

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/m²). 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.

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).

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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, copper, 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) = $21.8 \times \text{Cr (g/day)}$) (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) $\times 100$.

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 \pm 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/m²). 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/m²) ($r = -0.20$, $p = 0.007$).

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		
	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/m², %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 1,200 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 (RCTs) 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 RCTs 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 1,25(OH)₂D₃ (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)₂D 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 1,25(OH)₂D₃ (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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