Effects of alfacalcidol on muscle strength, muscle fatigue, and bone mineral density in normal and ovariectomized rats

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ABSTRACT
Vitamin D affects not only bone but also muscle to prevent falls and osteoporotic fractures. However, these effects on muscle and the mechanisms of fall prevention are still unclear. The purpose of this study was to investigate the effects of alfacalcidol \([1\alpha(OH)D_3]\) on muscle strength, muscle fatigue, and bone mineral density (BMD) in ovariectomized rats. Seven-month-old female Wistar rats were orally administered \(1\alpha(OH)D_3\) or its vehicle everyday for 4 weeks after ovariectomy (OVX) or sham operation. Calf muscle strength and fatigue were evaluated by electrical stimulation of the sciatic nerve under general anesthesia. \(1\alpha(OH)D_3\) administration significantly increased the maximum muscle strength in the sham-operated \((P < 0.01)\) and the OVX \((P < 0.01)\) groups compared to their respective control groups. However, \(1\alpha(OH)D_3\) administration did not significantly affect muscle fatigue in these groups. The BMD of the femur in the \(1\alpha(OH)D_3\)-treated OVX group was significantly higher than that in the vehicle-treated OVX group \((P = 0.04)\). These results suggested that \(1\alpha(OH)D_3\) increases muscle strength but does not affect muscle fatigue in this rat model. The effectiveness of activated vitamin D in preventing bone fractures may be partly owing to its effect on muscle strength in addition to its known effect on bone metabolism.

The active, hormonal form of vitamin D \(1,25\text{-dihydroxyvitamin D}_3\) \([1,25(OH)_2D_3\text{ or calcitriol}]\) and its prodrug \(1\alpha\text{-hydroxyvitamin D}_3\) \([1\alpha(OH)D_3\text{ or alfacalcidol}]\) have been widely used to treat a variety of metabolic bone diseases, such as rickets/osteomalacia, renal osteodystrophy, and osteoporosis \((8, 41)\). Activated vitamin D can prevent fractures by improving bone quality, bone metabolism, and bone mineral density (BMD) \((7, 22, 40, 45, 50)\). However, its preventive effects on fractures were more significant than its effects on bone quality or BMD. Several recent meta-analyses have demonstrated that native or activated vitamin D has preventive effects on falls \((2, 16)\). This preventive effect on falls contributes to a partial reduction of fractures by vitamin D in elderly osteoporotic individuals \((3, 37)\).

However, the mechanisms by which activated vitamin D prevents falls remain unknown. Several factors have been reported as physical risk factors for falls, including decreased muscle strength or function of lower extremity, impaired physical function such as single-leg standing, and postural imbalance with aging \((26, 28, 34, 36)\). Vitamin D may act directly on muscle tissue to exert its preventive effect on falls. It has been known that osteomalacia and rickets cause muscular atrophy and decrease muscle strength and that the muscular symptoms associated with these diseases quickly improve with vitamin D treatment \((39, 43)\). These findings indicate a potential association between vitamin D and muscle tissue. Therefore, the effects of vitamin D on muscle tissue with regard to fall prevention have been of focus in aged and osteoporotic patients.

Muscle function is defined by several factors, including muscle strength, fatigue, or volume. It is
well recognized that muscle weakness of the lower extremities is one of the important risk factors for falls (32). Several previous studies have reported increases in postural sway due to muscle fatigue of the lower extremities (12, 20), which is linked to an increased risk of falling (29, 30). This evidence indicates that muscle weakness or muscle fatigue increases the risk of falls. However, it remains unclear whether the effects of vitamin D on muscle function with regard to fall prevention in aged, osteoporotic people is via affecting muscle strength, muscle fatigue, or muscle volume. To clarify the mechanisms of preventive effects of activated vitamin D on falls in addition to its effect on BMD of the femur, the effects of 1α(OH)D₃ on the muscle strength or fatigue and the histomorphometric changes in muscle tissues of normal and ovariectomized rats as a model for aged, osteoporotic women were examined in the present study.

MATERIALS AND METHODS

Animals and experimental protocol. Seven-month-old female Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were housed in a controlled environment with temperature maintained at 23°C ± 2°C and humidity at 50 ± 10% under a 12-hour light-dark cycle. The rats were allowed free access to tap water and commercial standard rodent chow (CE-7), containing 0.94% calcium, 0.96% phosphate, and 2.0 IU/g of vitamin D₃ (Clea Japan Inc., Tokyo, Japan) as in our previous studies (27, 35).

Rats underwent ovariectomy (OVX) or a sham operation under anesthesia with sodium pentobarbital (Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan). The rats were orally administered 1α(OH)D₃ (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) (0.1 μg/kg • day) or its vehicle (medium-chain triglyceride, MCT) everyday for 4 weeks after the operation. The 1α(OH)D₃ dose was determined on the basis of previous studies (46) showing that dose does not elevate serum calcium levels. The rats were divided into following 4 groups: 1) sham-vehicle group (n = 12), sham-operated rats treated with vehicle; 2) OVX-vehicle group (n = 12), OVX rats treated with vehicle; 3) sham-1α(OH)D₃ group (n = 12), sham-operated rats treated with 1α(OH)D₃; and 4) OVX-1α(OH)D₃ group (n = 12), OVX rats treated with 1α(OH)D₃.

The protocols for animal experimentation described in this paper were previously approved by the Animal Research Committee, Akita University. All subsequent animal experiments adhered to the “Guidelines for Animal Experimentation” of the University.

Muscle strength measurement (Fig. 1). Calf muscle strength and fatigue were measured on the day after the final administration of 1α(OH)D₃ or its vehicle (8 weeks after sham-operation or OVX) as previously described (23). General anesthesia was induced by intraperitoneal administration of sodium pentobarbital (30 mg/kg body weight). The right hindlimb was used for the measurement of muscle strength and fatigue. After opening the posterior surface of the leg to expose the sciatic nerve in the gluteal region, a bipolar cuff electrode (interelectrode distance, 5 mm; MD Giken, Tokyo, Japan) was attached to the sciatic nerve. Then, the rat was immobilized on a small platform with the knee in the fully extended position fixed with Kirschner wire. The distal end of the Achilles tendon was exposed and cut at the insertion to the calcaneal bone. A transducer (Orientec Co. Ltd., Tokyo, Japan) was attached and fixed next to the stump with a load of approximately 1 N. Signals transmitted from the force transducer during isometric muscular contraction were recorded on a force-time curve using a paper recorder (Nihon Koden Co. Ltd., Tokyo, Japan). To obtain a tetanic contraction, muscle contraction was induced by a monophasic rectangular pulse with a frequency of 100 Hz.

Fig. 1 Measurement of calf muscle strength and fatigue. A scheme of posterior right hindlimb indicates the procedure of measurement of muscle strength and fatigue. (A) Sciatic nerve, (B) Bipolar cuff electrode, (C) Kirschner wire fixing the knee joint with fully extended position, (D) Achilles tendon of calf muscle is exposed and cut at the insertion of the calcaneal bone, and (E) A stump of Achilles tendon is attached to a transducer and fixed with a load of 1 N.
Effects of alfacalcidol on muscle and bone

Statistical analysis. Data are expressed as the mean ± standard deviation (SD). The comparison of body weight in each group at the beginning and the end of experiment was performed using paired-sample t-test. Comparison between the vehicle and 1α(OH)D₃ groups was performed using Student’s t-test. Two-way analysis of variance was performed to evaluate the role of treatment and time point. All data were analyzed using a statistical software package (Statcel2; OMS Inc., Saitama, Japan), and P values less than 0.05 were considered statistically significant.

RESULTS

Body weight (Table 1)

There was no significant difference in body weight among the 4 groups at the beginning of the experiment. The body weights at the time of sacrifice changed significantly in the sham-1α(OH)D₃ (4% decrease, P < 0.001), OVX-vehicle (7% increase, P < 0.001), and OVX-1α(OH)D₃ (4% increase, P < 0.05) groups compared to those at the beginning of experiment in each group (paired t-test). 1α(OH)D₃ treatment significantly decreased the body weight of sham-operated rats (6% less, P < 0.001) compared to the sham-vehicle rats at the time of sacrifice (Table 1).

Muscle strength and fatigue

1α(OH)D₃ administration significantly increased maximum muscle strength (5–7% higher, P < 0.01) compared to that in the sham-vehicle group (Fig. 2A). 1α(OH)D₃ treatment also significantly increased maximum muscle strength (4–7% higher, P < 0.01) compared to that in the OVX-vehicle group (Fig. 2B). However, 1α(OH)D₃ administration did not significantly affect muscle fatigue during each stimulation cycle compared to that in the respective vehicle-treated control groups both in sham-operated and OVX rats (Fig. 3A and 3B).

BMD (Table 2)

In OVX-vehicle group, the total BMD and the BMD of the distal third of the femur were significantly

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<th>Table 1 Body weight (g) of four experimental groups</th>
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<td>Beginning of experiment</td>
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<td>Sham-vehicle</td>
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<td>Sham-1α(OH)D₃</td>
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<td>OVX-vehicle</td>
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<td>OVX-1α(OH)D₃</td>
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<td>Sacrifice</td>
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<td>Sham-vehicle</td>
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<td>Sham-1α(OH)D₃</td>
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<td>OVX-vehicle</td>
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<td>OVX-1α(OH)D₃</td>
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n = 12 in each group. Values are mean ± SD.

^{a}: P < 0.05, ^{c}: P < 0.001, vs. body weight at the beginning of experiment in each group (paired t-test).

^{a}: P < 0.05 vs. body weight of the sham-vehicle group at sacrifice (Student’s t-test).
lower than those in sham-vehicle group (7% and 8% lower, respectively, \(P < 0.01\)). 1α(OH)D₃ administration significantly increased the total BMD (4% higher, \(P = 0.04\)) and the BMD of the distal third of the femur (4% higher, \(P = 0.04\)) in OVX rats but not in sham-operated rats compared to those of the OVX-vehicle group. 1α(OH)D₃ administration did not completely recover the decreased BMDs of the femurs of OVX rats compared to those of sham-vehicle rats (\(P < 0.01\)). However, the BMD of the middle third of the femur was not significantly different among the 4 groups.

**DISCUSSION**

In the present study, 1α(OH)D₃ treatment for 4 weeks decreased rat body weights and significantly increased calf muscle strength adjusted for body weight, but not muscle fatigue, in aged or OVX rats. Although the cause of body weight loss is unclear, an increased activity of rats may lead to decrease the body weight after 1α(OH)D₃ treatment described in the previous report (45). To the best of our knowledge, there are no previous animal studies dealing with the effects of vitamin D on skeletal muscles, especially with regard to muscle strength and fatigue. It has been indicated that muscle strength of the lower extremities is a significant positive risk factor for falls in elderly people (18, 32). Pfeifer et al. reported that calcium and vitamin D supplementation caused a reduction in the number of falls and improved muscle strength and postural instability (38). The results of the present study also support the notion that vitamin D has positive effects

![Fig. 2 Muscle strength.](image1)

**Fig. 2** Muscle strength. 1α(OH)D₃ treatment (■) significantly increased the muscle strength normalized using body weight in the sham-operated (6–7%, \(P < 0.01\)) (A) and OVX (4–7%, \(P < 0.01\)) (B) groups when compared to that of respective vehicle-treated control rats (○) at all stimulations up to 20 cycles.

![Fig. 3 Muscle fatigue.](image2)

**Fig. 3** Muscle fatigue. Muscle fatigue was normalized using body weight, and evaluated as the percentage strength during each cycle. When compared to the initial contraction strength, the muscle fatigue was not significantly different between the 1α(OH)D₃ group (black bar) and the vehicle-treated control group (white bar) in the sham-operated (A) and OVX (B) groups.

Muscle fiber composition and lesser diameter of muscle fibers (Table 3)

Ovariectomy or 1α(OH)D₃ administration did not affect the lesser diameter of muscle fibers in any of the 4 groups. The compositions of type 1 fiber were from 83.6% to 88.3% in these 4 groups. There was no significant difference in the composition of muscle fiber types among the 4 groups.
on skeletal muscle strength, which may contribute to the prevention of falls in clinical situations.

To clarify the mechanisms of activated vitamin D effects on muscle function such as muscle strength or muscle fatigue, the muscle fiber type and diameter were evaluated in the present study. Previous clinical studies demonstrated that decreased muscle strength due to vitamin D deficiency was improved by vitamin D supplementation and that 25(OH)D level was significantly associated with quadriceps maximum voluntary contraction (19). In the prospective Longitudinal Study of Aging Amsterdam, lower serum 25(OH)D levels predicted a decrease in grip strength and appendicular muscle mass in elderly men and women over 3 years (49). Although 25(OH)D levels were not measured in the present study, treatment with 1α(OH)D$_3$, the activated form of 25(OH)D, increased muscle strength both in sham-operated and OVX rats in agreement with previous clinical studies.

In addition to the effects of vitamin D on muscle strength, Parijat et al. reported that localized muscle fatigue could be considered as a potential risk factor for slip-induced falls (36). Vitamin D deficiency causes myopathy with muscle pain, fatigue, muscle weakness, and gait disturbance in elderly people (17). Furthermore, treadmill locomotion training decreased muscle fatigue and increased muscle strength in spinal cord injured rats (48). Therefore, we speculated that the effect of vitamin D on muscle fatigue may be another factor related to the prevention of falls and fractures that occur in aged, osteoporotic patients. However, 1α(OH)D$_3$ treatment did not affect muscle fatigue in the present study.

With regard to muscle morphology, several previous studies have demonstrated that muscle exercise changed the composition of muscle fiber types (6), fiber size (24, 25), and muscle mass (14). These results suggest that morphological changes in muscle fibers result in recovery of muscle strength. In addition, vitamin D deficiency revealed type II muscle fiber atrophy in human (11). Two reports described the effects of vitamin D supplementation on muscle fiber composition. Sorenson et al. showed an increase in relative fiber composition and in fiber size of type IIa after treatment with 1α(OH)D$_3$ and calcium by analysis of muscle biopsies from elderly women (47). The other randomized, controlled study found that treatment of elderly stroke patients with 1000 IU of vitamin D$_2$ daily increased type II muscle fiber diameter and percentage of type II fiber over a 2-year period (42). However, in the present study, 4-week treatment with 1α(OH)D$_3$ did not affect the percentage of muscle fiber types or the lesser diameters of each muscle fiber type in either aged or OVX rats.

Other factors should be considered as regulators of the effects of vitamin D on muscle strength. In addition to the effects of vitamin D on muscle morphology, genomic and non-genomic effects of vitamin D in muscle have been reported. The genomic

<table>
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<th>Table 2 Bone mineral density (BMD) (g/cm$^2$)</th>
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<td>Sham-vehicle</td>
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<tr>
<td>Total</td>
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<td>Distal</td>
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<td>Middle</td>
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n = 12 in each group. Values are mean ± SD.
$^a$: P < 0.01 vs. sham-vehicle. $^b$: P < 0.05 vs. OVX-vehicle.

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<th>Table 3 Lesser diameter of muscle fibers and muscle fiber composition</th>
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<td>Sham-vehicle</td>
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<td>Lesser diameter (μm)</td>
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<td>Type IIA</td>
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<td>Composition (%)</td>
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n = 12 in each group. Values are mean ± SD.
effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} include regulating muscle calcium uptake (4), synthesis of muscle cytoskeletal proteins (5), and phosphate metabolism in myoblasts (5). Recently, it has been reported that 1,25(OH)\textsubscript{2}D\textsubscript{3} also affects muscle function through a transcription-enhancing role on proteins such as insulin like growth factor-I (IGF-I) and its binding proteins other than those involved directly in calcium metabolism to reveal an anabolic effect on muscle tissue (21). IGF-I induces proliferation, differentiation, and hypertrophy of skeletal muscle (1). On the other hand, the non-genomic effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} include activation of protein kinase C (PKC) to release Ca\textsuperscript{2+} into the cytosol (13, 33) and stimulation of protein synthesis in the muscle cells (44). These studies have suggested that 1α(OH)D\textsubscript{3} also has anabolic effects on muscle function via genomic and/or non-genomic effects.

There are several limitations in the present study. First, the treatment duration of 1α(OH)D\textsubscript{3} was only 4 weeks. With regard to the preventive effects of vitamin D on falls, Bischoff et al. reported in their 3-year randomized controlled trial that the fall preventing effect was noted after 12 months of vitamin D treatment and increased thereafter (2). The length of treatment appears to be an important factor, thus the effects of vitamin D treatment on muscle fatigue may be further clarified by increasing the duration of treatment in future studies. Second, although a frequency of 100 Hz for electrical stimulation of muscle was determined on the basis of a previous report (23), the frequency of stimulation was 100 Hz only in the present study. The 100 Hz stimulation is classified as fast switch stimulation. The other frequency of stimulation such as 20 Hz, slow switch stimulation, may be more appropriate to estimate muscle strength or fatigue in aged rats.

In conclusion, 1α(OH)D\textsubscript{3} increases muscle strength but does not affect muscle fatigue in the aged or ovariectomized rat model. The effectiveness of activated vitamin D in preventing falls and fall-related fractures may be partly owing to its effect on muscle strength in addition to its known effect on bone metabolism.

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