Effect of Vitamin D3 on Changes in Muscle Mass in the Elderly in Nursing Homes: Double-Blind Controlled Randomized Trial

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Abstract

The decline in muscle mass and strength begins at the age of 30 years by 40% within 30 years, then will decrease by 40% every ten years after the age of 60 years. Loss of muscle mass and strength in older people causes weakness and reduced muscle function, which can lead to a decrease in the ability to perform activities of daily living and an increased risk of falls. In addition, older people are prone to malnutrition; this condition will be accompanied by micronutrient deficiencies both in older people who are undernourished and overnourished. Giving vitamin D3 to increase muscle mass and ability and reduce fat mass in older people also has pros and cons. So, this study aims to explore the effect of giving Vitamin D3 on changes in muscle mass in older adults. The study will also examine changes in upper arm muscle strength and evaluate the impact of Vitamin D3 on changes in muscle mass. This research is a study with an Experimental Randomised Control Trial (RCT) research design. The population was elderly who were deficient in vitamin D, while the study sample amounted to 26 control subjects and 26 action subjects. Samples are taken by consecutive sampling technique. Based on research findings, most older adults (60–69 years) suffer from vitamin D deficiency and deficiency. However, vitamin D3 supplementation increases the muscle mass of older adults in nursing homes, especially in the muscles of the upper and lower extremities. However, the supplement weakens muscle mass and causes bruising and bleeding but does not weaken arm muscle mass.

Keywords: Elderly, Muscle Mass, Panti Werdha, Vitamin D3

INTRODUCTION

The elderly, according to Law Number 13 of 1998, is someone who has reached the age of 60 (sixty) years and over (Zaidi & Mutholaah, 2023). An increase in the number of older adults. Indonesia experienced a rise in the number of older adults from 18 million people (7.56%) in 2010 to 25.9 million people (9.7%) in 2019, and is expected to continue to increase where in 2035 to 48.2 million people (15.77%) (Badan Pusat Statistik, 2020). Indonesia itself has the eighth-largest elderly population in the world and fourth in Asia (Setiati et al., 2019). In Indonesia alone, 1 in 4 older adults have experienced illness in the past month. In 2020, half of the elderly experienced health complaints, both physical and psychological (Statistical Center Body, 2020); therefore, health problems faced by the elderly should also be given special attention because ageing itself is a complex phenomenon that has a detrimental effect on tissue homeostasis. Skeletal muscle is one of the earliest tissues affected and undergoes age-related changes such as

functional impairment and loss of muscle mass (Fernando, Drescher, Nowotny, Grune, & Castro, 2019).

The decline in muscle mass and strength begins at the age of 30 years by 40% within 30 years, then will decrease by 40% every ten years after the age of 60 years. As a result, muscle mass becomes a quarter of the total body mass in people in their late 70s (Widajanti et al., 2020). The process of decreasing muscle mass and ability in the elderly is due to chronic conditions, excessive adipokine tissue, decreased activity and ability to move, telomere erosion/disorders, neuron degeneration, insufficient intake, anorexia, and malabsorption, hormonal factors, inflammatory processes, and mitochondrial dysfunction (Widajanti et al., 2020). Loss of muscle mass and strength in older people causes weakness and decreased muscle function, which can lead to a decrease in the ability to perform activities of daily living and an increased risk of falls. The prevalence of muscle weakness in older people was 36.7% (men 5.2%, women 31.5%) (Rezuş et al., 2020).

In addition, older people are prone to malnutrition due to physiological changes related to ageing (they tend to choose foods that are easily digested due to the ability to chew and digest food that decreases) and limitations in accessing healthy nutritious foods due to less diverse food sources, and older people tend to adjust their food menu with motor and cognitive abilities so that it becomes a menu that is always the same. This malnutrition condition will be accompanied by micronutrient deficiencies in both undernourished and overnourished elderly. Low micronutrient intakes have been reported in both developing and developed countries. 4 out of 5 elderly have inadequate intake of vitamin D, calcium, vitamin E, and folate. In contrast, the intake of macronutrient foods ranges from 85% - 110% of RDA (Aaron, Ekawanti, and Josafat, 2020). In Indonesia, a study states that 96% of older people in South Lampung nursing homes experience vitamin D deficiency (Hermawan & Andoko, 2018). Vitamin D deficiency that occurs in older people is often associated with a decrease in muscle mass and ability because vitamin D deficiency affects Vitamin D Receptors (VDR) located mainly in fast-twitch muscle fibres, where the muscle will respond first in quick action so that adequate vitamin D can improve muscle strength and coordination, and allow fall prevention (Ponti et al., 2020; Sun, Lee, Yim, Won, & Co., 2019). Similarly, research conducted in Bandung and Sumedang showed that vitamin D deficiency was significantly associated with muscle mass, muscle strength, and fat mass in older people (Biben, Defi, Nugraha, & Setiabudia, 2017).

Giving vitamin D3 to increase muscle mass and ability and reduce fat mass in older people also has pros and cons. Some studies said that Vitamin D3 supplementation had no effect on body composition, with the administration of 2000 IU of Vitamin D3 and observation (sub-

cohort) for two years, but in the study was not followed by observations about diet, sun exposure and activity of each subject, besides that the subjects in the study had various variants both age, race, sex, and history of the subject's disease, besides that in the study body composition measurements were carried out at the beginning of the study. After two years, the subjects took vitamin D3 supplements without looking at the subject's vitamin D3 status (Suryadinata & Lorensia, 2020). Another study showed that giving 4000 ui per day to 60,000ui per week for four weeks to 6 months in adult and elderly subjects can increase muscle fibre size, intranuclear VDR concentration and increase upper and lower body muscle strength in elderly subjects and no symptoms of toxicity (Ceglia et al., 2013; Dzik & Kaczor, 2019; Tomlinson, Joseph, & Angioi, 2015). These differences of opinion need to be researched by taking into account the nutritional intake and uniformity of research subjects.

This study aims to delve deeper into the effects of vitamin D3 supplementation on changes in muscle mass in older people. Vitamin D is a group of vitamins that includes ergokalsiferol (D2) and kolekalsiferol (D3). Of these, vitamin D3 is easier for human bodies to absorb and is typically found in fat-soluble forms. To achieve this goal, several aspects will be examined, including socio-demographic characteristics, Vitamin D status, nutritional status, nutritional intake, as well as muscle and fat mass in older people in nursing homes. In addition, this study will also examine changes in upper arm muscle strength and evaluate the effect of Vitamin D3 on changes in muscle mass. Vitamin D3 referred to here is vitamin D3 supplementation in the form of pre-active vitamin D. The benefits of this study include further understanding of the vitamin D3 status, nutritional status, and muscle mass of older people for the study subjects. For researchers, the study provided insight into the impact of vitamin D3 administration on muscle mass. Meanwhile, for institutions and governments, the results of this study can be the basis for the formulation of health policies and intervention programs related to vitamin D deficiency in older people.

METHODS

This study is a study with an Experimental Randomised Control Trial (RCT) research design or a randomised controlled trial with double disguise where neither the research subject nor the researcher knows which subject is the control or treatment group. The first outcome of this study was to see changes in muscle mass increase influenced by vitamin D3 supplementation in the elderly and changes in the subject's blood serum vitamin D status, and the second observation was on changes in the grip strength of subjects affected by vitamin D3 supplementation. Older people in the nursing home will be conducted for a short interview regarding the personal data of the research subject. Then, a blood

draw examination will be carried out to check vitamin D levels in the blood with Chemiluminescent Immunoassay (CLIA), examination of muscle mass and fat mass using the BIA Tanita tool and examination of upper extremity muscle strength using the Handgrip "Jamar" tool. After the examination of blood samples, muscle mass, and fat mass as well as upper extremity muscle strength. The inclusion criteria were the same between the control and treatment groups, namely age >=60 years, insufficiency or deficiency of vitamin D blood serum, not being treated for cancer or autoimmune, not bedridden, and not malnutrition. Then, randomisation was carried out to determine the subjects of the control group or treatment group. The study subjects in the control group will be given a placebo, and the treatment group will be given vitamin D3 of 4000 IU in a single dose every day. It will be monitored for two months of administration. Two months after vitamin D3 administration, muscle mass, fat mass, and muscle strength of the upper extremities of the study subjects will be re-examined.

The research was conducted at the Tresna Werdha Budi Mulia 1 Social Institution, East Jakarta. Data collection of research subjects will be carried out from April 2023 to June 2023. The population was elderly who were deficient in vitamin D, while the study sample amounted to 26 control subjects and 26 action subjects. The determination of the sample size in this study was carried out using a sample calculation formula to test the average difference hypothesis in 2 dependent groups. Inclusion and exclusion criteria have been set, including being over 60 years old, staying for a minimum period of three months, and not taking vitamin D3 supplements before. Research subjects who met the inclusion criteria were 70 subjects, and subjects who refused to participate in the study were seven subjects for fear of blood sampling. There were 63 research subjects randomised to 31 subjects in the control group and 32 subjects in the treatment group. The data collection process is carried out through a series of health and laboratory examinations, as well as formal evaluations throughout the process of purchasing supplies. After the second month, muscle mass, fat, and strength will be measured again to assess the effect of vitamin D3 supplementation on changes in muscle mass, fat mass, and upper arm muscle strength. Samples are taken by consecutive sampling technique. Patients who meet the inclusion and exclusion criteria will be taken as research samples until they meet the total minimum sample number set and simple randomisation is carried out.

Measurements of muscle mass and fat mass were carried out using *Bio Impedance Analyzer* (BIA) Karada Scan. They obtained four points of muscle mass check, namely muscle mass throughout the body, torso, upper arms, lower arms, and lower and upper limbs. Muscle mass was measured before and after both the control and treatment groups.

RESULTS

Characteristics

In the second month of the study, a total of 32 subjects in the treatment group and 29 subjects in the control group carried out further data analysis using SPSS to see the meaningfulness of each group and the effect of vitamin D3 administration on muscle mass in the treatment group

Table 1. Socio-demographic characteristics in older people at Tresna Werdha Budi Mulia I Social Institution

Variable	Control (n=31)	Treatment (n=32)	p
Gender, n (%)			
Male	15 (48,4)	6 (18,8)	0,055
Female	16 (51,6)	26 (81,3)	
Age, n (%)			
60-69 years old	23 (74,2)	20 (62,5)	0,700
70-79 years old	5 (16,1)	8 (25,0)	
>80 years	3 (9,7)	4 (12,5)	
Education Level, n (%)			
Unknown	5 (16,1)	8 (25,0)	0,170
End of SD	11 (35,5)	12 (37,5)	
Graduated from junior high school	5 (16,1)	7 (21,9)	
Graduated from high school	10 (32,3)	3 (9,4)	
D3/D4/S1/S2	0 (0)	2 (6,3)	
Religion, n (%)			
Islamic	27 (87,1)	30 (93,8)	0,411*
Christian	4 (12,9)	2 (6,3)	
History of the disease, n=34, n (%)			
Diabetes Melitus	0 (0)	2 (10,5)	0,429
Hypertensive	10 (62,5)	11 (57,9)	
Other	6 (37,5)	6 (31,6)	
Status Gizi			
Good Nutrition	9 (29,0)	9 (28,1)	1,000
Risk of Malnutrition	22 (71,0)	23 (71,9)	
GDS, n (%)			
0	3 (9,7)	3 (9,4)	0,168
1	6 (19,4)	11 (34,4)	
2	11 (35,5)	5 (15,6)	
3	5 (16,1)	10 (31,3)	
4	6 (19,4)	3 (9,4)	
Frail, n (%)			
0	23 (74,2)	23 (71,9)	0,997
1	4 (12,9)	5 (15,6)	
2	3 (9,7)	3 (9,4)	
3	1 (3,2)	1 (3,1)	

Uji Chi Square; *Uji Fisher Exact

Based on the results of the analysis of characteristics between the control group and the treatment, p>0.05 showed that the characteristics of the control group and homogeneous treatment did not differ in meaning. The study subjects were male in the control group, amounting to 15 subjects (48.4%), and women, amounting to 16 subjects (51.6%), while in the treatment group, the number of subjects was six people (18.8%) and women 26 people (81.3%).

The age of 60-69 years in the control group amounted to 23 subjects (74.2%), and in the treatment group, 20 subjects (62.5%). In the age range of 70-79 years in the control group, there were five subjects (16.1%) and in the treatment group 8 subjects (25.0%), and in the age range of more than 80 years, there were three subjects (9.7%) in the control group and four subjects (12.5%) in the treatment group. Nutritional status of subjects In the control group, nine subjects (29%) had good nutritional status, and 22 subjects (71%) had nutritional status at risk of malnutrition. In contrast, in the treatment group, nine subjects (28.1%) had good nutritional status, and 23 subjects (71.9%) had nutritional status at risk of malnutrition. All subjects had a GDS score of less than 5, indicating that the subject's psychological state was not in a state of depression. When the assessment of the fragility of the subjects was carried out, 23 subjects (74.2%) were not at risk of brittle/old, and seven subjects (22.6%) were in pre-fragile/ageing conditions. One subject (3.2%) was in fragile/ageing conditions, while in the treatment group, there were 23 subjects (71.9%) who were not at risk of weak/old, eight subjects (25%) in pre-fragile/ageing conditions and one subject (3.1%) in soft/ageing conditions.

Muscle mass and fat mass

The mean/median muscle mass of the control group before the study was conducted on whole body muscle mass was 23.93% (2.54), in the torso 17.35% (2.94), muscle mass in the arms 31.6% (7.2) and muscle mass in the legs 39.19% (4.55). The average/median muscle mass after research was conducted on whole body muscle mass was 22.3% (4.4), in the torso 16.57% (3.72), muscle mass in the arms 28.18% (5.74) and muscle mass in the legs 37.01% (4.26). Analysis of muscle mass data before and after the study was carried out with paired t-tests for torso and leg muscle mass and the Wilcoxon test for arm and whole body muscle mass. In all tests, there was no significant difference in each muscle mass ($p > 0.05$).

The average/median muscle mass of the treatment group before the study was carried out on whole body muscle mass was 22.6% (4.3), in the torso 16.45% (3.9), muscle mass in the arms 26.4% (8.8), and average muscle mass in the legs 36.34% (5.8). The average muscle mass after research on whole body muscle mass was 22.03% (3.67), in the torso 15.67% (4.11), muscle mass in the arms 27.07% (5.2), and muscle mass in the legs 36.01% (5.5). Analysis of muscle mass data before and after the study was carried out with paired t-tests for leg muscle mass and Wilcoxon tests for the rest. There was no significant difference in each muscle mass ($P>0.05$).

The mean/median fat mass of the control group before the study was conducted on whole body fat mass was 23.49% (SD 6.74), in the torso 21.9% (interquartile range 10), fat mass in the arms 32.8% (19.3) and fat mass in the legs 30.88% (8.94). The average/median fat mass after the study was conducted on whole body muscle mass was 25.18% (7.35), in the torso 22.35% (6.90),

in the arms 38.2% (19.2) and fat mass in the legs 32.3% (14.8). Analysis of fat mass data before and after the study was carried out with a paired t-test, and the Wilcoxon test was continued, and no significant difference in each muscle mass was found ($p > 0.05$).

The mean/median fat mass of the treatment group before the study was conducted on whole body fat mass was 26.8% (5.9), in the torso 24% (6.1), fat mass in the arms 42.5% (16.7) and fat mass in the legs 34.6% (8.5). The average/median fat mass after the study was conducted on whole body muscle mass was 26.75% (5), on the torso 23.55% (4.4), on the arms 41.05% (17), and fat mass on the legs 34.9% (7.2). Analysis of fat mass data before and after the study was carried out with a paired t-test, and the Wilcoxon test was continued, and no significant difference in each muscle mass was found ($p > 0.05$).

Table 2. Data analysis of changes in muscle mass and fat mass of control and treatment groups Paired T Test followed by Wilcoxon Test

Variable	Control			Treatment		
	Before	After	P value	Before	After	P value
Fat Mass						
WB	23,49% (SD 6,74)	25,18% (SD 7,35)	0,093	26,8% (5,9)	26,75% (5)	0,399*
Trunks	21,9% (10)	22,35% (SD 6,90)	0,086*	24% (6,1)	23,55% (4,4)	0,53*
Arms	32,8% (19,3)	38,2% (19,2)	0,161*	42,5% (16,7)	41,05% (17)	0,381*
Legs	30,88% (SD 8,94)	32,3% (14,8)	0,158*	34,6% (8,5)	34,9% (7,2)	0,721*
Muscle Mass						
WB	23,93% (SD 2,54)	22,3% (4,4)	0,058*	22,6% (4,3)	21,8 (19,3 – 24,45)	0,029*
Trunks	17,35% (SD 2,94)	16,57% (SD 3,72)	0,094	16,45% (3,9)	16,1 (13,1 – 17,6)	0,416*
Cute	31,6% (7,2)	28,18% (5,74)	0,187*	26,4% (8,8)	26,35 (23,97 – 31,35)	0,704*
Legs	39,19% (SD 4,55)	37,04 (4,26)	0,061	36,34% (SD 5,82)	35,15 (31,80 – 39,85)	0,676

T-test paired test;* Wilxocon test

Upper arm muscle strength

Upper arm muscle strength was measured using the *Handgrip* "Jamar" tool, which measured the subject's hand grip to see the strength of the subject's upper arm muscles. The normality test was carried out with the Shapiro-Wilk test, obtaining abnormal data distribution on the results of *handgrip* measurements in the control group before the study. The distribution of data was normal in the control group after the study, as well as in the treatment group both before and after the study. The median, upper arm muscle strength before the survey in the control group was 8kg (interquartile range 10), and the average muscle strength after the study was 11.17kg (SD 6.07). Analysis of upper arm muscle strength data before and after the study was

carried out with a paired t-test and continued with the Wilcoxon test; There was no significant difference (p>0.05). The average upper arm muscle strength after study in the treatment group was 9.48kg (SD 5.61), and after the study, it was 11.81kg (SD 6.20). Data analysis of upper arm muscle strength before and after the study was carried out with a paired t-test and continued with the Wilcoxon test found a significant difference (p < 0.05).

Table 3. T-Test: Paired test and Wilcoxon test

Variable	Control			Treatment		
	Before	After	P value	Before	After	P value
Muscle Strength	8 (10)	11,17 (6,07)	0,241*	9,48 (SD 5,60)	11,81 (SD 6,20)	<0,0001

T-test paired test;* Wilxocon test

a. The effect of vitamin D3 on changes in fat mass, muscle mass, and upper arm muscle strength in older people in nursing homes

Table 4. Effect of vitamin D3 supplementation on muscle mass and fat mass Unpaired T-Test Results and Mann Withney Test

Variable	Group		
	Control	Treatment	P value
Muscle Mass			
WB	1,69% (SD 5,23)	0% (3,67)	0,347*
Trunks	1,45% (SD 4,95)	-0,15% (3,67)	0,348*
Arms	2,7% (SD 10,74)	-1,75% (SD 11,97)	0,129
Legs	1,89 (SD 6,64)	-0,35% (4,87)	0,355*
Muscle Mass			
WB	-0,4% (3,3)	0,1% (3,63)	0,712*
Trunks	-0,78% (SD 2,43)	0,1% (3,77)	0,891*
Arms	2,7% (SD 10,74)	-1,75% (SD 11,97)	0,961
Legs	-2,18% (SD 5,6)	-0,33% (SD 4,49)	**0,045**
Muscle Strength	-0,4% (3,3)	0,1% (3,63)	0,647*

Test t-test is not paired; *Test Mann Whitney.

The average difference in muscle mass in the control group before and after the study was -0.82% (SD 2.13) in the whole body, -0.78% (SD 2.43) in the torso, 2.75% (SD 10.74) in the arms, and -2.17% (SD 5.6) in the limbs. In the treatment group, the median/mean difference in muscle mass before and after the study was 0.1% (interquartile range 3.63) throughout the body, 0.1% (interquartile range 3.77) in the torso, -1.75% (SD 11.97) in the arms, and -0.34% (SD 4.49) in the limbs. The Mann-Whitney test on the difference in whole body and torso muscle mass between the control and treatment groups showed no significant results, nor did the T-Test on arm muscle mass. In the T-test, effective results were found regarding the difference in leg muscle mass between the control and treatment groups (p = 0.045).

Researchers conducted a normality test to measure the difference in upper arm muscle strength before and after the study using a normality test with the Shapiro-Wilk test. In the difference in upper arm muscle strength in the control and treatment groups, an abnormal

distribution ($p < 0.05$) was found so that the data would be reported in the median form with the interquartile range. The median difference in upper arm muscle strength in the control group was 2 (interquartile range 4), and the median difference in upper arm muscle strength in the treatment group was 2 (interquartile range 4).

The difference in upper arm muscle strength in the control group was -0.4% (3.3), and in the treatment group was 0.1% (3.63). The Mann-Whitney test on the difference in upper arm strength of the control and treatment groups found no significant difference ($p = 0.647$).

Appendicular skeletal muscle index (ASMI)

Researchers conducted a normality test on ASMI difference data before and after the study with the Shapiro-Wilk test. In the normality test, the distribution of data is found to be normal so that the data will be displayed in average (SD).

Table 5. ASMI control group and treatment

Variable	Control		Treatment	
	Before	After	Before	After
ASMI				
Man	7,15 (0,63)	7,06 (0,3)	6,71 (0,78)	6,78 (0,85)
Woman	6,69 (0,87)	6,84 (0,85)	6,73 (0,31)	6,72 (0,35)

Table 6. Effect of vitamin D3 supplementation on ASMI changes

Variable	Group		P value
	Control	Treatment	
ASMI Changes	0,041 (0,331)	0,661 (0,335)	0,039*

From the results of calculating ASMI and the difference in ASMI before and after treatment, a bivariate test was carried out with the Pearson test to determine the relationship between treatment and changes in ASMI. It was found that the administration of therapy had a significant effect on the difference in ASMI with $p = 0.039$. Results from the statistical analysis show that vitamin D3 supplementation can significantly increase extremity muscle mass

b. Elderly fat in nursing homes

Researchers conducted normality tests on fat mass and visceral fat mass data before and after the study using normality tests with the Shapiro-Wilk test. In fat mass in the control group, a normal distribution ($p>0.05$) was found in measurements before and after the study so that the data would be reported in average form with standard deviations. In the fat mass of the treatment group, the distribution was found to be abnormal before the study so that the data would be reported in the median form with the interquartile range and normal after the study so that the data would be written in mean with standard deviation. In visceral fat mass, the distribution was found to be abnormal ($p<0.05$) in the control and treatment groups, both before and after the study, so the data were reported in medians with interquartile ranges.

In the control group, researchers performed a paired T-test on fat measurement data and a Wilcoxon test on visceral fat. The two found no significant differences. In the treatment group, statistical tests were carried out with the Wilcoxon test on fat and visceral fat measurement data, and a significant difference was obtained in the fat measurement results ($p < 0.05$).

Table 7. Analysis of whole body mass and visceral fat mass data in both groups paired t-test on fat measurement data and Wilcoxon test on visceral fat

Variable	Control			Treatment		
	Before	After	P	Before	After	P
Fat	31,97 (SD 7,80)	31,78 (SD 7,36)	0,845	36,7 (32,52 – 39,1)	34,15 (29,65 – 39)	**0,045***
VF	7,5 (5,75 – 10)	7 (4,5 – 9,7)	0,352*	6,5 (3,62 – 11,37)	6 (3,87 – 13,62)	0,212*

T-test paired test;* Wilxocon test

c. The effect of vitamin D3 on changes in Fat and Visceral Fat in older people in nursing homes

Researchers conducted normality tests on data on the difference in overall fat mass and visceral fat mass before and after the study using normality tests with the Shapiro-Wilk test. In the difference in fat mass in the control group, a normal distribution ($p>0.05$) was found so that the data would be reported in average form with standard deviations. In the difference in fat mass of the treatment group, the distribution was found to be abnormal so that the data would be reported in the median form with the interquartile range. In the difference in visceral fat mass in both the control and treatment groups, the distribution of data is abnormal so that the data will be reported in the median with the interquartile range. The Mann-Whitney test on the difference in fat mass and visceral fat mass between the control and treatment groups found no significant difference.

Table 8. Normality Test With Shapiro-Wilk Test

Variable	Group		
	Control	Treatment	P value
Fat	0 (-2,9 – 3,5)	-1,25 (-4,37 – 0,72)	0,257*
VF	-0,5 (-1,5 – 0,75)	0,25 (-0,87 – 0,75)	0,111*

*Uji Mann Whitney

Status Vitamin D

The median result in the control group of vitamin D status before the study was 23.5ng/ml, with an interquartile range of 7.15. After the survey, the average vitamin D status in the control group was 27.38 ng/ml, with a standard deviation of 7.46. In the treatment group, vitamin D status before the study was 17.9 ng/ml with SD 4.38. After the survey, the average vitamin D level was 36.07 ng/ml with SD 9.83. The Wilcoxon test in the control group found significant differences in vitamin D status before and after the study with $p<0.0001$. The paired T-test in the treatment group found significant differences between vitamin D status before and after the

survey with p<0.0001. In these results, there was an increase in blood serum vitamin D levels in both the control group and the treatment group, both of which had significant or significant results. The limitation of this study was that no analysis was carried out on the amount of sun exposure, food intake in the subjects and physical activity of the subjects so that the increase in the control group could be due to improvements in the behaviour of the subjects as a result of Counseling on the importance of vitamin D for health before researching all subjects.

Table 9. Analysis of blood serum vitamin D data of both groups Median Test Results and T-Test

Variable	Control (n=29)			Treatment		
	Before	After	P value	Before	After	P value
Vitamin D	23,5 (7,15)	27.38 (SD 7.46)	<0.0001	17.9 (SD 4.38)	36.07 (SD 9.83)	<0.0001

Test T-Test pairing

DISCUSSION

1. Effects of vitamin D supplementation on muscle mass and strength

Based on the results of measuring vitamin D status before and after the study, subjects from both the control and treatment groups experienced an increase in vitamin D status after the survey. Vitamin D insufficiency is defined as serum 25 D levels less than 30ng/ml, and vitamin D deficiency is defined as serum 25 D levels less than 20ng/ml. In this study, all subjects from both the control and treatment groups experienced vitamin D insufficiency and deficiency. Before the study, a total of 5 people from the control group and 23 people from the treatment group had vitamin D deficiency, as well as 24 people from the control group and nine people from the treatment group had vitamin D deficiency. The increase in vitamin D levels in the treatment group was greater than in the control group. Vitamin D status in both groups experienced a significant increase, which can be caused by the Hawthorne Effect, which is a phenomenon when a person changes his behaviour because he feels he is being observed. This can happen because, before the research, each subject was educated through counselling about the importance of vitamin D3 supplementation for the body and what things can help improve it, such as eating foods rich in vitamin D and sunbathing in the morning when the sun has begun to scorch by increasing the area of skin exposed to sunlight.

A randomised controlled study by El Hajj et al. examined the effect of vitamin D supplementation on muscle strength in elderly pre-sarcopenia. The study found a significant impact on arm and leg muscle mass but not on muscle strength. (El Ness, Boiigny, Chardigny, Boirie, & Walrand, 2019) This finding is slightly different from the findings in this study, which saw an increase in muscle strength without an increase in muscle mass in the upper limbs.

The opposite findings were put forward by Bislev et al. in their meta-analysis of RCT studies of vitamin D supplementation by monotherapy in populations of all ages. Bislev et al. found that vitamin D3 supplementation significantly decreased performance on the timed up and go (TUG) test, maximal knee flexion strength, and SPPB test performance. In a subgroup analysis of groups with low vitamin D status, there was also no significant difference in performance between the supplemented group and the placebo group. Bislev concluded that vitamin D3 supplementation can harm muscles, so vitamin D3 supplementation recommendations need to be considered carefully. (Bislev, Grove-Laugesen, & Rejnmark, 2021)

For a study of vitamin D supplementation accompanied by protein supplementation, a study by Bo et al. in 2018 found that supplements high in whey protein, leucine, vitamin D, and vitamin E can maintain muscle mass, muscle strength, and quality of life in older people with sarcopenia. (Bo et al., 2019) The findings of Bo et al.'s study are consistent with the findings of this study, in which vitamin D supplementation slowed the decline in muscle mass. In addition to Bo et al., there are other similar studies examining vitamin D supplementation accompanied by protein supplementation or amino acid in patients with sarcopenia, namely studies by Bauer et al., Rondanelli et al., Lin et al., and Chang et al., with similar findings. (Bauer et al., 2015; Chang & Choo, 2023; Lin, Shih, Chen, & Yeh, 2021; Rondanelli et al., 2016)

Meanwhile, in studies that conducted vitamin D supplementation accompanied by physical exercise programs and protein supplementation, more consistent findings resulted in a significant effect on muscle mass and muscle strength. A systematic review by Cochet et al. in older people with sarcopenia or malnutrition found that vitamin D supplementation therapy accompanied by BCAAs significantly maintained upper and lower leg muscle mass, strength *handgrip*, *gait speed*, *short physical performance battery* and *chair stand test*, where monotherapy with BCAAs or PUFAs without vitamin D3 does not increase muscle mass or strength significantly. (Cochet, Belloni, Buondonno, Chiara, & D'Amelio, 2023) Cochet attributed these findings to mitochondrial activity as well as redox reactions in muscle cells, where BCAAs such as isoleucine and valine play a role in activating the mammalian target of rapamycin (mTOR) network in mitochondria, which ultimately increases resistance to oxidative stress. In contrast, vitamin D plays a role in improving biogenesis as well as mitochondrial function and regulating the expression of genes associated with this process in muscle cells. (Salles et al., 2022)

Thus, to increase muscle mass and muscle performance in older people, vitamin D3 supplementation is recommended, along with other therapies such as resistance training and supplementation with BCAAs.

2. Effects of vitamin D supplementation on fat mass

In addition to significant results on muscle strength and muscle mass, significant findings in this study were also obtained on measurements of the difference in overall fat mass. A significant difference was obtained between the control and treatment groups when measuring the difference in fat mass. Both groups experienced a decrease in overall fat mass, but the treatment group experienced a significantly greater decrease (0.19% vs. 1.25%). This result was obtained even though the difference in visceral and subcutaneous fat mass at all measurement points was not found to be significantly different. These findings indicate a role for vitamin D3 supplementation in lowering overall fat mass.

Existing studies have shown a significant influence between vitamin D status and fat mass, especially in the elderly population and post-menopausal women. A survey by Vázquez-Lorente et al. in post-menopausal women found an impact of vitamin D status on BMI, waist circumference, arm circumference, and fat mass. (Vázquez-Lorente et al., 2020) Pinkas et al., also in postmenopausal women, found a significant association between vitamin D status and BMI and dyslipidemia. (Pinkas et al., 2017) In the elderly population in general, similar findings were also put forward by Araghi et al., Zhao et al., and Vitezova et al. in studies in the elderly population, where vitamin D status was inversely related to fat mass. (Oliai Araghi et al., 2015; Vitezova et al., 2017; Zhao et al., 2021)

Although vitamin D status was found to be associated with fat mass, findings related to the effect of vitamin D3 supplementation on fat mass varied. The study by Chou et al. found no significant impact between vitamin D3 supplementation and body composition in the elderly population. (Chou et al., 2021) A meta-analysis by Golzarand et al. found that 25(OH) D levels were inversely related to fat mass. However, vitamin D3 supplementation did not significantly reduce the percentage of fat mass (- 0.31%, IK95%: - 1.07 - 0.44). (Golzarand, Hollis, Mirmiran, Wagner, & Shab-Bidar, 2018).

In these results, there was an increase in blood serum vitamin D levels in both the control group and the treatment group, both of which had significant or significant results. The limitation of this study was that no analysis was carried out on the amount of sun exposure, food intake in the subjects and physical activity of the subjects so that the increase in the control group could be due to improvements in the behaviour of the subjects as a result of counselling about the importance of vitamin D for health before researching all subjects.

CONCLUSIONS

From the results of research analysis and discussion, we give the following conclusions: The elderly in this study are mostly aged 60-69 years, education levels vary, are Muslim, the majority have comorbid hypertension, and the majority are not in a frail condition. All study subjects experienced an increase in vitamin D levels in blood serum, with the greatest increase in the treatment group influenced by vitamin D3 supplementation. The majority of the study subjects had a nutritional status at risk of malnutrition, according to an assessment of MNA tests. Muscle mass and fat mass of the study subjects were measured in 4 examination points, namely the mass of the whole body, torso, upper extremities, and lower extremities. In contrast, ASMI calculations were still classified as good in all subjects. Grip strength in the treatment group increased with Vitamin D3 supplementation, although delta in both the control and treatment groups there was no significant difference. Vitamin D3 supplementation affects the increase in muscle mass, especially muscle extremity. This is evidenced by the calculation of substantial ASMI changes in the treatment group, and the group given vitamin D3 supplementation experienced a smaller decrease in leg muscle mass.

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