Relationship between age-related decreases in serum 25-hydroxyvitamin D levels and skeletal muscle mass in Japanese women

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Abstract : A clearer understanding of skeletal muscle mass (SMM) in middle-aged and elderly individuals is important for maintaining functionality. In the present study, age-related changes in SMM, the threshold of SMM with walking difficulty, intestinal nutrient absorption rate, and various serum factors were examined in Japanese populations of different ages. We used 24-h creatinine excretion as a measure of total body SMM. Age-related decreases in SMM, intestinal nutrient absorption rates, and serum 25-hydroxyvitamin D [25(OH)D] concentrations were significantly higher in women than in men. The cut-off values for SMM (kg), its percentage of total body weight (BW), the SMM index [SMMI] (Kg / m²), and creatinine height index (CHI) (%) in elderly individuals with walking difficulty were approximately 8-10 kg, 17-20% of BW, 3.9-4.6 kg / m², and 44%, respectively. Serum 25(OH)D concentrations were closely associated with SMM (kg, % of BW, kg / m²) and CHI (%) as well as the intestinal absorption rates of nitrogen (%) and phosphorus (%) in women, but not in men. The present results demonstrate that vitamin D is an important metabolic factor in skeletal muscle, and contributes to the optimal management of skeletal muscle and the prevention of sarcopenia. J. Med. Invest. 67 : 151-157, February, 2020

Keywords : Skeletal muscle mass, 24-h urinary creatinine method, serum 25-hydroxyvitamin D level, walking difficulty, intestinal absorption rate

INTRODUCTION
The aging of society in developed and developing countries has resulted in an increase in the number of elderly individuals who need human and social support. Older individuals with frailty are at a higher risk of falls and disability as well as nursing home placement and mortality (1). Skeletal muscle is the most abundant tissue in the human body, comprising 40-50% of the body mass, and plays vital roles in locomotion and glucose and energy metabolism (2). Skeletal muscle mass (SMM) has been identified as a reliable marker of frailty and a poor prognosis among the very elderly (3).

Creatine synthesized in the kidney and liver and from the diet is transported into the sarcoplasm against a concentration gradient. Up to 98% of total body creatine is found in skeletal muscle (4). The turnover of skeletal muscle creatine occurs as a result of the non-enzymatic, irreversible conversion of a small proportion of creatine to creatinine, and creatinine is rapidly and fully excreted in the urine. The creatine turnover rate constant for humans is reportedly 1.7%/day (5, 6). Therefore, 24-h creatinine excretion is used as a measure of total body SMM because urinary creatinine appears to be proportional to SMM and a reliable measure of SMM in patients with advanced renal failure, in children and adolescents, in the elderly, and in patients with wasting conditions (7-12).

The loss of SMM is a common issue in the elderly worldwide, with a prevalence ranging between 7% and more than 50% (13). A clearer understanding of SMM in middle-aged and elderly individuals is important for maintaining functionality, providing adequate rehabilitation and proper nursing care, and preventing a bedridden state. Sarcopenia is characterized by the progressive and generalized loss of skeletal mass and strength with a risk of adverse outcomes, such as physical disability and a poor quality of life (14-17). However, a correlation has not yet been found between muscle mass and muscle function (18, 19). Nutrition in SMM also plays an important role (20). In the present study, age-related changes in SMM, the threshold of muscle mass on walking difficulty, the nutrient absorption rate, and serum electrolyte, mineral, and vitamin levels were examined in Japanese populations of various ages.

MATERIALS AND METHODS

Subjects
This was a cross-sectional study that investigated age-related changes in SMM and nutritional factors. The numbers of male and female subjects in the age groups of 21-30 years, 31-50 years, 51-75 years and 76 years and older were 13 and 10, 13 and 11, 8 and 10, 3 and 1, respectively (Tables 1 and 2). Among them, the numbers of male and female subjects with walking difficulty
In nursing homes were 1 and 1 in the age group of 51-75 years and 2 and 16 in the age group of 76 years and older, respectively. These subjects were being cared for mainly by registered care workers and dietitians. Another 68 subjects aged between 21-75 years old were healthy registered care workers and physical and occupational therapists in nursing homes, and teaching and administration staff in Keshokai Gokuen College of Health and Welfare. None of the healthy subjects were engaged in high levels of exercise training or were 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 protocol.

Height and weight measurements were performed with the participants wearing light clothing and no shoes. BMI was calculated as weight (kg) divided by the square of height (m). In clinical laboratory tests, blood samples were collected from subjects who had undertaken a more than 8-hour overnight fast, immediately refrigerated, transported in cold storage to the SRL Laboratory in Tokyo, and analyzed within 24 hours. Serum 25-hydroxyvitamin D [25(OH)D] levels, as an indicator of the vitamin D status, were measured by an electrochemiluminescent immunoassay (ECLIA).

Measurement of SMM
Each subject provided two 24-h urine samples at inclusion on 2 consecutive days. Creatinine excretion was calculated as the mean of the two 24-h urine collections. SMM was calculated from the 24-h urinary creatinine amount based on this equation (SMM (kg) = 21.8×Cr (g / day)) (10). The SMM index (SMMI) was calculated as body weight (BW) (kg) divided by the square of height (m). SMM (% of body weight (kg)) was calculated as weight (%) divided by BW (kg). The creatinine height index (CHI) was calculated from the following formula:
\[ \text{CHI} = \frac{\text{24-h urine creatinine excretion (mg)}}{\text{expected 24-h urine creatinine excretion (23 mg/kg in males and 18 mg/kg in females)}} \times 100. \]

Estimated nitrogen balance was calculated as the difference between total nitrogen intake and total nitrogen output in urine. Nitrogen intake was calculated from nitrogen provided in food for each subject. The following formula was used to calculate the nitrogen balance: 
\[ \text{Nitrogen balance} = \frac{\text{24-h protein uptake}}{6.25} - \frac{\text{24-h nitrogen excretion}}{0.8}. \]

The putative intestinal absorption rate of nitrogen, sodium, calcium, and phosphorus balance were assessed as the urinary excretion rate (%) defined as the amount of urinary excretion divided by the amount of oral food intake.

Statistical analysis
Data were expressed as the mean ± SD of male and female subjects in different age groups. The Kruskal-Wallis test (non-parametric) was used to compare the groups.

### Table 1. General characteristics, skeletal muscle mass, and predicted intestinal nutrient absorption rate in male subjects

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Height (cm)</th>
<th>Body weight (kg)</th>
<th>Body mass index (kg/m²)</th>
<th>Skeletal muscle mass (kg)</th>
<th>Skeletal muscle mass (% of body weight)</th>
<th>Skeletal muscle mass index (kg/m²)</th>
<th>Creatinine height index (%)</th>
<th>Predicted intestinal absorption rate</th>
<th>Serum electrolyte, mineral, and vitamin levels</th>
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<tr>
<td>21-30 yr group (n = 13)</td>
<td>170.8 ± 5.7</td>
<td>69.9 ± 13.6</td>
<td>24.4 ± 5.9</td>
<td>34.5 ± 3.9</td>
<td>50.2 ± 9.0</td>
<td>11.9 ± 1.7</td>
<td>99.9 ± 17.9</td>
<td>Nitrogen (%)</td>
<td>Sodium (mEq/L)</td>
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<td>31-50 yr group (n = 13)</td>
<td>171.7 ± 3.3</td>
<td>68.4 ± 8.7</td>
<td>23.2 ± 3.1</td>
<td>32.3 ± 8.2</td>
<td>47.7 ± 12.7</td>
<td>11.0 ± 2.8</td>
<td>78.1 ± 15.4</td>
<td>Sodium (%)</td>
<td>141.2 ± 1.7</td>
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<td>51-75 yr group (n = 8)</td>
<td>163.5 ± 7.9</td>
<td>64.9 ± 8.0</td>
<td>24.2 ± 1.5</td>
<td>29.1 ± 9.3</td>
<td>44.8 ± 13.8</td>
<td>10.9 ± 3.7</td>
<td>97.0 ± 29.3</td>
<td>Calcium (%)</td>
<td>4.2 ± 0.2</td>
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<td>≥ 76 yr group (n = 2)</td>
<td>149.5 ± 0.7</td>
<td>43.2 ± 7.1</td>
<td>19.4 ± 3.0</td>
<td>7.8 ± 3.1</td>
<td>17.5 ± 4.2</td>
<td>22.9 ± 10.2</td>
<td>89.3 ± 25.1</td>
<td>Phosphorus (%)</td>
<td>103.6 ± 1.4</td>
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<td>3.7 ± 0.4</td>
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<td>6.0 ± 1.5</td>
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ns: not significant
metric method) was used to assess the significance of differences between age groups in male and female subjects. Pearson’s analysis was used to examine the significance of differences between serum 25(OH)D levels and various values for SMM and the intestinal nutrition absorption rate. A p value less than 0.05 was considered to indicate significance.

**Ethical considerations**

The protocol of this project was approved by the Institutional Review Board of the 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 SMM

The general characteristics of the different age groups in male and female subjects were shown in Tables 1 and 2, respectively. Significant age-related decreases in height, SMM (kg), the calcium absorption rate (%), and serum calcium levels were observed in men. In contrast, more significant age-related decreases in BW, height, SMM (kg, % of BW, kg/m²), CHI (%), serum sodium, calcium, phosphorus, and zinc levels, and the intestinal absorption rates of nitrogen (%), sodium (%), calcium (%), and phosphorus (%) were observed in women.

The values for SMM (kg, % of BW, kg/m²) and CHI (%) in the 21-30 year group were 34.5 ± 3.9 kg, 50.2 ± 9.0% of BW, 11.9 ± 1.7 kg/m², and 100.0 ± 10.0% in males, and 25.3 ± 6.2 kg, 47.5 ± 9.0% of BW, 10.1 ± 2.7 kg/m², and 116.2 ± 23.2% in females, respectively. In comparisons with SMM (kg, % of BW, kg/m²) and CHI (%) in the 21-30 year group, those in males were 93.6, 95, 92.4, and 97.1% in the 31-50 year group, 84.3, 89.2, 90.6, and 85.3% in the 51-75 year group, and 22.6, 34.8, 29.4, and 33.8% in the 76 years and older group, while those in females were 74.3, 73.3, 75.2, and 74.0% in the 31-50 year group, 77.1, 65.9, 76.2, and 65.8% in the 51-75 year group, and 31.2, 36.2, 37.6, and 36.1% in the 76 years and older group, respectively.

Thus, a more rapid decrease in SMM after the age of 31 years was observed in women, whereas a marked reduction in SMM was noted after the age of 76 years in men. Furthermore, age-related reductions in serum 25(OH)D concentrations were observed in women, but not in men.

2) Cut-off values for SMM with walking difficulty

Cut-off values for SMM (kg), % of BW, kg/m², and CHI (%) in male and female subjects with walking difficulty were 10.8 ± 5.8 and 8.1 ± 3.3 kg, 20.8 ± 6.5 and 17.5 ± 6.3% of BW, 4.6 ± 2.2 and 3.9 ± 1.7 kg/m², and 44.3 ± 9.8 and 44.6 ± 16.1%, respectively. SMM (kg), % of BW, kg/m², and CHI (%) were approximately...
differences were noted among men in the different age groups. SMM in those with hypovitaminosis. However, no significant differences were observed in SMM based on the 25(OH)D status and phosphorus (%) in women, but not in men. A significant difference was observed in SMM based on the 25(OH)D status in women regardless of age, suggesting a lower mean value for SMM in those with hypovitaminosis. However, no significant differences were noted among men in the different age groups.

3) Relationships between the vitamin D status, SMM, and intestinal nutrient absorption rates

The factors associated with differences in the 25(OH)D status with gender are shown in Table 3. Serum 25(OH)D concentrations were closely associated with SMM (kg, % of BW, kg/m²), CHI (%), and the intestinal absorption rates of nitrogen (%) and phosphorus (%) in women, but not in men. A significant difference was observed in SMM based on the 25(OH)D status in women regardless of age, suggesting a lower mean value for SMM in those with hypovitaminosis. However, no significant differences were noted among men in the different age groups.

### Table 3. Index associated with serum 25-hydroxyvitamin D levels by Pearson’s analysis

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle mass (kg)</td>
<td>0.23</td>
<td>ns</td>
</tr>
<tr>
<td>Skeletal muscle mass index (kg/m²)</td>
<td>0.15</td>
<td>ns</td>
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<tr>
<td>Skeletal muscle mass (%) of body weight</td>
<td>0.16</td>
<td>ns</td>
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<tr>
<td>Creatinine height index (%)</td>
<td>0.14</td>
<td>ns</td>
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<tr>
<td>Predicted intestinal absorption rate</td>
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<td></td>
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<tr>
<td>Nitrogen (%)</td>
<td>0.09</td>
<td>ns</td>
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<tr>
<td>Sodium (%)</td>
<td>-0.19</td>
<td>ns</td>
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<tr>
<td>Calcium (%)</td>
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<td>Phosphorus (%)</td>
<td>0.11</td>
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ns: not significant

DISCUSSION

The recommended cut-off values for SMM diagnosed as sarcopenia were obtained using a bioelectric impedance analysis (BIA) and dual-energy X-ray absorptiometry lean mass (DXA) in Asian and European populations (13, 21). BIA measures body conductivity or resistance to a small electrical current through the body or across a limb. Resistance is strongly related to total body water and is used to assess lean body mass. Thus, BIA does not directly measure skeletal muscle mass (22). DXA estimates body composition using a three compartment model. Fat and bone mineral contents are directly measured through the body or across a limb. Resistance is strongly related to total body water and is used to assess lean body mass. Thus, BIA does not directly measure skeletal muscle mass (22). DXA estimates body composition using a three compartment model. Fat and bone mineral contents are directly measured through the differential absorption of two photon energies, and then by subtraction, lean mass is obtained (23). Soft-tissue lean mass includes muscle mass, water, organ weight, and all other non-bone and non-fat soft tissue. Therefore, DXA does not measure muscle mass specifically and does not provide an accurate measurement of SMM in practice. In addition, computed tomography (CT) and magnetic resonance imaging (MRI) measurements of the cross-sectional area or whole body skeletal muscle mass are precise and applicable in small studies; however, due to very high costs and radiation exposure (for CT), they are impractical for field studies.

The use of creatinine excretion provides a direct assessment of the whole body creatine pool size and, as a result, of muscle mass. This method has been used as an estimate of muscle mass (7-13, 24, 25). CHI is another valid and very simple tool for assessing the protein status in patients with various diseases, including malnutrition. In the present study, CHI in the 21-30 year male and female groups was approximately 100-120%. Furthermore, the intestinal sodium absorption rate was constantly 80-110% in both males and females of different ages; therefore, the measurement of SMM using the amount of creatinine in 24-h urine collection may reflect total body creatine and, consequently, total SMM.

The present study also showed that reduced SMM was positively associated with a vitamin D deficiency in women. Previous findings indicated that the vitamin D receptor (VDR) for 1,25-dihydroxyvitamin D (1,25(OH)₂D) was expressed in skeletal muscle and was a crucial mediator of 1,25(OH)₂D, thereby affecting muscle contractility (26). Age-related reductions in the expression of VDR in both muscle and bone are one feature suggesting that a decline in the vitamin D status plays an integral role in the functional link between sarcopenia and osteoporosis (27). 25(OH)D levels are generally accepted to decrease longitudinally with age (28). This is attributed to both a decrease in the ability of cholecalciferol in the skin to make 25(OH)D and a decline in vitamin D absorption, as well as reduced sun exposure and the use of sunblock (29).

There is a general consensus that serum 25(OH)D concentrations of < 20 ng/mL, 20 to < 30 ng/mL, and ≥ 30 ng/mL indicate a vitamin D deficiency, vitamin D insufficiency, and vitamin D sufficiency, respectively (30). Serum 25(OH)D concentrations in Japanese individuals aged 83.8 ± 7.6 and 85.4 ± 8.5 years were previously reported to be 9.7 ± 1.8 and 9.7 ± 3.7 ng/mL, respectively (31). In another study that examined institutionalized elderly patients from Buenos Aires, 41% of residents had a serum 25(OH)D level of less than 25 nmol/L (10 ng/mL) (32). In a study of 1585 osteoporotic Japanese women aged 70-95 years, a multivariate linear regression analysis indicated that a low serum 25(OH)D level (< 20 ng/mL) was an independent determinant of the total QOL score quartile (33). Their mean serum 25(OH)D level was 23.7 ng/mL. The numbers of subject with serum 25(OH)D levels of < 20 ng/mL, 20 to 30 ng/mL, and ≥ 30 ng/mL were 376 (23.7%), 982 (63.0%), and 227 (14.3%), respectively. Lower serum 25(OH)D levels were associated with a lower QOL score, and this relationship was clearly observed in patients with levels < 20 ng/mL.

Previous studies reported an inconsistent relationship between vitamin D and muscle mass in different sexes and age groups (34-40). Serum 25(OH)D levels in 40 healthy Japanese volunteers aged 42.0 ± 10.6 years old were 15.2 ± 5.4 ng/mL. This level was identified as a significant positive predictor for both the appendicular skeletal muscle index (kg/m²) and lower limb muscle strength, and female subjects had significantly lower serum 25(OH)D levels than males (41). The prevalence of a vitamin D sufficiency (plasma 25(OH)D concentration ≥ 30 ng/mL) was extremely low (9.1%) in 9084 Japanese adults, and male gender was identified as a significant positive predictor (42). Thus, female gender is a significant risk factor for both a vitamin D deficiency and insufficiency. The higher frequency of sunscreen use in females may decrease serum 25(OH)D concentrations (43) because lower sun exposure has been identified as a significant contributor to a vitamin D deficiency. In (44), 1,25(OH)₂D receptor-mediated signaling is age-dependently impaired in intestinal cells. The relationship between duodenal TRPV6 expression and 1,25(OH)₂D was previously reported to differ in men and women (45). A linear regression showed a strong relationship with 1,25(OH)₂D, which was unaffected by age in men. In contrast, there was no significant overall relationship with 1,25(OH)₂D, whereas a significant decrease was observed with age in women. The effects of age with lower median VDR levels were more significant in women than in men. These findings may explain the age-related reduction in serum 25(OH)D levels, intestinal nutrient absorption, including calcium, and SMM.
predominantly observed in women.

The present study indicated that 87% (20 out of 23 people) of participants living in nursing homes had walking difficulty because their serum 25(OH)D levels were extremely low. The results obtained support the hypothesis that muscle mass is strongly associated with the vitamin D nutritional status, particularly in women. These 20 residents with less 25(OH)D had lower serum 25(OH)D levels than those with normal muscle mass (51). Among diet-related factors, a low vitamin D level has been shown to influence declined muscle function and increase the risk of falling in the elderly (52, 53).

The present study had several limitations. The number of subjects recruited, particularly in the 76 years and older male group, was small. This may reflect the different p values between male and female subjects. Furthermore, the lower serum 25(OH)D concentrations in elderly subjects with a sufficient food intake may be explained by lower sun exposure in nursing homes. In addition, intestinal nutrient absorption rates obtained from these urinary excretion rates may need to be calculated from their amounts in stool samples. However, the present results suggest that vitamin D is a key metabolic regulator in muscle cells that will contribute to the prevention and optimal management of sarcopenia.

CONFLICTS OF INTEREST AND ACKNOWLEDGMENTS

The present study was financially supported by Meiji Co., Ltd. and the Food Science Institute Foundation (Ryushokuhou-kenukyu-kai) and is one of the cooperating programs in the Keshokai Group promoting welfare society. We express special thanks to all volunteers and nursing homes (Egao, Heart, Shoenburn) in the Keshokai Group for their kind support of this study.

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