INTRODUCTION

Several factors are involved in the maintenance of activities of daily living (ADL) in older adults (1). Loss of muscle mass is highly prevalent in older adults (2), and represents an impaired state of health with mobility disorders, impaired ability to be well and perform ADL, and loss of independence (3-5). Age-related changes in skeletal muscle strength and physical function, in addition to social costs (6, 7). Muscle strength is an important component in maintaining physical function, mobility and vitality with age (8, 9).

Although there is a growing interest in welfare in ageing societies regarding such conditions, information on sarcopenia and frailty-related QOL in nursing homes, and their relationship with physical function is lacking. Understanding skeletal muscle function and muscle mass is important to provide sufficient rehabilitation and proper care in nursing homes. In our previous study, age-related decreases in skeletal muscle mass and cut-off levels for walking difficulty were calculated using 24-h creatinine excretion as a measure of total-body skeletal muscle mass in the Japanese population, including nursing home residents (10, 11). Furthermore, the serum 25-hydroxyvitamin D [25(OH)D] level was closely related to skeletal muscle mass. This study investigated the age-related changes in muscle strength and physical functions, and their association with vitamin D levels.

MATERIALS AND METHODS

Subjects

Age-related changes in skeletal muscle strength and physical functions, and their association with serum 25(OH)D levels were analyzed in this cross-sectional study. The numbers of male and female subjects in age groups of 21-30 years, 31-50 years, 51-75 years and 76 years or older were 13 and 11, 13 and 11, 5 and 6, and 2 and 1, respectively. Among them, the numbers of male subjects with walking difficulty in nursing homes were 1 and 1 in the 51-75 years group, and 2 and 1 in the 76 years or older group. The other 68 subjects aged between 21-75 years old were healthy registered care workers, physical and occupational therapists in 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.

Assessment of physical performance and serum 25(OH)D levels

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 (12). The BI is a 10-item measure of basic ADL (13). It is used in clinical practice to inform rehabilitation and care planning, and in research to describe outcomes and as a case-mix adjuster (14).

Serum 25(OH)D levels, as an indicator of vitamin D status, were measured by Electro Chemiluminescent Immunoassay...
(ECLIA), as previously reported (11).

**Statistical analysis**

Data were expressed as the mean ± SD of the male and female subjects of different age groups. Spearman's analysis was used for age-related changes in skeletal muscle functions, and association between serum 25(OH)D levels and skeletal muscle functions. The Mann-Whitney U test (nonparametric analysis) was used to assess differences between male and female values in each group, and Steel's multiple comparison test was used to assess differences between the 21-30 year group and the other groups. A P-value less than 0.05 was evaluated as significant.

**Ethical considerations**

The protocol of this project was approved by the institutional review board of Hinomine Medical Center (Komatsushima, Tokushima, Japan). The procedures were fully explained to subjects and an informed consent form was signed.

**RESULTS**

1) Age-related changes in skeletal muscle functions and serum 25(OH)D levels

The skeletal muscle functions, such as the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI, and serum 25(OH)D levels of men and women in different age groups are shown in Table 1. A significant aging-related reduction in all skeletal muscle functions and serum 25(OH)D levels was observed in both men and women.

Comparing these indices with those of the 21-30 year group, the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI values were lower in both men and women in the 76 year or older group, but only the BI was lower in men in the 51-75 year group. Handgrip strength (kg) as muscle torque in the 31-50 year, 51-75 year and 76 year or older groups was 119.3%, 55.1% and 31.4% of that of males in the 21-30 year group, and 98.5%, 92.3% and 36.3% of females in the 21-30 year group, respectively.

On comparison of the values for the TUG test (sec), walking speed (m/sec) and BI in males in the 31-50 year, 51-75 year and 76 year or older groups, the TUG test (sec) was 92.9%, 32.1% and 14.7%, walking speed (m/sec) was 78.2%, 65.2% and 21.7%, handgrip strength (kg) was 119.3%, 55.1% and 31.4%, and BI was 100%, 68.3% and 50% of those of males in the 21-30 year group, respectively. The values in females in the 31-50 year, 51-75 year and 76 year or older groups for the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI were 84.7%, 86.2% and 12.6%, 90.6%, 86.4% and 18.2%, 98.5%, 92.3% and 36.3%, and 100%, 91.0% and 39.0%, respectively, of those of females in the 21-30 year group.

The walking speed and grip strength of males in the 21-30 year and 31-50 year groups were higher than those of women. In contrast, the BI of women in the 51-75 group was higher than those of women.

On comparison of the values for the TUG test (sec), walking speed (m/sec) and BI in males in the 31-50 year, 51-75 year and 76 year or older groups, the TUG test (sec) was 92.9%, 32.1% and 14.7%, walking speed (m/sec) was 78.2%, 65.2% and 21.7%, handgrip strength (kg) was 119.3%, 55.1% and 31.4%, and BI was 100%, 68.3% and 50% of those of males in the 21-30 year group, respectively. The values in females in the 31-50 year, 51-75 year and 76 year or older groups for the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI were 84.7%, 86.2% and 12.6%, 90.6%, 86.4% and 18.2%, 98.5%, 92.3% and 36.3%, and 100%, 91.0% and 39.0%, respectively, of those of females in the 21-30 year group.

The walking speed and grip strength of males in the 21-30 year and 31-50 year groups were higher than those of women. In contrast, the BI of women in the 51-75 group was higher than those of women.

### Table 1. Skeletal muscle function and serum 25-hydroxyvitamin D level

| Age Group     | Male                | Female               |
|---------------|---------------------|----------------------|
|               | 21-30 year group    | 31-50 year group     | 51-75 year group | 76 year~ group | Spearman's analysis | Walking difficulty |
|               | (n = 13)            | (n = 13)             | (n = 8)          | (n = 2)        |                      | (n = 3)            |
| Timed up and go test (sec) | 5.2 ± 1.8           | 5.6 ± 1.7            | 16.2 ± 19.7      | 35.3 ± 21.8     | *                     | p < 0.001          | 34 ± 30.7          |
| (of 21-30 year group) | 100                 | 92.9                 | 32.1             | 14.7           |                       |                    |                   |
| Walking speed (m/sec) | 2.3 ± 0.9           | 1.8 ± 0.8            | 1.5 ± 0.7        | 0.5 ± 0.1       | *                     | p < 0.001          | 0.4 ± 0.1          |
| (of 21-30 year group) | 100                 | 78.2                 | 65.2             | 21.7           |                       |                    |                   |
| Handgrip strength (kg) | 41.0 ± 7.9          | 48.9 ± 8.4           | 22.6 ± 19.0      | 12.9 ± 1.8      | *                     | p < 0.05           | 8.2 ± 7.3          |
| (of 21-30 year group) | 100                 | 119.3                | 55.1             | 31.4           |                       |                    |                   |
| Barthel Index | 100 ± 0             | 100 ± 0              | 68.3 ± 36.7      | 50.0 ± 26.5    | ***                   | p < 0.001          | 45 ± 32.8          |
| (of 21-30 year group) | 100                 | 100                  | 68.3             | 50             |                       |                    |                   |
| Serum 25-hydroxyvitamin D level (ng/ml) | 17.8 ± 3.2          | 18.1 ± 4.4           | 16.8 ± 7.1       | 15.7 ± 9.5     | ns                    | 13.2±8.0           |
| 21-30 year group | (n = 10)            | 31-50 year group     | (n = 11)         | 51-75 year group | (n = 15)             |                      |                    |
| Timed up and go test (sec) | 5.0 ± 0.8           | 5.9 ± 0.9            | 5.8 ± 2.2        | 41.3 ± 20.4    | **                    | p < 0.001          | 54.0 ± 22.4        |
| (of 21-30 year group) | 100                 | 84.7                 | 86.2             | 12.6           |                       |                    |                   |
| Walking speed (m/sec) | 2.20 ± 0.4          | 2.0 ± 0.6            | 1.9 ± 0.5        | 0.4 ± 0.2      | **                    | p < 0.001          | 0.3 ± 0.1          |
| (of 21-30 year group) | 100                 | 90.6                 | 86.4             | 18.2           |                       |                    |                   |
| Handgrip strength (kg) | 27.3 ± 9.4          | 26.9 ± 3.8           | 25.2 ± 6.1       | 9.9 ± 3.2      | ***                   | p < 0.001          | 9.1 ± 5.2          |
| (of 21-30 year group) | 100                 | 98.5                 | 92.3             | 36.3           |                       |                    |                   |
| Barthel index | 100 ± 0             | 100 ± 0              | 91.0 ± 21.1      | 39.0 ± 27.3    | ***                   | p < 0.001          | 34.4 ± 24.9        |
| (of 21-30 year group) | 100                 | 100                  | 91               | 39             |                       |                    |                   |
| Serum 25-hydroxyvitamin D level (ng/ml) | 17.0 ± 4.9          | 15.3 ± 4.3           | 12.9 ± 3.5       | 8.7 ± 2.3      | p < 0.001             | 8.5 ± 2.4          |

* Different from the 21-30 year group (p < 0.05, ** : p < 0.01, *** : p<0.001)

ns : not significant
that of men. Furthermore, the serum 25(OH)D level was closely related to handgrip strength (kg) and BI in men, and with the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI in women.

2) Cut-off values for skeletal muscle functions with walking difficulty

Cut-off values for the TUG test, walking speed, handgrip strength and BI for detecting walking difficulties in the ROC analysis were 11.1 sec, 0.60 m/sec, 17.0 kg and 90.0 in males, and 28.6 sec, 0.43 m/sec, 13.9 kg and 67.5 in females, respectively (Table 2). All 20 subjects with walking difficulty were living in nursing homes, and the rate was 60% (3 in 5) for men and 94.4% (17 in 18) for women.

DISCUSSION

In general, muscle mass and strength increase with growth during childhood and young adulthood, are maintained in midlife and then decrease with age. In young adulthood up to 40 years, maximal levels are observed (15). Beyond the age of 50 years, loss of leg muscle mass and strength was reported to occur at a rate of 1-2% per year and 1.5-5% per year, respectively (16). Frailty is a geriatric syndrome associated with adverse health outcomes such as physical disability, hospitalization and mortality (17). One of the key features of frailty is marked muscle weakness and a decline in functional capabilities (18).

Age-related loss of muscle strength and functions were observed in both men and women in this study. The skeletal muscle functions, skeletal muscle mass (kg, kg/m², %BW) and creatine height index were also closely related to serum 25(OH)D levels in present and previous studies (11). These results are consistent with earlier studies suggesting that a decline in muscle function is strongly correlated with the loss of muscle mass due to aging (19-21). Aging results in a decline in strength, which is primarily attributed to a loss of muscle mass (22-24). Of note, several cross-sectional studies involving adults and younger humans found that sufficient vitamin D levels positively affect muscle strength (25-27). Recent studies demonstrated adipose tissue accumulation around and between muscle fibers concomitant with reductions in the muscle cross-sectional area due to aging, and this skeletal muscle attenuation is inversely associated with muscle performance (28, 29).

Corroborating these findings in the present study, subjects in the 76 year or older group and nursing home residents were more likely to be at risk for physical dependence than those in younger groups. In addition, the loss of strength was much greater than the loss of muscle mass, which suggests a decrease in muscle quality (30, 31). Furthermore, we calculated cut-off values for the TUG test (sec), walking speed (m/sec), handgrip strength (kg) and BI for walking difficulty. In the initial stages of sarcopenia development, an individual may be above the threshold of low physical performance and is likely to be above the threshold of disability. Handgrip strength was reported to be a reliable and cost-effective surrogate of overall muscle strength (32, 33), and is a robust indicator of subsequent functional limitations and future disease status (31, 34, 35).

The Asian Working Group for Sarcopenia (AWGS) 2019 retained the original cut-off values for height-adjusted muscle mass: dual-energy X-ray absorptiometry, < 7.0 kg/m² in men and < 5.4 kg/m² in women; and bioimpedance, < 7.0 kg/m² in men and < 5.7 kg/m² in women, but revised the diagnostic algorithm, protocols and some criteria: low muscle strength is defined as handgrip strength <28 kg for men and <18 kg for women (30). These are intermediate values between the young, healthy situation and old, handicapped situation with walking difficulty. Therefore, it is recommended that the cut-off values for muscle mass and handgrip strength for walking difficulty be kept in mind when aged persons are trained in rehabilitation to avoid physical disability and independence.

Although the cause of this loss in strength and function is multifactorial, serum 25(OH)D levels were significantly associated with physical performance in this study. Previously reported surveys revealed that vitamin D levels are positively correlated with muscle mass, strength and physical performance in older adults (37-40). Handgrip strength has gained attention as a simple, noninvasive marker of muscle strength and function in both epidemiological and clinical studies (41). In addition, several studies reported an association between vitamin D deficiency and reduced handgrip strength (42, 43). Thus, handgrip strength is more effective than muscle mass as a predictor of adverse outcomes such as falls, decreased physical performance and mortality (44-47).

Vitamin D deficiency is globally widespread, particularly in older populations (48-50). Therefore, based on the present study, optimizing the vitamin D status may be a practical and cost-effective approach to support skeletal muscle function, although sufficient intervention study using vitamin D supplementation will be required.

| Table 2. Cut-off values for detecting walking difficulty in ROC analysis |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Male                        | Sensitivity (%)     | Specificity (%)    | Cut-off value | AUC                  |
| Timed up and go test (sec)  | 100.0             | 88.6              | 11.08         | 0.94                  |
| Walking speed (m/sec)       | 88.6              | 100.0             | 0.60          | 0.94                  |
| Handgrip strength (kg)      | 86.5              | 100.0             | 16.95         | 0.93                  |
| Barthel Index               | 84.2              | 100.0             | 90.00         | 0.93                  |
| Female                      | Sensitivity (%)     | Specificity (%)    | Cut-off value | AUC                  |
| Timed up and go test (sec)  | 100.0             | 81.6              | 28.55         | 0.92                  |
| Walking speed (m/sec)       | 81.6              | 100.0             | 0.43          | 0.92                  |
| Handgrip strength (kg)      | 63.5              | 80.0              | 13.85         | 0.79                  |
| Barthel Index               | 61.1              | 88.2              | 67.50         | 0.78                  |

ROC : Receiver operating characteristic, AUC : area under the curve.
CONFLICT OF INTEREST AND ACKNOWLEDGEMENT

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