ORIGINAL

Effects of daily 1,000-IU vitamin D-fortified milk intake on skeletal muscle mass, power, physical function and nutrition status in Japanese

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Abstract : An intervention study was conducted to investigate the effects of daily 1,000-IU vitamin D-fortified milk intake on skeletal muscle mass, power, physical function and nutrition status in 26 healthy people and 8 older adults living in a nursing home. The serum 25-hydroxyvitamin D [25(OH)D] level was 13.4 ± 0.8 ng/mL and it markedly increased to 29.6 ± 0.9 ng/mL after daily 1000-IU vitamin D-fortified milk intake for 6 months. Handgrip strength (kg) also significantly increased in the 21-50 years and total groups, and male subjects, and the timed up and go test significantly improved in the 21-50 years and total groups, and female subjects after 6-month vitamin D intake. However, there were no significant differences between baseline and post-treatment in the Barthel Index (BI), walking speed (m/sec) or skeletal muscle mass (kg, % of BW, kg/m^2). Therefore, the present study suggested that vitamin D-fortified milk intake is effective at improving muscle strength and physical function in Japanese, although further studies are needed, particularly for older adults. J. Med. Invest. 68 : 249-255, August, 2021

Keywords **:** *Vitamin D supplementation, Serum 25-hydroxyvitamin D level, Skeletal muscle mass, Muscle strength*

INTRODUCTION

Sarcopenia is the most common cause of musculoskeletal disability in the elderly. It is characterized by the gradual age-related loss of muscle mass, muscle strength and physical performance, which leads to reduced mobility and an increased risk of falls, and is associated with premature death (1-3). In our previous studies, an age-related decrease in total-body skeletal muscle mass based on 24-hour creatinine excretion and age-related loss of muscle power and function were observed in a Japanese population including nursing home residents (4-6). Furthermore, a low vitamin D level is positively associated with reduced skeletal muscle mass, muscle function and strength. As vitamin D deficiency is prevalent and is associated with poor physical performance in older populations (7-9), correcting this deficiency may be important for protecting muscle function with age (10).

The beneficial effects of vitamin D supplementation on muscle mass, muscle strength and physical performance have been reported in older people with a low level of serum vitamin D (11-13). However, some reports of vitamin D supplementation noted no improvement in muscle strength or physical function (14-16). A daily dose vitamin D of 800-2000 IU was recommended to reach a serum 25(OH)D level of 75 nmol/L in study participants with a low vitamin D status (17). Therefore, the effects of daily 1,000-IU vitamin D-fortified milk intake on skeletal muscle mass, power, physical function and nutrition status were investigated in a population of young to old Japanese subjects, including older nursing home residents.

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MATERIALS AND METHODS

Subjects

This was an intervention study that investigated the effects of daily 1,000-IU vitamin D-fortified milk intake in 200 ml of cow milk for 6 months on skeletal muscle mass, physical function, intestinal nutrient absorption rate, and vitamin and mineral status. The milk was manufactured by Meiji Co., Ltd., Tokyo, Japan and contains 137 kcal, 9.9 g of carbohydrates, 6.8 g of protein, 7.8 g of fat, 227 mg of calcium and 1000 IU of vitamin D. The numbers of male and female subjects in the age groups of 21-50 years, 51-75 years and 76 years or older were 11 and 6, 5 and 8, and 0 and 4, respectively. Among them, male and female residents in nursing homes accounted for 4 and 0 in the 51-75 years group and 0 and 4 in the 76 years or older group, respectively. The other 26 subjects aged between 21-75 years old were healthy registered care workers or physical and occupational therapists at nursing homes, or teaching and administration staff at Kenshokai Gakuen College for Health and Welfare. None of the healthy subjects were engaged in high levels of exercise training or taking any medications just before or during the study. Routine blood studies, including electrolytes, liver tests and hematological indexes, confirmed the health status of each subject before entry into the study. Height and weight measurements were performed with the participants wearing light clothing and no shoes. The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

Clinical laboratory tests

Blood samples were collected from subjects who fasted for more than 8 hours overnight, immediately refrigerated, transported in cold storage to the SRL Laboratory in Tokyo, and analyzed within 24 hours. Serum levels of sodium, potassium, chloride, calcium, phosphorus, zinc, iron, cupper, folic acid, b-carotene, 25-hydroxyvitamin D [25(OH)D] and intact parathyroid hormone (PTH) were measured. Serum 25(OH)D levels, as an indicator of the vitamin D status, were measured by electro-chemiluminescent immunoassay (ECLIA) as previously reported (5, 6). There is a general consensus that a serum 25(OH)D concentration of <20 ng/ml, 20 to <30 ng/mL and ≥30 ng/mL indicates vitamin D deficiency (VDD), insufficiency (VDI), and sufficiency (VDS), respectively (18).

Measurement of skeletal muscle mass (SMM)

Each subject collected two 24-hour urine samples at inclusion on two consecutive days. Creatinine excretion was calculated as the mean of the two 24-hour urine samples. SMM was calculated from the 24-hour urinary creatinine amount based on this equation (SMM (kg) = 21.8 × Cr (g/day)) (19). The SMM index (SMMI) was calculated as weight (kg) divided by square of height (m). SMM (% of body weight (kg)) was calculated as weight (%) divided by body weight (kg). Creatinine height index (CHI) was calculated from the following formula :
CHI = 24-hour urine creatinine excretion (mg)/ expected 24-hour urine creatinine excretion (23 mg/kg in males and 18 mg/kg in females) × 100.

The estimated nitrogen balance was calculated as the difference between total nitrogen intake and total nitrogen output in the urine. Nitrogen intake was calculated from the nitrogen provided in food for each subject. The following formula was used in calculating the nitrogen balance : Nitrogen balance = 24-hour protein uptake/6.25-(24-hour nitrogen excretion/0.8). Putative intestinal absorption rates of nitrogen, sodium, calcium and phosphorus balance were calculated as the urinary excretion rate (%) among the amount of oral intake from food.

Assessment of physical performance

Physical performance was evaluated through several physical tests such as the timed up and go (TUG) test (sec), walking speed (m/sec), handgrip strength (kg) and Barthel Index (BI). Muscle strength was assessed as handgrip strength using a dynamometer (Takei Scientific, Tokyo, Japan). Both hands were measured twice and the maximum value of either hand was analyzed. For the TUG test, individuals were asked to rise from a standard chair, walk to a marker 3 m away, turn around, walk back and sit down again (20). The BI is a 10-item measurement tool of basic activities of daily living (ADL) (21). It is used in clinical practice to inform rehabilitation and care planning, and in research both to describe outcomes and as a case-mix adjuster (22).

Statistical analysis

Using BellCurve for Excel (version 3.20) (Social Survey Research Information Co., Ltd., Tokyo, Japan), the Wilcoxon signed rank test was performed to assess changes in skeletal muscle function, muscle mass and intestinal nutrition absorption rates after vitamin D supplementation, and the association between serum 25(OH)D levels and skeletal muscle mass and body functions.

Data are expressed as the mean ± SE of male and female subjects in different age groups.

Ethical considerations

Ethics approval was obtained from the clinical research ethics committee at Tokushima University Hospital (approval number 384). Informed consent to participate in the study was also received from participants or from an authorized surrogate. This trial was registered as UMIN000038105.

RESULTS

1. Effects of vitamin D fortified milk intake in different age groups (Table 1)

At baseline, the serum 25(OH)D level in the 34 male and female subjects was 13.4 ± 0.8 ng/mL. Thirty participants (88.2%) had VDD and 4 (11.8%) had VDI. After 1,000-IU vitamin D-fortified milk intake per day for 6 months, there was a significant increase in the serum 25(OH)D level to 29.6 ± 0.9 ng/mL. In different age groups, the serum 25(OH)D level markedly improved in the 21-50 years (n = 17), 51-75 years (n = 13), 21-75 years (n = 30) and total (n = 34) groups, but not in the 76 years or older (n = 4) group. Only two participants (5.8%) had VDD, 16 (47.1%) had VDI, and 16 (47.1%) had VDS. After vitamin D-fortified milk intake for 6 months, serum calcium and phosphorus levels increased, and PTH level decreased in these subjects.

Handgrip strength and TUG significantly improved after vitamin D supplementation in the 21-50 years and total groups, but there were no significant differences after treatment in BI, walking speed (sec/m) or skeletal muscle mass (kg, % of BW, kg/m^2). A significant increase in skeletal muscle mass (kg) was observed only in the 51-75 years group. Regarding the intestinal absorption rate after vitamin D-fortified milk intake, those of nitrogen and calcium decreased, and that of phosphorus increased in the 21-50 years and total groups, but that of sodium was unaffected.

2. Effects of vitamin D fortified milk intake in male and female groups (Table 2)

At baseline, the serum 25(OH)D level in 16 male and 18 female subjects was 14.6 ± 1.5 ng/mL and 12.4 ± 0.8 ng/mL, respectively. Thirteen (81%) and 3 (19%) male, and 17 (94%) and 1 (6%) female subjects had VDD and VDI, respectively. After 1,000-IU vitamin D-fortified milk intake per day for six months, the serum 25(OH)D level significantly increased to 30.0 ± 1.2 ng/mL (9 (56%) VDI and 7 (44%) VDS) in male subjects and 29.2 ± 1.4 ng/mL (2 (11%) VDD, 7 (39%) VDI and 9 (50%) VDS) in female subjects. After vitamin D-fortified milk intake for 6 months, serum calcium and phosphorus levels increased in female subjects.

Although skeletal muscle mass was unaffected by vitamin D supplementation in both male and female groups, handgrip strength in males and TUG in females significantly improved. Regarding the intestinal absorption rate after vitamin D-fortified milk intake, that of nitrogen decreased in males and that of phosphorus increased in females.

3. Association of 25(OH)D with skeletal muscle mass, skeletal muscle function and predicted intestinal absorption rate

A positive correlation between serum 25(OH)D levels and the BI, grip-hand strength, and nitrogen and calcium absorption rates were observed at baseline ($r = -0.32$, $p < 0.001$). After vitamin D-fortified milk intake, the serum 25(OH)D level was also positively correlated with skeletal muscle mass (kg, kg/m^2) ($r = -0.20$, $p = 0.007$).

DISCUSSION

Aging results in a decrease in muscle strength and this is

Table 1. Effects of vitamin D-fortified milk intake in different age groups

	21-50 years				51-75 years				76 years～				Total			
	Baseline		6 months		Baseline		6 months		Baseline		6 months		Baseline		6 months	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (years)	37.8 ± 1.5				60.3 ± 2.1				86.5 ± 2.7				52.1 ± 3.1			
n (male/female)	17(11/6)				13(5/8)				4(0/4)				34(16/18)			
SMM																
SMM (kg)	28.1 ± 2.4		29.2 ± 2.2		20.7 ± 1.8		23.8 ± 3.2	*	8.9 ± 1.9		6.0 ± 1.5		23.0 ± 1.7		24.4 ± 2.0	
SMM (% of body weight)	42.9 ± 3.5		44.2 ± 3.5		35.1 ± 3.5		39.4 ± 5.6		18.4 ± 3.1		13.0 ± 3.4		37.0 ± 2.6		38.7 ± 3.2	
SMM (kg/m^2)	10.7 ± 0.9		10.9 ± 0.9		8.2 ± 0.8		8.9 ± 1.2		4.1 ± 0.9		2.7 ± 0.7		9.0 ± 0.7		9.2 ± 0.8	
Creatinine height index (%)	92.4 ± 7.3		93.7 ± 6.9		82.4 ± 7.2		93.9 ± 13.4		46.9 ± 8.0		33.1 ± 8.7		83.2 ± 5.2		86.7 ± 7.0	
SMF																
Timed up and go test (sec)	6.3 ± 0.4		5.3 ± 0.1	*	9.8 ± 3.7		11.7 ± 4.4		-		-		8.3 ± 1.5		8.4 ± 1.8	**
Walking speed (m/sec)	3.3 ± 0.2		3.2 ± 0.2		4.1 ± 0.9		4.3 ± 1.1		-		-		3.8 ± 0.4		3.7 ± 0.4	
Handgrip strength (kg)	40.7 ± 2.7		42.3 ± 2.8	**	23.6 ± 3.3		25.1 ± 2.6		8.4 ± 1.6		6.4 ± 3.2		31.0 ± 2.7		32.3 ± 2.7	*
Barthel index	100.0 ± 0.0		100.0 ± 0.0		84.2 ± 7.9		83.5 ± 7.9		31.3 ± 13.1		30.0 ± 13.7		85.9 ± 4.9		85.4 ± 5.0	
Predicted intestinal absorption rate																
Nitrogen (%)	76.6 ± 5.1		56.7 ± 3.2	**	65.2 ± 4.5		50.3 ± 4.1		36.5 ± 11.8		20.3 ± 9.2		67.6 ± 3.9		50.0 ± 3.1	***
Sodium (%)	86.4 ± 6.8		77.3 ± 4.7		82.3 ± 9.7		86.0 ± 9.7		70.4 ± 10.0		80.8 ± 22.0		83.0 ± 5.1		81.1 ± 4.9	
Calcium (%)	15.2 ± 1.9		11.7 ± 1.3	*	22.4 ± 3.8		20.8 ± 2.8		6.8 ± 3.3		4.0 ± 1.7		17.0 ± 1.9		14.3 ± 1.6	**
Phosphorus (%)	59.4 ± 4.3		70.5 ± 4.6	*	51.9 ± 4.4		61.5 ± 7.3		22.8 ± 6.0		23.3 ± 5.5		52.2 ± 3.4		61.5 ± 4.4	***
Concentration in serum																
Calcium (mg/dL)	9.3 ± 0.1		9.4 ± 0.1	*	9.2 ± 0.1		9.4 ± 0.1	**	8.6 ± 0.1		8.8 ± 0.1		9.2 ± 0.1		9.3 ± 0.1	***
Phosphorus (mg/dL)	3.6 ± 0.1		3.8 ± 0.1		3.5 ± 0.1		3.8 ± 0.1	*	3.6 ± 0.1		4.2 ± 0.1		3.5 ± 0.1		3.8 ± 0.1	***
25(OH)D (ng/mL)	14.5 ± 1.0		29.7 ± 1.3	***	12.8 ± 1.6		28.1 ± 1.5	**	10.8 ± 0.5		33.9 ± 0.4		13.4 ± 0.8		29.6 ± 0.9	***
Intact PTH (pg/mL)	41.7 ± 3.1		37.5 ± 4.0		40.5 ± 6.3		34.7 ± 2.5		41.5 ± 4.3		34.3 ± 6.5		41.2 ± 2.8		36.1 ± 2.3	*

SMM : Skeletal muscle mass, SMF : skeletal muscle functioning, 25(OH)D : 25-hydroxy vitamin D, Intact PTH : intact parathyroid hormone
* : p < 0.05, ** : p < 0.01, *** : p < 0.001

Table 2. Effects of vitamin D-fortified milk intake between the sexes

	Male				Female			
	Baseline		6 months		Baseline		6 months	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (years)	46.4 ± 3.9				57.2 ± 4.4			
n	16				18			
SMM								
SMM (kg)	29.8 ± 2.3		29.8 ± 2.5		16.96 ± 1.6		19.6 ± 2.7	
SMM (% of body weight)	44.1 ± 3.6		43.7 ± 4.7		30.7 ± 3.0		34.2 ± 4.1	
SMM (kg/m^2)	11.3 ± 0.9		10.8 ± 1.1		6.9 ± 0.6		7.7 ± 0.9	
Creatinine height index (%)	88.7 ± 6.9		87.7 ± 9.2		78.3 ± 7.6		85.8 ± 10.6	
SMF								
Timed up and go test (sec)	9.6 ± 2.8		10.3 ± 3.5		7.1 ± 1.3		6.6 ± 1.3	*
Walking speed (m/sec)	4.4 ± 0.7		4.3 ± 0.8		3.3 ± 0.5		3.1 ± 0.3	
Handgrip strength (kg)	36.2 ± 4.7		39.4 ± 4.2	**	26.2 ± 2.3		25.5 ± 2.5	
Barthel index	87.2 ± 6.6		86.6 ± 6.5		84.7 ± 7.4		84.4 ± 7.6	
Predicted intestinal absorption rate								
Nitrogen (%)	80.3 ± 4.2		56.5 ± 4.0	**	56.2 ± 5.2		44.2 ± 4.3	
Sodium (%)	91.4 ± 7.1		80.9 ± 7.2		75.5 ± 7.0		81.2 ± 6.8	
Calcium (%)	20.3 ± 3.1		16.2 ± 2.5		14.0 ± 2.2		12.5 ± 1.9	
Phosphorus (%)	61.6 ± 4.0		67.2 ± 6.0		43.9 ± 4.5		56.5 ± 6.2	*
Concentration in serum								
Calcium (mg/dL)	9.3 ± 0.1		9.4 ± 0.1		9.1 ± 0.1		9.3 ± 0.1	**
Phosphorus (mg/dL)	3.5 ± 0.1		3.6 ± 0.1		3.5 ± 0.1		4.0 ± 0.1	**
25(OH)D (ng/mL)	14.6 ± 1.5		30.0 ± 1.2	***	12.4 ± 0.8		29.2 ± 1.4	***
Intact PTH (pg/mL)	40.1 ± 3.3		36.1 ± 4.2		42.2 ± 4.6		36.1 ± 2.3	

SMM : Skeletal muscle mass, SMF : skeletal muscle functioning, 25(OH)D : 25-hydroxy vitamin D, Intact PTH : intact parathyroid hormone
* : p < 0.05, ** : p < 0.01, *** : p < 0.001

primarily due to the loss of muscle mass (23-25). Muscle function decreases through the progression of sarcopenia, linking vitamin D and different symptoms (26-29) because vitamin D receptor expression decreases with age (30, 31). In our previous study, an age-related decrease in skeletal muscle mass and its cut-off levels for walking difficulty were clarified using 24-hour creatinine excretion as a measure of total-body skeletal muscle mass in a Japanese population including nursing home residents (4, 5). The skeletal muscle function, skeletal muscle mass (kg, kg/m^2, %BW) and CHI were also closely related to serum 25(OH)D levels (6).

In this study, the mean serum 25(OH)D concentration was low in the study population at baseline and it markedly improved after daily 1000-IU vitamin D-fortified milk intake for 6 months in the 21-50 years, 51-75 years and total groups in both males and females. The mean value (29.6 ng/mL) exceeded the recommendation for older adults of >50 nmol/L (20 ng/mL) (32), but 47% of the participants achieved VDS, whereas 47% and 6% had VDI and VDD, respectively. Vitamin D-fortified milk intake also improved handgrip strength and physical performance in the 21-50 years group, but not in older groups. Vitamin D treatment also did not improve muscle mass or nitrogen or calcium absorption rates. Regarding the relevance of vitamin D for muscle (33), an improvement in the plasma 25(OH)D concentration may also have played a role in the observed increase in muscle mass.

Japanese mean plasma 25(OH)D levels were 22.2 ng/mL (55.6 ± 14.6 nmol/L) in 600 home-dwelling postmenopausal women (34), 21 ng/mL (54.2 ± 29.0 nmol/L winter, 53.3 ± 30.3 nmol/L summer) in non-institutionalized elderly subjects requiring care (35) and 12 ng/mL (29.9 ± 13.1 nmol/L) in 133 physically inactive elderly subjects (84.6 ± 8.2 years old) living in nursing homes (36). The average dietary intake of vitamin D was around 300 IU/d, which is approximately 150% and 88% of the adequate intake by the Japan Dietary Reference Intake 2005 and 2020, respectively (37). Supplementation with 5 μg (200 IU) and 20 μg (800 IU) of vitamin D daily for 30 days increased the plasma 25(OH)D level from 11.1 ± 3.2 ng/mL to 14.7 ± 3.6 ng/mL in 33 institutionalized subjects (87.3 ± 7.3 years old) (37) and 9.7 ± 2.8 ng/mL to 19.3 ± 4.1 ng/mL in 32 physically disabled elderly (83.8 ± 7.6 years old) subjects living in nursing homes (38). Chel *et al*. (39) assessed the effects of daily supplementation with 600 IU of vitamin D for 4 months in elderly nursing home residents (84.3 ± 6.3 years old). The serum 25(OH)D level increased from 9.2 ng/mL to 28 ng/mL, and the percentage of subjects with a serum 25(OH)D level below 20 ng/mL was only 10.9%. Furthermore, Chapuy *et al*. (40) reported that daily supplementation with 800 IU of vitamin D in combination with l,200 mg of calcium increased the serum 25(OH)D level from 9.2 ng/mL to above 30.0 ng/mL after 6 months in older subjects (85.2 ± 7.1 years old). These reports suggest that the daily administration of 800 IU of vitamin D for several months is required for the correction of a low vitamin D status in the institutionalized elderly.

Furthermore, vitamin D-fortified milk intake significantly improved the handgrip strength and TUG, particularly in the 21-50 years group. Of note, several cross-sectional studies involving adults and younger humans demonstrated that sufficient vitamin D levels positively affected muscle strength (41-43). Similarly, a positive association between sufficient vitamin D status and higher handgrip muscle strength was reported in a study involving adolescent girls, revealing that girls with a sufficient vitamin D status have a significantly higher handgrip muscle strength than those with a poor vitamin D status (41). Meta-analysis of randomized controlled studies (RTCs) using vitamin D revealed vitamin D supplementation to be effective for the global muscle strength, which was more marked in those with a 25(OH)D level <30 nmol/l and those >65 years of age (44).

Vitamin D supplementation at 1000 IU per day for one year improved the hip muscle strength and TUG test in older women with VDI (45), increased the mean type II muscle fiber diameter and percentage of type II fibers in elderly patients with post-stroke hemiplegia (46), and increased the intra myonuclear vitamin D receptor (VDR) concentration by 30% and total (type I and II) muscle fiber size by 10% in mobility-limited elderly women (47). On the other hand, increased 25(OH)D levels in the 51-75 years and 76 year or older groups were not related to the improvement of muscle function in this study. The lack of effect may be explained by our participants being vitamin D resistant and thus unable to improve their muscle strength. However, the pooled data from meta-analysis of RTCs suggested that vitamin D supplementation increases upper and lower limb strength in the healthy adult population ; therefore, vitamin D may have a significantly positive effect on overall muscle function in humans (48).

Aging is associated with reduced serum vitamin D metabolites (49, 50), intestinal calcium absorption (51, 52) and age-related intestinal resistance to 1α,25(OH)$_2$D$_3$ (53-55). Regardless of vitamin D supplementation, the intestinal calcium absorption rate in this study decreased from 17.0 ± 1.9% to 14.3 ± 1.6% and the calcium absorption rate increased from 78 mg/day at baseline to 98 mg/day. Intestinal fractional calcium absorption (FCA) was previously assessed using a double isotope method in post-menopausal osteoporosis patients (74.2 ± 2.9 years old) (56). The baseline FCA value of the participants was 21.5 ± 7.9% and was significantly correlated with the serum 1,25(OH)$_2$D concentration, but not with the serum 25(OH)D concentration. After treatment for 4 weeks, the FCA significantly increased by 59.5% in the eldecalcitol (ELD) (0.75 μg/day) group and by 45.9% (27.9 to 63.8%) in the 1α hydroxyl calcidiol (ALF) (1 μg/day) group, whereas no significant change in the plain vitamin D3 (800 IU/day) group was noted. Therefore, the stimulation of FCA by plain vitamin D3 requires a dose greater than 800 IU. Plain vitamin D3 administration was also not effective or induced only small increases in FCA in a high-dose group (57, 58).

The age-related loss of skeletal muscle mass and function is partly the result of impaired activation of postprandial muscle protein synthesis. A post hoc study (59) demonstrated that participants with higher baseline 25(OH)D concentrations (>50 nmol/L) and dietary protein intake (>1.0 g/kg/day) had greater gains in appendicular muscle mass, skeletal muscle index and relative appendicular muscle mass in response to the nutritional intervention. Protein synthesis is tightly controlled by the Akt/mTOR pathway, which is activated by anabolic factors such as insulin and amino acids (60). Vitamin D may interfere with the insulin signaling pathway and insulin sensitivity (61, 62), and promote skeletal muscle protein anabolism (61). Vitamin D- and leucine-enriched nutrition may aid in the prevention and treatment of muscle mass and strength loss *in vivo* because of synergistic effects of leucine and vitamin D on protein synthesis in C2C12 myotubes (61). Therefore, synergy between vitamin D and milk protein may help to prevent or counteract the loss of muscle mass and strength during aging. The improved handgrip force and TUG test may be explained by the increased calcium absorption and anabolic utility of nitrogen by vitamin D administration in this study.

This study has several limitations. The controlled before–after design of this study did not include a control group. The lack of randomization is also a weak point related to the effects of vitamin D-fortified milk intake. The small sample size may prevent us from establishing relevance. However, a minimum level of 50 nmol/L (20 ng/mL) of 25(OH)D must be reached in the general elderly population and 75 nmol/L (30 ng/mL) is considered the

target in fragile study participants who have a higher risk of falls and fractures (63, 64). Therefore, daily 1,000-IU vitamin D-fortified milk intake is a practical method to maintain a serum 25(OH)D level of 30 ng/mL, and to improve muscle strength and physical function in the Japanese population.

CONFLICTS OF INTEREST AND ACKNOWLEDGEMENT

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REFERENCES

1. Yeung SSY, Reijnierse EM, Pham VK, Trappenburg MC, Lim WK, Meskers CGM, Maier AB : Sarcopenia and its association with falls and fractures in older adults : a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle 10 : 485-500, 2019
2. Fuggle N, Shaw S, Dennison E, Cooper C : Sarcopenia. Best Pract Res Clin Rheumatol 31 : 218-242, 2017
3. Morley JE, Anker SD, von Haehling S : Prevalence, incidence, and clinical impact of sarcopenia : facts, numbers, and epidemiology-update 2014. J Cachexia Sarcopenia Muscle 5 : 253-259, 2014
4. Morishita T, Sato M, Kume H, Sakuma M, Arai H, Katayama T, Katoh S, Sairyo K, Takeda E : Skeletal muscle mass of old Japanese women suffering from walking difficulty in nursing home. J Med Invest 65(1.2) : 122-130, 2018
5. Sato M, Morishita T, Katayama T, Satomura S, Okuno H, Sumita N, Sakuma M, Arai H, Katoh S, Sairyo K, Kawaura A, Takeda E : Relationship between age-related decreases in serum 25-hydroxyvitamin D levels and skeletal muscle mass in Japanese women. J Med Invest 67(1.2) : 151-157, 2020
6. Morishita T, Sato M, Katayama T, Sumida N, Omae H, Satomura S, Sakuma M, Arai H, Kawaura A, Takeda E, Katoh S, Sairyo K : Cut-off values of skeletal muscle strength and physical functions in Japanese elderly with walking difficulty. J Med Invest 68(1.2) : 48-52, 2021
7. Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P : Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab 92 : 2058-2065, 2007
8. Laird E, O'Halloran AM, Carey D, Healy M, O'Connor D, Moore P, Shannon T, Molloy AM, Kenny RA : The prevalence of vitamin D deficiency and the determinants of 25(OH)D concentration in older Irish adults : data from The Irish Longitudinal Study on Ageing (TILDA). J Gerontol A Biol Sci Med Sci 73(4) : 519-525, 2018
9. Aspell N, Laird E, Healy M, Shannon T, Lawlor B, O'Sullivan M : The prevalence and determinants of vitamin D status in community-dwelling older adults : results from the English Longitudinal Study of Ageing (ELSA). Nutrients 11(6) : 1253, 2019
10. Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington DC : The National Academies Press ; 2011
11. Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H : Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. Osteoporos Int 20 : 315-322, 2009
12. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA, Conzelmann M : Effects of vitamin D and calcium supplementation on falls : A randomized controlled trial. J Bone Miner Res 18 : 343-351, 2003
13. Capatina C, Caragheorgheopol A, Berteanu M, Poiana C : Short-term administration of alphacalcidol is associated with more significant improvement of muscular performance in women with vitamin D deficiency compared to native vitamin D. Exp Clin Endocrinol Diabetes 124 : 461-465, 2016
14. Kenny AM, Biskup B, Robbins B, Marcell G, Burleson JA : Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men J Am Geriatr Soc 51 : 1762-1767, 2003
15. Janssen HC, Samson MM, Verhaar HJ : Muscle strength and mobility in vitamin D-insufficient female geriatric patients : A randomized controlled trial on vitamin D and calcium supplementation. Aging Clin Exp Res 22 : 78-84, 2010
16. Knutsen KV, Madar AA, Lagerlov P, Brekke M, Raastad T, Stene LC, Meyer HE : Does vitamin D improve muscle strength in adults? A randomized, double-blind, placebo-controlled trial among ethnic minorities in norway. J Clin Endocrinol Metab 99 : 194-202, 2014
17. Kubiak J, Thorsby PM, Kamycheva E, Jorde R : Vitamin D supplementation does not improve CVD risk factors in vitamin D-insufficient subjects. Endocr Connect 7(6) : 840-849, 2018
18. Okazaki R, Ozono K, Fukumoto S, Inoue D, Yamauchi M, Minagawa M, Michigami T, Takeuchi Y, Matsumoto T, Sugimoto T : Assessment criteria for vitamin D deficiency/insufficiency in Japan‥ proposal by an expert panel supported by the Research Program of Intractable Diseases, Ministry of Health, Labour and Welfare. Japan, the Japanese Society for Bone and Mineral Research and the Japan Endocrine Society [Opinion]. J Bone Miner Metab 35 : 1-5, 2017
19. Wang ZM, Gallagher D, Nelson ME, Matthews DE, Heymsfield SB : Total-body skeletal muscle mass : evaluation of 24-h urinary creatinine excretion by computerized axial tomography. Am J Clin Nutr 63 : 863-869, 1996
20. Podsiadlo D, Richardson S : The timed 'Up & Go' : a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 39 : 142-148, 1991
21. Mahoney FI, Barthel DW : Functional evaluation : the BARTHEL Index. Md State Med J 14 : 61-65, 1965
22. Granger CV, Dewis LS, Peters NC, Sherwood CC, Barrett JE : Stroke rehabilitation : analysis of repeated Barthel index measures. Arch Phys Med Rehabil 60 : 14-17, 1979
23. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R : Aging of skeletal muscle : a 12-yr longitudinal study. J Appl Physiol 88(4) : 1321-1326, 2000
24. Kent-Braun JA, Ng AV : Specific strength and voluntary muscle activation in young and elderly women and men. J Appl Physiol 87(1) : 22-29, 1999
25. Klein CS, Rice CL, Marsh GD : Normalized force, activation, and coactivation in the arm muscles of young and old men. J Appl Physiol 91(3) : 1341-1349, 2001
26. Vitale G, Cesari M, Mari D, Vitale G : Aging of the endocrine system and its potential impact on sarcopenia. Eur J Intern Med 35 : 10-15, 2016
27. McCabe PS, Pye SR, Beth JM, Lee DM, Tajar A, Bartfai G, Boonen S, Bouillon R, Casanueva F, Finn JD, Forti G,

Giwercman A, Huhtaniemi IT, Kula K, Pendleton N, Punab M, Vanderschueren D, Wu FC, O'Neill TW ; EMAS Study Group : Low vitamin D and the risk of developing chronic widespread pain : results from the European male ageing study. BMC Musculoskelet Disord 16 ; 17 : 32, 2016
28. Salminen M, Saaristo P, Salonoja M, Vaapio S, Vahlberg T, Lamberg-Allardt C, Aarnio P, Kivelä SL : Distal lower limb strength is reduced in subjects with impaired glucose tolerance and is related to elevated intramuscular fat level and vitamin D deficiency. Arch Gerontol Geriatr 61(3) : 419-424, 2015
29. Almurdhi MM, Reeves ND, Bowling FL, Boulton AJ, Jeziorska M, Malik RA : Vitamin D Status and Physical Function in Older Finnish People : A One-Year Follow-Up Study. Diabet Med 34(3) : 356-363, 2017
30. Olsson K, Saini A, Strömberg A, Alam S, Lilja M, Rullman E, Gustafsson T : Evidence for Vitamin D Receptor Expression and Direct Effects of $1\alpha,25(OH)_2D_3$ in Human Skeletal Muscle Precursor Cells. Endocrinology 157(1) : 98-111, 2016
31. Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS, Salisbury JL, Nair KS, Terzic A, Sieck GC, Kumar R : 1α,25-Dihydroxyvitamin D3 Regulates Mitochondrial Oxygen Consumption and Dynamics in Human Skeletal Muscle Cells. J Biol Chem 291(3) : 1514-1528, 2016
32. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA : The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine : what clinicians need to know. J Clin Endocrinol Metab 96 : 53-58, 2011
33. Ceglia L, Harris SS : Vitamin D and its role in skeletal muscle. Calcif Tissue Int 92 : 151-162, 2013
34. Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, Maruyama K, Oshiki R, Kobayashi R, Nashimoto M, Yoshihara A, Ozaki R, Okano T, Yamamoto M : Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women : Yokogoshi study. Bone 42 : 271-277, 2008
35. Nakamura K, Nishiwaki T, Ueno T, Yamamoto M : Serum 25-hydroxyvitamin D levels and activities of daily living in noninstitutionalized elderly Japanese requiring care. J Bone Miner Metab 23 : 488-494, 2005
36. Nashimoto M, Nakamura K, Matsuyama S, Hatakeyama M, Yamamoto M : Hypovitaminosis D and hyperparathyroidism in physically inactive elderly Japanese living in nursing homes : relationship with age, sunlight exposure and activities of daily living. Aging Clin Exp Res 14 : 5-12, 2002
37. Himeno M, Tsugawa N, Kuwabara A, Fujii M, Kawai N, Kato Y, Kihara N, Toyoda T, Kishimoto M, Ogawa Y, Kido S, Noike T, Okano T, Tanaka K : Effect of vitamin D supplementation in the institutionalized elderly. J Bone Miner Metab 27(6) : 733-737, 2009
38. Nashimoto M, Nakamura K, Matsuyama S, Hatakeyama M, Yamamoto M : Hypovitaminosis D and hyperparathyroidism in physically inactive elderly Japanese living in nursing homes : relationship with age, sunlight exposure and activities of daily living. Aging Clin Exp Res 14 : 5-12, 2002
39. Chel V, Wijnhoven HA, Smit JH, Ooms M, Lips P : Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporos Int 19 : 663-671, 2008
40. Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, Garnero P, Meunier PJ : Combined calcium and vitamin D3 supplementation in elderly women : confirmation of reversal of secondary hyperparathyroidism and hip fracture risk : the Decalyos II study. Osteoporos Int 13 : 257-264, 2002
41. Foo LH, Zhang Q, Zhu K, Ma G, Hu X, Greenfield H, Fraser DR : Low Vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. J Nutr 139(5) : 1002-1007, 2009
42. Hurst Pv, Conlon C, Foskett A : Vitamin D status predicts hand-grip strength in young adult women living in Auckland, New Zealand. J Steroid Biochem Mol Biol 136 : 330-332, 2013
43. Ward KA, Das G, Roberts SA, Berry JL, Adams JE, Rawer R, Mughal MZ : A Randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. J Clin Endocrinol Metab 95(10) : 4643-4651, 2010
44. Beaudart C, Buckinx F, Rabenda V, Gillain S, Cavalier E, Slomian J, Petermans J, Reginster JY, Bruyère O : The effects of vitamin D on skeletal strength, muscle mass, and muscle power : a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 99(11) : 4336-4345, 2014
45. Zhu K, Austin N, Devine A, Bruce D, Prince RL : A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. J Am Geriatr Soc 58 : 2063-2068, 2010
46. Sato Y, Iwamoto J, Kanoko T, Satoh K : Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke : A randomized controlled trial. Cerebrovasc Dis 20 : 187-192, 2005
47. Ceglia L, Niramitmahapanya S, da Silva Morais M, Rivas DA, Harris SS, Bischoff-Ferrari H, Fielding RA, Dawson-Hughes B : A randomized study on the effect of vitamin D(3) supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. J Clin Endocrinol Metab 98 : E1927-E1935, 2013
48. Tomlinson PB, Joseph C, Angioi M : Effects of vitamin D supplementation on upper and lower body muscle strength levels in healthy individuals. A systematic review with meta-analysis. J Sci Med Sport 18(5) : 575-580, 2015
49. Armbrecht HJ, Forte LR, Halloran BP : Effect of age and dietary calcium on renal 25(OH)D metabolism, serum 1,25(OH)2D, and PTH. Am J Physiol 246(3 Pt 1) : E266-270, 1984
50. Liang CT, Barnes J, Takamato S, Sacktor B : Effect of Age on Calcium Uptake in Isolated Duodenum Cells : Role of 1,25-dihydroxyvitamin D3. Endocrinology 124(6) : 2830-2836, 1989
51. Bullamore JR, Wilkinson R, Gallagher JC, Nordin BE, Marshall DH : Effect of age on calcium absorption. Lancet 2(7672) : 535-537, 1970
52. Armbrecht HJ : Effect of age on calcium and phosphate absorption. Role of 1,25-dihydroxyvitamin D. Miner Electrolyte Metab 16(2-3) : 159-166, 1990
53. Wood RJ, Fleet JC, Cashman K, Bruns ME, Deluca HF : Intestinal calcium absorption in the aged rat : evidence of intestinal resistance to 1,25(OH)2 vitamin D. Endocrinology 139(9) : 3843-3848, 1998
54. Pattanaungkul S, Riggs BL, Yergey AL, Vieira NE, O'Fallon WM, Khosla S : Relationship of Intestinal Calcium Absorption to 1,25-dihydroxyvitamin D [1,25(OH)2D] Levels in Young Versus Elderly Women : Evidence for Age-Related Intestinal Resistance to 1,25(OH)2D Action. J Clin Endocrinol Metab 85(11) : 4023-4027, 2000
55. Song Y, Fleet JC : Intestinal Resistance to 1,25

Dihydroxyvitamin D in Mice Heterozygous for the Vitamin D Receptor Knockout Allele. Endocrinology 148(3) : 1396-1402, 2007
56. Uenishi K, Tokiwa M, Kato S, Shiraki M : Stimulation of intestinal calcium absorption by orally administrated vitamin D3 compounds : a prospective open-label randomized trial in osteoporosis. Osteoporos Int 29(3) : 723-732, 2018
57. Aloia JF, Dhaliwal R, Shieh A, Mikhail M, Fazzari M, Ragolia L, Abrams SA : Vitamin D supplementation increases calcium absorption without a threshold effect. Am J Clin Nutr 99(3) : 624-631, 2014
58. Hansen KE, Johnson RE, Chambers KR, Johnson MG, Lemon CC, Vo TNT, Marvdashti S : Treatment of vitamin D insufficiency in postmenopausal women. A randomized clinical trial. JAMA Intern Med 175(10) : 1612-1621, 2015
59. Verlaan S, Maier AB, Bauer JM, Bautmans I, Brandt K, Donini LM, Maggio M, McMurdo MET, Mets T, Seal C, Wijers SLJ, Sieber C, Boirie Y, Cederholm T : Sufficient levels of 25-hydroxyvitamin D and protein intake required to increase muscle mass in sarcopenic older adults - The PROVIDE study. Clin Nutr 37(2) : 551-557, 2018
60. Anthony JC, Anthony TG, Kimball SR, Jefferson LS : Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. J Nutr 131(3) : 856S-860S, 2001
61. Salles J, Chanet A, Giraudet C, Patrac V, Pierre P, Jourdan M, Luiking YC, Verlaan S, Migné C, Boirie Y, Walrand S : 1,25(OH)2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. Mol Nutr Food Res 57(12) : 2137-2146, 2013
62. Teegarden D, Donkin SS : Vitamin D : emerging new roles in insulin sensitivity. Nutr Res Rev 22(1) : 82-92, 2009
63. René Rizzoli, John C Stevenson, Jürgen M Bauer, Luc J C van Loon, Stéphane Walrand, John A Kanis, Cyrus Cooper, Maria-Luisa Brandi, Adolfo Diez-Perez, Jean-Yves Reginster, ESCEO Task Force : The Role of Dietary Protein and Vitamin D in Maintaining Musculoskeletal Health in Postmenopausal Women : A Consensus Statement From the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Maturitas 79(1) : 122-132, 2014
64. Rizzoli R, Boonen S, Brandi ML, Bruyère O, Cooper C, Kanis JA, Kaufman JM, Ringe JD, Weryha G, Reginster JY : Vitamin D supplementation in elderly or postmenopausal women : a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Curr Med Res Opin 29(4) : 305-313, 2013