)	HOMA-IR, FPG, HbA1c	Inverse association, men only	M r= -0.19	
Del Gobbo ⁹⁷	Negative	510	>18 M,F	Canadian Cree	52 (21)	HOMA-IR	No association	r= 0.03	BMI, age, sex, activity, education, ETOH, smoking
Gannage' - Yared ⁹⁸	Positive, subgroup	381	24 M,F	Lebanon	77 (31)	HOMA-IR, FPG	Association with FPG	r= -0.15	Sex and BMI
Baynes ⁹⁹	Positive	142	70-88 M		42 (17)	Insulin at OGTT	Inverse association	r= -0.18 to -0.23	age, BMI, activity, month, smoking, ETOH
Manco ¹⁰⁰	Negative	116	F	Italian	39 (16)	EH clamp	No association		
Chiu ⁸³	Positive	126	~25 M,F		47-70 (19-28)	ISI on H clamp	Significant association	r = 0.25	Age, sex, ethnicity, BMI, WHR, BP, season

Abbreviations, BMI = Body mass index, BP = Blood pressure, EH clamp= euglycaemic hyperinsulinaemic clamp, ETOH= Alcohol consumption, FPG = Fasting Plasma Glucose, H clamp= hyperglycaemic clamp, ISI= Insulin Sensitivity Index, IR = Insulin Resistance, MetS = Metabolic Syndrome, IGT = Impaired Glucose Tolerance, OGTT = Oral Glucose Tolerance Test, HbA1C = Glycated haemoglobin, HOMA-IR = Homeostasis Model Assessment of insulin Resistance, NR = Not Reported, PE = Parameter estimates, SES = Socioeconomic status, WC = Waist circumference, WHR = Waist to hip ratio

Table 2: Interventional Studies Assessing the Effects of Vitamin D Supplementation on Parameters of Insulin Sensitivity

Study	Positive or Negative	No.	Age, Sex	Other	Basal 25OHD nmol/l (ng/ml)	Intervention D in IU/day, Ca in mg	↑ VitD ?	Follow -Up	Outcomes	Findings	Adjustments
Non-Diabetic at Baseline											
de Boer ¹⁰¹	Negative	33951	50-79 F		<80 (32) in 89%	D ₃ 400+ Ca 1000 vs. placebo	No	7y	T2D onset	No change	Nil
Menas ⁹² et al	Negative	238	45-54 F		NR	D ₃ 2000, 1 α -OHD 0.25 μ g, 1,25OHD 0.25-0.5 μ g or placebo	NR	2y, 1yr	BGL, weight	No change	Nil
At risk of Diabetes											
Pi ⁸⁵⁻⁸⁷ et al	Positive, subgroup	314	~ 71 M,F	92 IFG, 222 NGT	> 65 (26) in most	D ₃ 700+Ca 500 or placebo	Yes	3y	FPG, HOMA-IR	IFG arm ↓ rises in FPG and HOMA-IR	Age, sex, BMI, activity, smoking

Mitri ⁸⁵	Positive	92	57 M,F	BMI>25, IGT &/or IFG	61 (24.5)	D ₃ 2000 or Ca 800mg	Yes	16w	Clamp	↑insulin release and DI	Age, sex, BMI, baseline values, race, time
Nagpal ⁸⁶	Positive	71	~ 45 M	Central obesity, Indian	<50 (20)	D ₃ 120,000 two weekly or placebo	Yes	6 w	OGIS	Improvement	Age, WHR, baseline 25OHD, betel-nut use
Ljunghall ¹⁰³	Negative	65	61- 65 M	IGT	'Normal'	1αOHD ₃ (0.75 μg) or placebo	Yes	3 m	FPG, IVGTT, HbA _{1c}	No change	Nil
von Hurst ¹⁰⁴	Positive	81	23- 68 F	South Asian	<50 (20)	D ₃ 400 or placebo	Yes	6 m	HOMA-IR, Insulin	Improved. More effect if 25OHD >32ng/ml.	Baseline values

Subjects with Diabetes

Nikooyeh ⁸⁸	Positive	90	51 M,F	Iranian	~44 (18)	Yoghurt: plain vs. 1000 D ₃ vs. D ₃ 1000+Ca 300	Yes	12 w	HbA _{1c} , HOMA-IR	↓ HbA1c and HOMA-IR	Nil
Sabherwal ¹⁰⁵	Positive	52	33- 73 M,F	South Asian Retro.	15 (6)	D ₃ 400 and Ca1200 No placebo group.	Yes	3 m	HbA _{1c}	ΔHbA1c and Δ25OHD correlated. r = -0.305	Nil
Jorde ¹⁰⁶	Negative	36	~55 M,F	Metform in + insulin	~60 (24)	D ₃ 40,000 /w or placebo	Yes	6 m	C-peptide HbA _{1c} , fasting insulin	No change	Age, sex, BMI, time, baseline D

Corissova 97	Positive: insulin secretion; Negative: IR	27	? F	10 T2DM, 17 control	35 (14)	T2DM: Vitamin D ₃ 1332	Yes	1 m	IVGTT, Peripheral IR	↑ 1st-phase insulin. Nil No change in IR
-----------------	---	----	-----	------------------------------	---------	--------------------------------------	-----	-----	-----------------------------	---

Ca=calcium, IGT= Impaired Glucose tolerance, IFG= Impaired fasting glucose, FPG= Fasting Plasma Glucose, HbA1c= Glycated haemoglobin, IR = Insulin Resistance, DI = disposition index, OGIS = Oral glucose insulin sensitivity, NR = Not reported, OGTT= Oral Glucose tolerance testing, IVGTT = Intravenous glucose tolerance testing, HOMA-IR = Homeostasis Model Assessment of insulin Resistance, Clamp= Euglycaemic hyperinsulinaemic clamp, QUICKI= Quantitative Insulin Sensitivity Check Index, FIRI= Fasting Insulin Resistance Index, doses of vitamin D and calcium are daily unless specified otherwise. Retro=retrospective.

Table 3: Conclusions
Vitamin D deficiency is associated with muscle weakness, falls and sarcopaenia.
In general, RCT evidence supports the use of supplemental vitamin D to prevent falls in elderly, institutionalised individuals.
RCT evidence suggests a beneficial effect of supplemental vitamin D in proximal muscle strength in subjects with severe vitamin D deficiency. However this area is clouded by non-uniformity in end-points and muscle parameters.
Although vitamin D deficiency is associated with myalgia, the single RCT addressing this question shows no effect of vitamin D supplementation in alleviating myalgia in deficient subjects.
Vitamin D deficiency is surprisingly common in athletes. Two recent RCTs suggest that higher serum 25OHD levels (~ 100 nmol/l) may be necessary to produce effects in athletic performance.
Vitamin D deficiency is associated with insulin resistance but this is confounded by adiposity and sedentary, indoor living. There is limited RCT evidence examining the effects of supplemental vitamin D in those with/at risk of insulin resistance but more studies are currently underway.
VDR polymorphisms are associated with differences in muscle function. This weighs in to the debate regarding the presence of VDR in skeletal muscle.
Animal studies support a combined role for vitamin D and its associated minerals (calcium/phosphate) in the development of muscle defects in vitamin D deficiency. However mineral defects appear to be important.
In culture, muscle cells respond directly to 1,25(OH) ₂ D supporting a molecular basis for its <i>in vivo</i> effects.
Two intriguing studies have recently emerged, one reporting the activation of VDR in regenerating muscle and another demonstrating that muscle serves as a storage site for 25OHD. The elucidation of vitamin D's effects in muscle promises to remain a vibrant and evolving field.

REFERENCES

1. Mayer, E. (1932) *The Curative Value of Light: Sunlight and Sun Lamp in Health and Disease*. In Book (ed. Editor), pp. Kessinger Publishing.
2. Hess, A.F. (1922) The Prevention and Cure of Rickets by Sunlight. *American Journal of Public Health*, **12**, 104-7.
3. Windhaus, A., Linsert, O., Luttringhaus, A. *et al.* (1932) Uber Das Krystallisierte Vitamin D2. *Justus Liebigs Ann Chem*, **492**, 226-231.
4. Girgis, C.M., Clifton-Bligh, R.J., Hamrick, M.W. *et al.* (2013) The Roles of Vitamin D in Skeletal Muscle: Form, Function, and Metabolism. *Endocrine Reviews*, **34**, 33-83.
5. Daly, R.M., Gagnon, C., Lu, Z.X. *et al.* (2012) Prevalence of Vitamin D Deficiency and Its Determinants in Australian Adults Aged 25 Years and Older: A National, Population-Based Study. *Clinical Endocrinology*, **77**, 26-35.
6. Forrest, K.Y.& Stuhldreher, W.L. (2011) Prevalence and Correlates of Vitamin D Deficiency in Us Adults. *Nutrition research*, **31**, 48-54.
7. Holick, M.F. (2007) Vitamin D Deficiency. *New England Journal of Medicine*, **357**, 266-81.
8. Chalmers, J., Conacher, W.D., Gardner, D.L. *et al.* (1967) Osteomalacia--a Common Disease in Elderly Women. *The Journal of bone and joint surgery*, **49**, 403-23.
9. Murad, M.H., Elamin, K.B., Abu Elnour, N.O. *et al.* (2011) The Effect of Vitamin D on Falls: A Systematic Review and Meta-Analysis. *Journal of Clinical Endocrinology and Metabolism*, **96** 1911-30.
10. Stockton, K.A., Mengersen, K., Paratz, J.D. *et al.* (2011) Effect of Vitamin D Supplementation on Muscle Strength: A Systematic Review and Meta-Analysis. *Osteoporosis International*, **22**, 859-71.
11. Bischoff-Ferrari, H.A., Borchers, M., Gudat, F. *et al.* (2004) Vitamin D Receptor Expression in Human Muscle Tissue Decreases with Age. *Journal of Bone and Mineral Research*, **19**, 265-9.
12. Wang, Y.& DeLuca, H.F. (2011) Is the Vitamin D Receptor Found in Muscle? *Endocrinology*, **152**, 354-63.
13. Srikuea, R., Zhang, X., Park-Sarge, O.K. *et al.* (2012) Vdr and Cyp27b1 Are Expressed in C2c12 Cells and Regenerating Skeletal Muscle: Potential Role in Suppression of Myoblast Proliferation. *American Journal of Physiology. Cell Physiology*, **303**, C396-405.

14. Bunout, D., de la Maza, M.P., Barrera, G. *et al.* (2011) Association between Sarcopenia and Mortality in Healthy Older People. *Australasian journal on ageing*, **30**, 89-92.
15. Visser, M., Deeg, D.J., Lips, P. (2003) Low Vitamin D and High Parathyroid Hormone Levels as Determinants of Loss of Muscle Strength and Muscle Mass (Sarcopenia): The Longitudinal Aging Study Amsterdam. *The Journal of clinical endocrinology and metabolism*, **88**, 5766-72.
16. Flicker, L., Mead, K., MacInnis, R.J. *et al.* (2003) Serum Vitamin D and Falls in Older Women in Residential Care in Australia. *Journal of the American Geriatrics Society*, **51**, 1533-8.
17. Snijder, M.B., van Schoor, N.M., Pluijm, S.M. *et al.* (2006) Vitamin D Status in Relation to One-Year Risk of Recurrent Falling in Older Men and Women. *Journal of Clinical Endocrinology and Metabolism*, **91**, 2980-5.
18. Wicherts, I.S., van Schoor, N.M., Boeke, A.J. *et al.* (2007) Vitamin D Status Predicts Physical Performance and Its Decline in Older Persons. *Journal of Clinical Endocrinology and Metabolism*, **92**, 2058-65.
19. Verreault, R., Semba, R.D., Volpato, S. *et al.* (2002) Low Serum Vitamin D Does Not Predict New Disability or Loss of Muscle Strength in Older Women. *Journal of the American Geriatrics Society*, **50**, 912-7.
20. Serrano-Urrea, R.& Garcia-Meseguer, M.J. (2013) Malnutrition in an Elderly Population without Cognitive Impairment Living in Nursing Homes in Spain: Study of Prevalence Using the Mini Nutritional Assessment Test. *Gerontology*, **Aug 14 [Epub ahead of print]**,
21. Sambrook, P.N., Cameron, I.D., Chen, J.S. *et al.* (2011) Does Increased Sunlight Exposure Work as a Strategy to Improve Vitamin D Status in the Elderly: A Cluster Randomised Controlled Trial. *Osteoporosis International*,
22. Flicker, L., MacInnis, R.J., Stein, M.S. *et al.* (2005) Should Older People in Residential Care Receive Vitamin D to Prevent Falls? Results of a Randomized Trial. *Journal of the American Geriatrics Society*, **53**, 1881-8.
23. Bischoff, H.A., Stahelin, H.B., Dick, W. *et al.* (2003) Effects of Vitamin D and Calcium Supplementation on Falls: A Randomized Controlled Trial. *Journal of bone and mineral research*, **18**, 343-51.
24. Bischoff-Ferrari, H.A., Orav, E.J., Dawson-Hughes, B. (2006) Effect of Cholecalciferol Plus Calcium on Falling in Ambulatory Older Men and Women: A 3-Year Randomized Controlled Trial. *Archives of internal medicine*, **166**, 424-30.
25. Sato, Y., Iwamoto, J., Kanoko, T. *et al.* (2005) Low-Dose Vitamin D Prevents Muscular Atrophy and Reduces Falls and Hip Fractures in Women after Stroke: A Randomized Controlled Trial. *Cerebrovascular diseases*, **20**, 187-92.

26. Porthouse, J., Cockayne, S., King, C. *et al.* (2005) Randomised Controlled Trial of Calcium and Supplementation with Cholecalciferol (Vitamin D3) for Prevention of Fractures in Primary Care. *British medical journal*, **330**, 1003.
27. Grant, A.M., Avenell, A., Campbell, M.K. *et al.* (2005) Oral Vitamin D3 and Calcium for Secondary Prevention of Low-Trauma Fractures in Elderly People (Randomised Evaluation of Calcium or Vitamin D, Record): A Randomised Placebo-Controlled Trial. *Lancet*, **365**, 1621-8.
28. Sanders, K.M., Stuart, A.L., Williamson, E.J. *et al.* (2010) Annual High-Dose Oral Vitamin D and Falls and Fractures in Older Women: A Randomized Controlled Trial. *Journal of the American Medical Association*, **303**, 1815-22.
29. Moreira-Pfrimer, L.D., Pedrosa, M.A., Teixeira, L. *et al.* (2009) Treatment of Vitamin D Deficiency Increases Lower Limb Muscle Strength in Institutionalized Older People Independently of Regular Physical Activity: A Randomized Double-Blind Controlled Trial. *Annals of nutrition & metabolism*, **54**, 291-300.
30. Zhu, K., Austin, N., Devine, A. *et al.* (2010) A Randomized Controlled Trial of the Effects of Vitamin D on Muscle Strength and Mobility in Older Women with Vitamin D Insufficiency. *Journal of the American Geriatrics Society*, **58**, 2063-8.
31. Latham, N.K., Anderson, C.S., Reid, I.R. (2003) Effects of Vitamin D Supplementation on Strength, Physical Performance, and Falls in Older Persons: A Systematic Review. *Journal of the American Geriatrics Society*, **51**, 1219-26.
32. Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B. *et al.* (2009) Fall Prevention with Supplemental and Active Forms of Vitamin D: A Meta-Analysis of Randomised Controlled Trials. *British medical journal*, **339**, b3692.
33. Ross, A., Taylor, C., Yaktine, A., Del Valle, H. , *Dietary Reference Intakes for Calcium and Vitamin D. Institute of Medicine Report.*, in *Institute of Medicine (Us) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium*2011, The National Academies Press: Washington DC.
34. Murad, M.H., Elamin, K.B., Abu Elnour, N.O. *et al.* (2011) The Effect of Vitamin D on Falls: A Systematic Review and Meta-Analysis. *Journal of Clinical Endocrinology & Metabolism*,
35. Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A. *et al.* (2011) Evaluation, Treatment, and Prevention of Vitamin D Deficiency: An Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism*, **96**, 1911-30.
36. Takeda, E., Yokota, I., Kawakami, I. *et al.* (1989) Two Siblings with Vitamin-D-Dependent Rickets Type II: No Recurrence of Rickets for 14 Years after Cessation of Therapy. *European journal of pediatrics*, **149**, 54-7.

37. Chalmers, J., Conacher, W.D., Gardner, D.L. *et al.* (1967) Osteomalacia--a Common Disease in Elderly Women. *Journal of Bone and Joint Surgery. British Volume*, **49**, 403-23.
38. Russell, J.A. (1994) Osteomalacic Myopathy. *Muscle & nerve*, **17**, 578-80.
39. Gorkin Z, G.M., Teslenko NE (1938) The Effect of Ultraviolet Irradiation Upon Training for 100m Sprint [Article in Russian]. *Fiziol Zh USSR*, **25**, 695-701.
40. Lehmann G, M.E. (1944) Ultraviolet Irradiation and Altitude Fitness [Article in German]. *Lufahrt Medizin*, **9**, 37-43.
41. Allen R, C.T. (1945) Effects of Ultraviolet Radiation on Physical Fitness. *Arch Phys Med.*, **10**, 641-4.
42. Hamilton, B., Grantham, J., Racinais, S. *et al.* (2010) Vitamin D Deficiency Is Endemic in Middle Eastern Sportsmen. *Public Health Nutrition*, **13**, 1528-34.
43. Close, G.L., Leckey, J., Patterson, M. *et al.* (2013) The Effects of Vitamin D(3) Supplementation on Serum Total 25[Oh]D Concentration and Physical Performance: A Randomised Dose-Response Study. *British journal of sports medicine*, **47**, 692-6.
44. Lovell, G. (2008) Vitamin D Status of Females in an Elite Gymnastics Program. *Clinical Journal of Sport Medicine*, **18**, 159-61.
45. Close, G.L., Russell, J., Cobley, J.N. *et al.* (2013) Assessment of Vitamin D Concentration in Non-Supplemented Professional Athletes and Healthy Adults During the Winter Months in the Uk: Implications for Skeletal Muscle Function. *Journal of Sports Sciences*, **31**, 344-53.
46. Sinha, A., Hollingsworth, K.G., Ball, S. *et al.* (2013) Improving the Vitamin D Status of Vitamin D Deficient Adults Is Associated with Improved Mitochondrial Oxidative Function in Skeletal Muscle. *The Journal of clinical endocrinology and metabolism*, **98**, E509-13.
47. Barker, T., Henriksen, V.T., Martins, T.B. *et al.* (2013) Higher Serum 25-Hydroxyvitamin D Concentrations Associate with a Faster Recovery of Skeletal Muscle Strength after Muscular Injury. *Nutrients*, **5**, 1253-75.
48. Ward, K.A., Das, G., Roberts, S.A. *et al.* (2010) A Randomized, Controlled Trial of Vitamin D Supplementation Upon Musculoskeletal Health in Postmenarchal Females. *Journal of Clinical Endocrinology & Metabolism*, **95**, 4643-51.
49. El-Hajj Fuleihan, G., Nabulsi, M., Tamim, H. *et al.* (2006) Effect of Vitamin D Replacement on Musculoskeletal Parameters in School Children: A Randomized Controlled Trial. *Journal of Clinical Endocrinology & Metabolism*, **91**, 405-12.
50. Glerup, H., Mikkelsen, K., Poulsen, L. *et al.* (2000) Hypovitaminosis D Myopathy without Biochemical Signs of Osteomalacic Bone Involvement. *Calcified Tissue International*, **66**, 419-24.

51. Plotnikoff, G.A.& Quigley, J.M. (2003) Prevalence of Severe Hypovitaminosis D in Patients with Persistent, Nonspecific Musculoskeletal Pain. *Mayo Clinic proceedings. Mayo Clinic*, **78**, 1463-70.
52. Al-Allaf, A.W., Mole, P.A., Paterson, C.R. *et al.* (2003) Bone Health in Patients with Fibromyalgia. *Rheumatology*, **42**, 1202-6.
53. Warner, A.E.& Arnsperger, S.A. (2008) Diffuse Musculoskeletal Pain Is Not Associated with Low Vitamin D Levels or Improved by Treatment with Vitamin D. *Journal of Clinical Rheumatology*, **14**, 12-6.
54. Uitterlinden, A.G., Fang, Y., Van Meurs, J.B. *et al.* (2004) Genetics and Biology of Vitamin D Receptor Polymorphisms. *Gene*, **338**, 143-56.
55. Hopkinson, N.S., Li, K.W., Kehoe, A. *et al.* (2008) Vitamin D Receptor Genotypes Influence Quadriceps Strength in Chronic Obstructive Pulmonary Disease. *American Journal of Clinical Nutrition*, **87**, 385-90.
56. Roth, S.M., Zmuda, J.M., Cauley, J.A. *et al.* (2004) Vitamin D Receptor Genotype Is Associated with Fat-Free Mass and Sarcopenia in Elderly Men. *The journals of gerontology. Series A, Biological sciences and medical sciences*, **59**, 10-5.
57. Geusens, P., Vandevyver, C., Vanhoof, J. *et al.* (1997) Quadriceps and Grip Strength Are Related to Vitamin D Receptor Genotype in Elderly Nonobese Women. *Journal of Bone and Mineral Research*, **12**, 2082-8.
58. Onder, G., Capoluongo, E., Danese, P. *et al.* (2008) Vitamin D Receptor Polymorphisms and Falls among Older Adults Living in the Community: Results from the ILSIRENTE Study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, **23**, 1031-6.
59. Grundberg, E., Brandstrom, H., Ribom, E.L. *et al.* (2004) Genetic Variation in the Human Vitamin D Receptor Is Associated with Muscle Strength, Fat Mass and Body Weight in Swedish Women. *European Journal of Endocrinology*, **150**, 323-8.
60. Wang, P., Ma, L.H., Wang, H.Y. *et al.* (2006) Association between Polymorphisms of Vitamin D Receptor Gene Apai, Bsmi and Taqi and Muscular Strength in Young Chinese Women. *International Journal of Sports Medicine*, **27**, 182-6.
61. Nimitphong, H., Saetung, S., Chanprasertyotin, S. *et al.* (2013) Changes in Circulating 25-Hydroxyvitamin D According to Vitamin D Binding Protein Genotypes after Vitamin D(3) or D(2)Supplementation. *Nutrition journal*, **12**, 39.
62. Clifton-Bligh, R.J., Nguyen, T.V., Au, A. *et al.* (2011) Contribution of a Common Variant in the Promoter of the 1-Alpha-Hydroxylase Gene (Cyp27b1) to Fracture Risk in the Elderly. *Calcified Tissue International*, **88**, 109-16.
63. Irani, P.F. (1976) Electromyography in Nutritional Osteomalacic Myopathy. *Journal of Neurology, Neurosurgery, and Psychiatry*, **39**, 686-93.

64. Yoshikawa, S., Nakamura, T., Tanabe, H. *et al.* (1979) Osteomalacic Myopathy. *Endocrinologia Japonica*, **26**, 65-72.
65. Sorensen, O.H., Lund, B., Saltin, B. *et al.* (1979) Myopathy in Bone Loss of Ageing: Improvement by Treatment with 1 Alpha-Hydroxycholecalciferol and Calcium. *Clinical Science*, **56**, 157-61.
66. Gilsanz, V., Kremer, A., Mo, A.O. *et al.* (2010) Vitamin D Status and Its Relation to Muscle Mass and Muscle Fat in Young Women. *Journal of Clinical Endocrinology & Metabolism*, **95**, 1595-601.
67. Tagliafico, A.S., Ameri, P., Bovio, M. *et al.* (2010) Relationship between Fatty Degeneration of Thigh Muscles and Vitamin D Status in the Elderly: A Preliminary Mri Study. *AJR. American journal of roentgenology*, **194**, 728-34.
68. Samuel, V.T.& Shulman, G.I. (2012) Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell*, **148**, 852-71.
69. Boland, R., Buitrago, C., De Boland, A.R. (2005) Modulation of Tyrosine Phosphorylation Signalling Pathways by 1alpha,25(OH)₂-Vitamin D₃. *Trends in endocrinology and metabolism: TEM*, **16**, 280-7.
70. Costa, E.M., Blau, H.M., Feldman, D. (1986) 1,25-Dihydroxyvitamin D₃ Receptors and Hormonal Responses in Cloned Human Skeletal Muscle Cells. *Endocrinology*, **119**, 2214-20.
71. Simpson, R.U., Thomas, G.A., Arnold, A.J. (1985) Identification of 1,25-Dihydroxyvitamin D₃ Receptors and Activities in Muscle. *Journal of Biological Chemistry*, **260**, 8882-91.
72. Ceglia, L., Niramitmahapanya, S., Morais, M.D. *et al.* (2013) A Randomized Study on the Effect of Vitamin D₃ Supplementation on Skeletal Muscle Morphology and Vitamin D Receptor Concentration in Older Women. *The Journal of clinical endocrinology and metabolism*,
73. Kalueff, A.V., Lou, Y.R., Laaksi, I. *et al.* (2004) Impaired Motor Performance in Mice Lacking Neurosteroid Vitamin D Receptors. *Brain Research Bulletin*, **64**, 25-9.
74. Endo, I., Inoue, D., Mitsui, T. *et al.* (2003) Deletion of Vitamin D Receptor Gene in Mice Results in Abnormal Skeletal Muscle Development with Deregulated Expression of Myoregulatory Transcription Factors. *Endocrinology*, **144**, 5138-44.
75. Schubert, L.& DeLuca, H.F. (2010) Hypophosphatemia Is Responsible for Skeletal Muscle Weakness of Vitamin D Deficiency. *Archives of Biochemistry and Biophysics*, **500**, 157-61.
76. Bhat, M., Kalam, R., Qadri, S.S. *et al.* (2013) Vitamin D Deficiency Induced Muscle Wasting Occurs through the Ubiquitin Proteasome Pathway and Is Partially Corrected by Calcium in Male Rats. *Endocrinology*,

77. Ziambaras, K.& Dagogo-Jack, S. (1997) Reversible Muscle Weakness in Patients with Vitamin D Deficiency. *The Western journal of medicine*, **167**, 435-9.
78. Gagnon, C., Lu, Z.X., Magliano, D.J. *et al.* (2011) Serum 25-Hydroxyvitamin D, Calcium Intake, and Risk of Type 2 Diabetes after 5 Years: Results from a National, Population-Based Prospective Study (the Australian Diabetes, Obesity and Lifestyle Study). *Diabetes Care*, **34**, 1133-8.
79. Abboud, M., Puglisi, D.A., Davies, B.N. *et al.* (2013) Evidence for a Specific Uptake and Retention Mechanism for 25-Hydroxyvitamin D (25ohd) in Skeletal Muscle Cells. *Endocrinology*, **154**, 3022-30.
80. Ryder, J.W., Gilbert, M., Zierath, J.R. (2001) Skeletal Muscle and Insulin Sensitivity: Pathophysiological Alterations. *Frontiers in bioscience : a journal and virtual library*, **6**, D154-63.
81. Liu, E., Meigs, J.B., Pittas, A.G. *et al.* (2009) Plasma 25-Hydroxyvitamin D Is Associated with Markers of the Insulin Resistant Phenotype in Nondiabetic Adults. *The Journal of nutrition*, **139**, 329-34.
82. Kayaniyil, S., Vieth, R., Retnakaran, R. *et al.* (2010) Association of Vitamin D with Insulin Resistance and Beta-Cell Dysfunction in Subjects at Risk for Type 2 Diabetes. *Diabetes Care*, **33**, 1379-81.
83. Chiu, K.C., Chu, A., Go, V.L. *et al.* (2004) Hypovitaminosis D Is Associated with Insulin Resistance and Beta Cell Dysfunction. *American Journal of Clinical Nutrition*, **79**, 820-825.
84. Manco, M., Calvani, M., Nanni, G. *et al.* (2005) Low 25-Hydroxyvitamin D Does Not Affect Insulin Sensitivity in Obesity after Bariatric Surgery. *Obesity Research*, **13**, 1692-700.
85. Mitri, J., Dawson-Hughes, B., Hu, F.B. *et al.* (2011) Effects of Vitamin D and Calcium Supplementation on Pancreatic Beta Cell Function, Insulin Sensitivity, and Glycemia in Adults at High Risk of Diabetes: The Calcium and Vitamin D for Diabetes Mellitus (Caddm) Randomized Controlled Trial. *Am J Clin Nutr*, **94**, 486-94.
86. Nagpal, J., Pande, J.N., Bhartia, A. (2009) A Double-Blind, Randomized, Placebo-Controlled Trial of the Short-Term Effect of Vitamin D3 Supplementation on Insulin Sensitivity in Apparently Healthy, Middle-Aged, Centrally Obese Men. *Diabetic medicine : a journal of the British Diabetic Association*, **26**, 19-27.
87. Pittas, A.G., Harris, S.S., Stark, P.C. *et al.* (2007) The Effects of Calcium and Vitamin D Supplementation on Blood Glucose and Markers of Inflammation in Non-Diabetic Adults. *Diabetes Care*,
88. Nikooyeh, B., Neyestani, T.R., Farvid, M. *et al.* (2011) Daily Consumption of Vitamin D- or Vitamin D + Calcium-Fortified Yogurt Drink Improved Glycemic

- Control in Patients with Type 2 Diabetes: A Randomized Clinical Trial. *Am J Clin Nutr*, **93**, 764-71.
89. Zhou, Q.G., Hou, F.F., Guo, Z.J. *et al.* (2008) 1,25-Dihydroxyvitamin D Improved the Free Fatty-Acid-Induced Insulin Resistance in Cultured C2c12 Cells. *Diabetes/metabolism research and reviews*, **24**, 459-64.
90. de Boland, A.R.& Boland, R.L. (1993) 1,25-Dihydroxyvitamin D-3 Induces Arachidonate Mobilization in Embryonic Chick Myoblasts. *Biochimica et Biophysica Acta*, **1179**, 98-104.
91. Huhtakangas, J.A., Olivera, C.J., Bishop, J.E. *et al.* (2004) The Vitamin D Receptor Is Present in Caveolae-Enriched Plasma Membranes and Binds 1 Alpha,25(OH)₂-Vitamin D₃ in Vivo and in Vitro. *Molecular Endocrinology*, **18**, 2660-71.
92. Kositsawat, J., Freeman, V.L., Gerber, B.S. *et al.* (2010) Association of A1c Levels with Vitamin D Status in U.S. Adults: Data from the National Health and Nutrition Examination Survey. *Diabetes Care*, **33**, 1236-8.
93. Scragg, R., Sowers, M., Bell, C. (2004) Serum 25-Hydroxyvitamin D, Diabetes, and Ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care*, **27**, 2813-8.
94. Cheng, S., Massaro, J.M., Fox, C.S. *et al.* (2010) Adiposity, Cardiometabolic Risk, and Vitamin D Status: The Framingham Heart Study. *Diabetes*, **59**, 242-8.
95. Ford, E.S., Zhao, G., Tsai, J. *et al.* (2011) Associations between Concentrations of Vitamin D and Concentrations of Insulin, Glucose, and Hba1c among Adolescents in the United States. *Diabetes Care*, **34**, 646-8.
96. Pinelli, N.R., Jaber, L.A., Brown, M.B. *et al.* (2010) Serum 25-Hydroxy Vitamin D and Insulin Resistance, Metabolic Syndrome, and Glucose Intolerance among Arab Americans. *Diabetes Care*, **33**, 1373-5.
97. Del Gobbo, L.C., Song, Y., Dannenbaum, D.A. *et al.* (2011) Serum 25-Hydroxyvitamin D Is Not Associated with Insulin Resistance or Beta Cell Function in Canadian Cree. *The Journal of nutrition*, **141**, 290-5.
98. Gannage-Yared, M.H., Chedid, R., Khalife, S. *et al.* (2009) Vitamin D in Relation to Metabolic Risk Factors, Insulin Sensitivity and Adiponectin in a Young Middle-Eastern Population. *European journal of endocrinology / European Federation of Endocrine Societies*, **160**, 965-71.
99. Baynes, K.C., Boucher, B.J., Feskens, E.J. *et al.* (1997) Vitamin D, Glucose Tolerance and Insulinaemia in Elderly Men. *Diabetologia*, **40**, 344-7.
100. Manco, M., Calvani, M., Nanni, G. *et al.* (2005) Low 25-Hydroxyvitamin D Does Not Affect Insulin Sensitivity in Obesity after Bariatric Surgery. *Obes Res*, **13**, 1692-700.

101. de Boer, I.H., Tinker, L.F., Connelly, S. *et al.* (2008) Calcium Plus Vitamin D Supplementation and the Risk of Incident Diabetes in the Women's Health Initiative. *Diabetes Care*, **31**, 701-7.
102. Nilas, L.& Christiansen, C. (1984) Treatment with Vitamin D or Its Analogues Does Not Change Body Weight or Blood Glucose Level in Postmenopausal Women. *International journal of obesity*, **8**, 407-11.
103. Ljunghall, S., Lind, L., Lithell, H. *et al.* (1987) Treatment with One-Alpha-Hydroxycholecalciferol in Middle-Aged Men with Impaired Glucose Tolerance--a Prospective Randomized Double-Blind Study. *Acta medica Scandinavica*, **222**, 361-7.
104. von Hurst, P.R., Stonehouse, W., Coad, J. (2010) Vitamin D Supplementation Reduces Insulin Resistance in South Asian Women Living in New Zealand Who Are Insulin Resistant and Vitamin D Deficient - a Randomised, Placebo-Controlled Trial. *The British journal of nutrition*, **103**, 549-55.
105. Sabherwal, S., Bravis, V., Devendra, D. (2010) Effect of Oral Vitamin D and Calcium Replacement on Glycaemic Control in South Asian Patients with Type 2 Diabetes. *International Journal of Clinical Practice*, **64**, 1084-9.
106. Jorde, R.& Figenschau, Y. (2009) Supplementation with Cholecalciferol Does Not Improve Glycaemic Control in Diabetic Subjects with Normal Serum 25-Hydroxyvitamin D Levels. *European journal of nutrition*, **48**, 349-54.
107. Borissova, A.M., Tankova, T., Kirilov, G. *et al.* (2003) The Effect of Vitamin D3 on Insulin Secretion and Peripheral Insulin Sensitivity in Type 2 Diabetic Patients. *International journal of clinical practice*, **57**, 258-61.