Vitamin D Deficiency Induced Muscle Wasting

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Abstract

When it comes to animals and people alike, muscle loss is caused by a shortage in vitamin D. Male Sprague Dawley rats were used to establish a vitamin D-deficient rat model. Muscle atrophy caused by vitamin D insufficiency is linked to the ubiquitin proteasome and other proteolytic processes. A group of D-deficient rats was given high calcium alone to study the impact of hypocalcemia that comes along with D deficiency. HPLC was used to measure 3-methylhistidine in urine and the myosin ATPase staining procedure was used for muscle histology. Semiquantitative RTPCR was used to assess muscle gene expression. Three-methyl histidine concentrations in urine and total muscle protein breakdown were higher in the deficient group than the control group. Genes for muscle atrophy markers and proteasomal subunits were upregulated in D-deficient muscle, but genes for myogenic growth were downregulated. According to the findings, vitamin D deficiency-induced muscle protein breakdown is mostly caused via the ubiquitin proteasome pathway, and calcium supplementation alone, in the absence of vitamin D, partly corrects the abnormalities.

Keywords: *Vitamin-D, Muscle wasting, Dentistry, muscle weakness.*

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1. INTRODUCTION

Hand and foot tingling, as well as hurting muscles and bones, are some of the signs and symptoms to watch for. As a result of the muscular weakness, it is difficult to get out of a chair without using your arms or to walk up a flight of steps. Disturbances in gait are common, and the "penguin gait" is commonly used to describe the movement. Severe vitamin D deprivation has been linked to a myopathy, which has been substantiated by in vivo and in vitro investigations demonstrating histological and electrophysiological abnormalities. A link between muscle cell contraction and the vitamin D receptor (VDR) is established in the nucleus of muscle cells. Reduced VDR count is thought to be a contributing cause to aging-related muscular weakness. Vitamin D insufficiency may lead to secondary hyperparathyroidism (SHPT), which can have a negative impact on muscular function. Muscle strength is also enhanced by regular exercise. Even though most observational studies have been statistically adjusted for possible interactions between physical activity and vitamin D status on muscular strength, residual confounding may exist, and thus results from RCTs are imperative to draw valid conclusions on the effects of vitamin D status on muscle function.

2. METHODS

Chemicals

Unless otherwise noted, all reagents were purchased from Sigma-Aldrich. While the anti-E2-Ub-conjugating enzyme antibody was bought from Boston Biochem, Santa Cruz Biotechnology provided the anti-Ub antibody and HRP-conjugated secondary antibodies. Pierce Chemical Co. supplied the ECL chemiluminescent substrate. Enzo Life Sciences supplied all of the proteasome-related compounds.

Muscle protein turnover may be measured

The rate of tyrosine released into the media (TPD) was studied in the mixed fiber epitrochlearis muscle since muscle neither synthesizes nor degrades tyrosine, and tyrosine does not accumulate within the intracellular pool of muscle. (40) Preincubation of epitrochlearis muscles with cycloheximide (0.5 mM) in a water bath at 37°C in 95 percent O2-5 percent CO2 in a Kreb's Ringer bicarbonate buffer containing 10 mM glucose was used to prevent the utilization of amino acids released during protein breakdown, which was used to calculate the absolute rate of protein breakdown. Muscles were preincubated for 2 hours at 37°C, then transferred to new

Kreb's Ringer bicarbonate buffer. Peptides and proteins were removed using trichloroacetic acid (final concentration 10%) and the quantity of tyrosine released was measured fluorometrically in order to assess the rate of protein breakdown. In terms of tyrosine released nanomoles per hour per gram of muscle, the conclusion may be described as.

The quantity of [14C] tyrosine absorption by muscle was used to estimate total protein synthesis (41). Preincubation of epitrochlearis muscles with 0.5 mM cold tyrosine for 30 minutes at 37°C in DMEM (3 mL) with 95 percent O2-5 percent CO2 resulted in a tyrosine concentration of 0.5 mmol/L. After that, the muscles were blotted and treated for two hours at 37°C in fresh DMEM containing [14C] tyrosine (0.05 Ci/mL). An incubation time of 60 minutes at 60 degrees Celsius was used to dissolve the muscle homogenate in 10 percent trichloroacetic acid, and the pellet was then centrifuged four times in 10 percent trichloroacetic acid to remove any unincorporated [14C] tyrosine. The resulting solution was then dissolved in 0.3 N NaOH. The incorporation of tyrosine into protein was quantified by liquid scintillation counting. The amount of tyrosine absorbed per gram of muscle each hour is represented in picomoles.

Proteasomal enzyme activities in muscle

Extracts of Gastrocnemius (GM) and Soleus (SOL) muscles were utilized to assess the chymotrypsin-, trypsin-, and caspase like activities using substrates (final concentration of 50M) unique to the three enzymes as reported by Beyet et al. (43). Succinyl coumarin (AMC) was used to measure the activity of chromophore L, leucine arginine glutamate receptor (TLR), and leucine leucine glycine glutamate receptor (CpL). Clasto-lactacystin, a 26S proteasome inhibitor, was utilized at a final concentration of 10 M. Excitation and emission of the AMC group was measured at 380 nm (excitation) and 460 nm (emission) using a multi-mode reader for proteolytic activity (Spectramax-MS; Molecular Devices). Free AMC was used to create the standard curve. The difference in peptidase activity between the two conditions was determined, and the findings were represented in nanomoles/min/mg protein. The bichinchonic acid technique was used to determine the protein content of the muscle extract. All three proteasomal enzyme activity were significantly inhibited (>90%) by clastolactacystin.

Measurement of lysosomal and calpain activities in muscle

Cathepsin B/L activity was evaluated fluorometrically using Z-Phe-Arg-4-AMC as a substrate in the GM and SOL muscles as published by Furono et al. (44). Fluorescence was measured against a free AMC standard at 380 nm excitation and 460 nm emission wavelengths. Micromoles/hr/mg protein is the unit of measurement for these results.

According to Sultan et al, calpain activity was measured in the GM and SOL muscles (45). Using 10 mM EDTA and 10 mM EGTA, we carried out control experiments in the absence of CaCl2. Found to be the cause of Calpain activity Ca^{2+}- depending on calpain cleavage of Suc-LY-AMC, the calpain-specific substrate. Nanomoles/min/mg protein was used to measure its activity.

Muscle histology

Liquid nitrogen was used to chill the isopentane in which the GM muscle samples were frozen. Sections (10 µm) to be processed, the images were frozen in a cryostat (Leica-CM 3050S) and kept at -20° C until they were processed. The technique of Round et al. was used to perform ATPase staining for fiber typing (types I and II) (47). An Olympus 1X-51 inverted microscope and a Jenoptik CCD digital camera recorded the images (prog RESR C3). We used ProgRes capture pro version 2.8.8 to count the number and area of fibers at a magnification of 40x (Jenoptik AG).

3. RESULT

Vitamin D deficiency leads to a decrease in body weight, LBM, and muscle fiber area

Hypocalcemia and an absence of serum 25(OH)D3 (a marker of vitamin D deficiency) validated the diagnosis of vitamin D insufficiency. Vitamin D3's physiologically active hormone form, 1,25(OH)2D3, was also considerably elevated. ($P \leq .001$) vitamin D levels fell (10 pg/mL) in both the low-vitamin D and high-calcium groups, indicating that both groups were vitamin D deficient. The other two groups that received vitamin D-enriched meals had normal blood levels (>40 pg/mL) 1,25(OH)2D3 in the blood (Table 1). Increases in serum phosphorus and alkaline phosphatase were also linked to vitamin D insufficiency (Table 1). Vitamin D treatment corrected all of the serum values, as predicted. Serum calcium levels returned to normal when a high-

calcium diet was implemented. However, compared to the control group, levels of serum phosphorus and alkaline phosphatase were somewhat higher in this group. This suggests that the animals' general nutritional condition was maintained, since there were no significant variations in serum albumin and total protein levels across groups (Table 1). Despite the fact that rats in the deficient group ate much less food than rats in the control group, their body weights were significantly higher.

Table 1. Serum Parameters in the Different Groups of Rats

Parameter	Con (n = 6)	Def (n = 6)	HCa (n = 6)	SD (n = 6)
$25(OH)D_3$ (ng/mL)	22.8 ± 2.9^a	ND	ND	19.8 ± 2.5^a
$1,25(OH)_2D_3$ (pg/mL)	50.6 ± 1.64^a	10.3 ± 1.48^b	10.5 ± 1.46^b	42.0 ± 1.67^c
Calcium (mg/dL)	9.91 ± 0.37^a	6.45 ± 0.25^b	11.0 ± 0.44^a	10.2 ± 0.59^a
Phosphorus (mg/dL)	7.54 ± 0.09^a	9.30 ± 0.17^b	8.4 ± 0.12^c	7.75 ± 0.11^a
Alk. phosphatase (U/L)	238 ± 7.8^a	364 ± 8.5^b	267 ± 15.1^c	242 ± 10^a
Albumin (g/dL)	3.6 ± 0.2	4.3 ± 0.5	3.4 ± 0.2	3.6 ± 0.1
Total protein (g/dL)	7.84 ± 0.26	8.33 ± 0.4	8.01 ± 0.4	7.83 ± 0.2

Table 2. Body Weight, LBM, and Muscle Related Parameters in the Different Groups of Rats

Parameter	Con (n = 6)	Def (n = 6)	HCa (n = 6)	SD (n = 6)
Body weight (g)	432.14 ± 8.7^a	372.38 ± 9.9^b	408.57 ± 6.6^a	410.5 ± 7.2^a
Myofibrillar protein content (μg/mg muscle)	7.19 ± 0.13^a	6.79 ± 0.15^b	6.93 ± 0.17^a	7.03 ± 0.11^a
LBM (g)	364.9 ± 3.1^a	326.4 ± 5.3^b	339.7 ± 2.2^c	$349.1 \pm 4.1^{a,c}$
Epitrochlearis (mg)	215.12 ± 10^a	174.75 ± 7^b	$190 \pm 5^{b,c}$	$205.57 \pm 7^{a,c}$
GM (mg)	745 ± 23^a	625 ± 22^b	$689 \pm 25^{a,b}$	736 ± 36^a
SOL (mg)	235 ± 12^a	187 ± 8^b	215 ± 6^a	219 ± 9^a
Plantaris (mg)	188 ± 3^a	169 ± 8^b	$173 \pm 7^{a,b}$	$177 \pm 3^{a,b}$

In each group, there were six rats, and the data are presented as the mean SEM. Statistically significant differences between data with distinct superscripts (a, b, and c) may be found at $P < .05$. Calcium deficiency, excessive calcium, and D supplementation are all examples of groups ($P \leq .001$) lower than those of the controls A high-calcium diet and vitamin D supplements might reverse this deterioration. When vitamin D shortage occurred, muscle weights and LBM decreased significantly (Table 2). Taking vitamin D supplements helped to bring the muscle weights and lean body mass (LBM) back to near-control levels, however calcium supplementation alone was only able to partly reverse the effects. When compared to the control group, the deficient group had dramatically reduced Type II muscle fiber area (Figure 1, A–C). In the absence of vitamin D, calcium supplementation will not be able to address this shift in calcium metabolism. When it came to type I fiber area, however, there were no significant differences between the two groupings (data not shown).

Hypocalcemia and vitamin D deficiency lead to muscle atrophy by increasing muscle protein breakdown and reducing the production of new muscle protein

Tests were conducted to establish if vitamin D deficiency in rats led to an increase in muscle protein breakdown and/or a decrease in the pace at which protein synthesis was slowed. Muscle protein breakdown was found to be 40% higher in the epitrochlearis muscles of the deficient group than in the control group. Rescue with a high calcium diet resulted in an 18% rise in TPD relative to controls, demonstrating that calcium alone may partially repair the alteration (Figure 2A, $P \leq .01$). Myofibrillar proteins account for 50–60% of muscle protein and are more readily degraded under certain physiological settings, hence we looked at the myofibrillar fraction's protein concentration. There was a considerable increase in myofibrillar protein content ($P \leq .05$) the difference between inadequate and healthy muscle (Table 2). Both vitamin D supplementation and rescue with a high-calcium diet are viable options for reversing the trend. The minor amino acid 3-MH is also found in actin and myosin and is not degraded when released from myofibrillar protein.

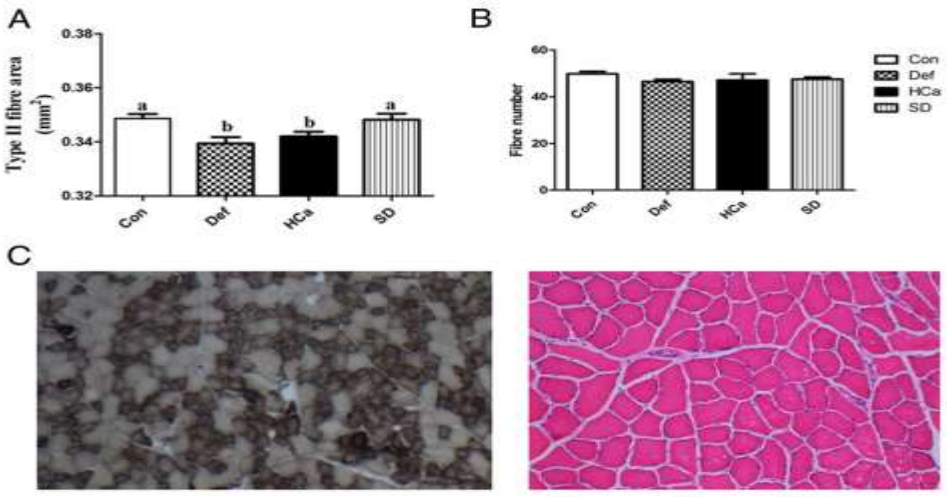

Figure 1. Histochemical analysis of GM muscle in the different experimental groups.

nor used in the production of protein. We found that the 3-MH concentration in the urine of rats with 3-MH deficiency was 53 percent higher than that of control rats (Figure 2B). In the presence of vitamin D supplementation, the 3-MH concentration returned to that of control subjects. A high-calcium diet, although reducing 3-MH concentration in urine, was not comparable to the control group's urine 3-MH content. With regard to protein production, a 17 percent drop was seen in the deficient group compared to control (Figure 2C; $P \leq .01$).

muscle Wasting in vitamin D deficiency does not change lysosomal or calpain enzyme activity.

These enzymes were investigated utilizing fluorogenic substrates that were unique to the two enzymes in the GM and SOL muscles. There were no significant differences in lysosomal or calpain enzyme activity between the GM and SOL muscle groups (Figure 2, A–D).

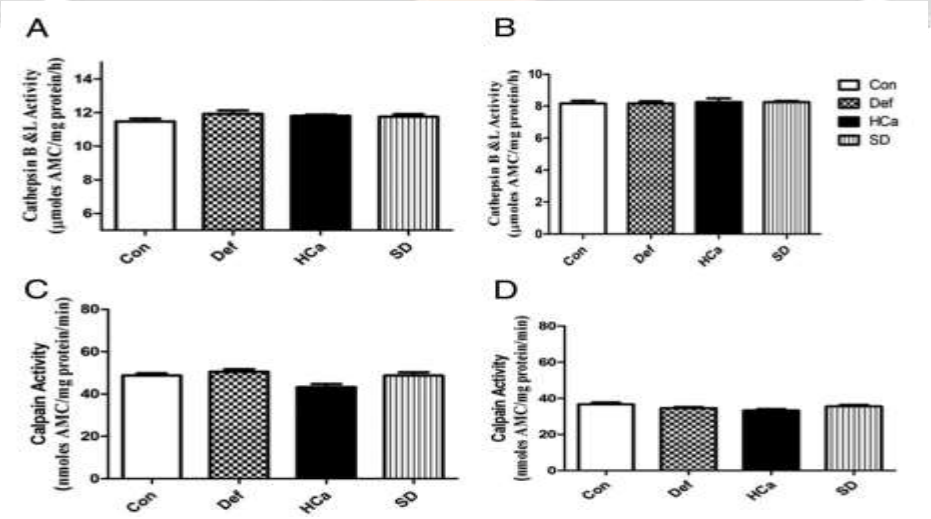

Figure 2. Vitamin D deficiency does not alter either lysosomal or calpain activities in muscle.

We initially studied the enzyme activities of the 20S catalytic core of the proteasome in two kinds of muscles since the UPP is responsible for the bulk of protein lysis in cells. Ch-L, T-L, and Cp-L enzyme activity in the 20S proteasome were all elevated in vitamin D-deficient rats' mixed fiber GM muscle. When it came to type I fibers in the SOL muscle, only the Ch-L activity increased. Recent investigations have shown that all three enzyme activities are necessary and contribute to protein degradation, even though the Ch-L activity was

previously assumed to be the rate-limiting one (60). Vitamin D supplementation and rescue with a high-calcium diet both have the ability to restore enzyme activity to that of control subjects. Rodents' ubiquitin-conjugating enzyme E2–14 kDa is the most important enzyme in this process (61). Vitamin D deficiency-induced muscle atrophy is further supported by the increased protein production of the E2 enzyme and high-molecular weight ubiquitin conjugates. The expression of two proteasome subunits, PSC2 and PSC8, was found to be higher in the deficient group compared to the vitamin D-supplemented group, and this was confirmed by measuring the mRNA levels. It was recently revealed that Atrogin-1 and MuRF1 are muscle-specific E3 ligases and that these molecules are molecular indicators of muscle atrophy (62). Both of the atrophy gene markers showed increased expression in the group with the deficiency. Vitamin D supplementation and a high-calcium diet may both correct the abnormalities in the proteasome in vitamin D-deficient individuals. Our findings imply that vitamin D deficiency-induced muscle atrophy activates the UPP.

4. CONCLUSION

There is strong evidence that vitamin D supplementation may improve muscular function in the elderly, and RCTs have demonstrated positive results. As a result, more studies have been published demonstrating that there is no impact than those showing that there is a positive benefit. Muscle wasting in vitamin D-deficient rats is caused by increased muscle protein breakdown despite a comparable food consumption to that of vitamin D adequate rats, according to our findings. The coordinated up-regulation of enzyme activity, gene and protein expression of several components of the UPP implies a substantial role for the route in vitamin D deficiency-induced muscle atrophy. Our work also shows that, in the absence of vitamin D, calcium alone may partly repair most of the muscular abnormalities. This study's findings look noteworthy from a therapeutic standpoint since they show the need of maintaining adequate levels of vitamin D and calcium for excellent musculoskeletal health.

5. REFERENCE

1. Elif Gül Aydın et.al "Vitamin D and Dentistry" DOI: 10.5772/intechopen.98471
2. K. Dzik et.al "Vitamin D Deficiency Is Associated with Muscle Atrophy and Reduced Mitochondrial Function in Patients with Chronic Low Back Pain" DOI:10.1155/2019/6835341
3. Francesca Remelli et.al "Vitamin D Deficiency and Sarcopenia in Older Persons" doi: 10.3390/nu11122861
4. Boland RL. VDR activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol. 2011;347(1-2):11-16.
5. Hassan-Smith ZK, Jenkinson C, Hernandez I, et al. 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 exert distinct effects on human skeletal muscle function and gene expression. PLoS One. 2017; 12(2):e0170665. https://doi.org/10.1371/journal.pone.0170665
6. Pojednic RM, Ceglia L, Lichtenstein AH, Dawson-Hughes B, Fielding RA. Vitamin D receptor protein is associated with interleukin-6 in human skeletal muscle. Endocrine. 2015;49(2):512-520.
7. Endo I, Inoue D, Mitsui T, et al. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of my regulatory transcription factors. Endocrinology. 2003;144(12):5138-5144.
8. Boland R, de Boland AR, Marinissen MJ, Santillan G, Vazquez G, Zanello S. Avian muscle cells as targets for the secosteroid hormone 1,25-dihydroxyvitamin D3. Mol Cell Endocrinol. 1995;114(1–2):1–8.
9. Casey C, Woodside JV, McGinty A, Young IS, McPeake J, Chakravarthy U, et al. Factors associated with serum 25-hydroxyvitamin D concentrations in older people in Europe: the EUREYE study. Eur J Clin Nutr. 2019;73(2):319–28.
10. Heath, KM & Elovic, EP (2006) Vitamin D deficiency: implications in the rehabilitation setting. Am J Phys Med Rehabil 85, 916–923.CrossRefGoogle Scholar PubMed
11. Daly, RM, Gagnon, C, Lu, ZX, et al. (2012) Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol (Oxf) 77, 26–35.
12. Chowdhury, R, Kunutsor, S, Vitezova, A, et al. (2014) Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. BMJ 348, g1903