Association between skeletal muscle mass and insulin secretion in patients with type 2 diabetes mellitus

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Abstract. Recent research has indicated a relationship between skeletal muscle mass and insulin resistance in patients with type 2 diabetes mellitus (T2DM). However, no study has examined the relationship between skeletal muscle mass and insulin secretion in patients with Japanese T2DM. This study aimed to fill this research gap by investigating the relationship between skeletal muscle mass and clinical parameters of T2DM with special reference to the effect of sex or age on the relationship. We examined 138 consecutive T2DM patients who presented at a single center. Anthropomorphic measurement was conducted and skeletal muscle mass was determined by bioelectrical impedance analysis for calculating skeletal muscle index (SMI) as the ratio of appendicular muscle mass (AMM) to total body weight. Fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) were levels, and values of stimulated C-peptide immunoreactivity (CPR) were determined by glucagon stimulation testing. Statistical analysis showed that AMM was negatively correlated with age in T2DM patients, whereas SMI had no correlation with either FPG or HbA1c levels. On the other hand, SMI was found to be negatively correlated with the log-transformed stimulated CPR values in male patients <65 years (\(r = –0.40, p < 0.05\)) and in female patients <65 years (\(r = –0.40, p < 0.05\)). The results of multivariate analysis suggest a strong association between the log-transformed stimulated CPR value and SMI. These findings indicate that increased endogenous insulin secretion is associated with lower skeletal muscle mass in T2DM patients who are <65 years of age.

Keywords: Skeletal muscle mass, Diabetes mellitus, Insulin secretion, Glucagon stimulation test

THE NUMBER of individuals diagnosed with type 2 diabetes mellitus (T2DM) continues to increase globally. The current estimate that 371 million individuals worldwide, including 7.1 million in Japan [1], have T2DM is expected to increase to 552 million by 2030 [2, 3]. Thus, prevention and treatment of diabetes will remain important issues for the foreseeable future. With the continued aging of populations worldwide, another critical issue is age-related reduction in muscle mass, the predominant change in body composition experienced by elderly populations. Defined as sarcopenia by Rosenberg in 1989 [4], this progressive loss of skeletal muscle mass has been related to physiological dysfunction, and thus to a greater risk of functional impairment and decrease in quality of life.

Many recent studies have examined the relationship between sarcopenia and diabetes. In a study of 1,840 adult subjects aged 70 to 79 years that measured leg and arm muscle mass and strength at baseline and 3 years later, those with T2DM showed greater declines in muscle mass and strength compared with adults without T2DM [5], suggesting that muscle quality declines more rapidly in older adults with T2DM. In a South Korean study of 810 subjects that compared the body composition parameters of subjects with and without T2DM [6], the skeletal muscle mass index of patients with T2DM significantly decreased compared with subjects without T2DM, suggesting that T2DM is associated with increased risk of sarcopenia. Furthermore, a National Health and Nutrition Examination Survey (NHANES) reported that sarcopenia was strongly associated with insulin resistance and higher HbA1c
levels in individuals 60 years or older, as well as with adverse glucose metabolism, regardless of obesity, in all individuals, and this association was the strongest in individuals under 60 years of age [7]. In a study of Japanese women, the HbA1c level in those with coexisting sarcopenia and metabolic syndrome was found to be higher than that in healthy controls or those with sarcopenia alone, suggesting that a high HbA1c level increases the risk of cardiovascular disease [8].

As the mean muscle mass of populations is known to differ by race, it is important to examine the relationship between skeletal muscle mass and clinical parameters in patients with T2DM within all national populations. However, to our knowledge, no study has examined the relationship between skeletal muscle mass and clinical parameters in T2DM patients in Japan. To fill this research gap, the present study investigated the relationship between skeletal muscle mass and parameters of T2DM and examined whether this relationship varied by sex or age in T2DM patients who presented for treatment at one hospital in Japan.

Materials and Methods

Patients

The study included consecutive 138 patients (86 male and 52 female) who had been admitted to the Osaka Medical College Hospital for treatment of T2DM between March 2011 and July 2013. None of the patients had any of the following: (1) detection of anti-glutamic acid decarboxylase antibodies, (2) history of gastrectomy, (3) use of a cardiac pacemaker and/or implantable cardiac defibrillator, (4) use of steroid hormones, (5) renal insufficiency (macroalbuminuria or an estimated glomerular filtration rate (eGFR) <60 mL/min • 1.73 m²), or (6) cachexia. Patients were treated with no medication (n = 13, 9.4%), an oral hypoglycemic agent (OHA; n = 58, 42.0%), insulin (n = 32, 23.2%), or OHA + insulin (n = 35, 25.4%). Approval to conduct this study was obtained by the Osaka Medical College Ethics Committee, and written informed consent was provided by all patients.

Anthropometric measurements

Body height and weight were measured while standing for calculation of body mass index (BMI) as body weight divided by height squared (kg/m²). Maximum waist circumference at the umbilical level was measured in the late exhalation phase while standing. Muscle mass was measured on the day following hospitalization before lunch and after urination by bioelectrical impedance analysis (BIA) using the Body Composition Analyzer MC-190 (Tanita Corp., Tokyo, Japan) [9]. The muscle mass data were used to calculate appendicular muscle mass (AMM) as the sum of the muscle mass of the arms and the legs and the skeletal muscle index (SMI) as the ratio of AMM to total body weight expressed as a percentage value [10, 11].

Laboratory measurements

Blood samples were drawn in the morning after an overnight fast for measurement of levels of fasting plasma glucose (FPG) by hexokinase enzymatic analysis, serum insulin (IRI) and serum C-peptide immunoreactivity (CPR) by chemiluminescence enzyme immunoassay, and glycosylated hemoglobin (HbA1c) by high-performance liquid chromatography. The value for HbA1c was converted from the Japan Diabetes Society (JDS) value to the equivalent National Glycohemoglobin Standardization Program (NGSP) value using the formula HbA1c (NGSP) = HbA1c (JDS) + 0.4% [12]. The homeostasis model assessment of insulin resistance (HOMA-IR) value was calculated using the following formula: HOMA-IR = FPG (mg/dL) × fasting IRI (μU/mL)/405. After the patients had fasted overnight, glucagon stimulation testing was performed by collection of blood 6 min subsequent to intravenous injection with 1 mg of glucagon (Novo Nordisk, Bagsvaerd, Denmark) for measuring plasma CPR level and determining stimulated CPR value [13, 14]. Those taking sulfonylureas were asked not to take these medications for 12 h before glucagon testing. Administration of insulin and OHAs was also withheld 12 h before glucagon testing. Insulin and OHAs were withheld before the glucagon test that day. Beta-cell function was subsequently evaluated by measurement of stimulated CPR values.

Statistical analysis

All measurements and calculated values are expressed as mean ± SD values. Male and female patients were compared using the Student’s t-test. The patients were compared by age by dividing all patients into 2 groups, those aged <65 years and those aged ≥65 years, for comparison using the Student’s t-test. The stimulated CPR values were log-transformed before further analysis into normally distributed values. The relationship between AMM and age and that between SMI and sev-
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dL and 9.9%, respectively, in male and 177 mg/dL and
9.9%, respectively, in female patients. The stimulated
CPR values in men were significantly higher than those
in women. The stimulated CPR values of male and
female patients aged <65 years were found to be sig-
nificantly higher than those of male and female patients
aged ≥65 years. AMM was found to be negatively cor-
related with age in both male and female patients (p <
0.01; Fig. 1). The correlation between SMI and the clin-
ical parameters of T2DM are shown in Tables 2 and 3.
In patients aged <65 years, SMI was found to be signif-
icantly negatively correlated with the log-transformed
stimulated CPR value in both men (r = –0.40, p < 0.05)
and women (r = –0.40, p < 0.05). In patients aged ≥65
years, SMI was not significantly correlated with any
of the clinical parameters of T2DM in either male or
female patients.

Results

The anthropometrical and clinical characteristics
of all the patients are shown in Table 1. As can be
observed, the mean anthropometric values for male
patients (mean age, 62.9 years) were found to be sig-
nificantly higher than those for female patients (mean
age, 61.6 years), except for BMI and waist circumfer-
ence. The mean FPG and HbA1c levels were 177 mg/

Table 1  Anthropometrical and clinical characteristics of all patients

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male patients</th>
<th></th>
<th>Female patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;65</td>
<td>≥65</td>
<td>Total</td>
<td>&lt;65</td>
</tr>
<tr>
<td>N</td>
<td>37</td>
<td>49</td>
<td>86</td>
<td>29</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.0 ± 9.9†</td>
<td>71.1 ± 4.4</td>
<td>62.9 ± 12.0</td>
<td>54.7 ± 8.6‡</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.0 ± 5.6†</td>
<td>165.0 ± 6.5</td>
<td>166.7 ± 6.4*</td>
<td>156.7 ± 5.2‡</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.9 ± 14.5†</td>
<td>66.8 ± 9.2</td>
<td>70.3 ± 12.4*</td>
<td>62.4 ± 15.9‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 4.0</td>
<td>24.6 ± 3.4</td>
<td>25.2 ± 3.7</td>
<td>26.1 ± 5.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.5 ± 11.1</td>
<td>92.6 ± 9.0</td>
<td>93.0 ± 9.9</td>
<td>91.1 ± 15.5</td>
</tr>
<tr>
<td>AMM (kg)</td>
<td>25.4 ± 4.0†</td>
<td>21.5 ± 3.2</td>
<td>23.2 ± 4.1*</td>
<td>17.0 ± 2.9‡</td>
</tr>
<tr>
<td>SMI (%)</td>
<td>34.2 ± 2.8†</td>
<td>32.3 ± 3.0</td>
<td>33.1 ± 3.1*</td>
<td>27.0 ± 3.1</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>172 ± 44</td>
<td>181 ± 54</td>
<td>177 ± 50</td>
<td>179 ± 54</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.8 ± 1.7</td>
<td>9.9 ± 1.7</td>
<td>9.9 ± 1.7</td>
<td>10.0 ± 1.4</td>
</tr>
<tr>
<td>HOMA-R valuea</td>
<td>4.0 ± 1.9</td>
<td>4.8 ± 5.9</td>
<td>4.4 ± 4.5</td>
<td>5.0 ± 3.1</td>
</tr>
<tr>
<td>Stimulated CPR (ng/mL)</td>
<td>5.6 ± 2.0†</td>
<td>4.4 ± 2.2</td>
<td>4.9 ± 2.2*</td>
<td>4.8 ± 2.4‡</td>
</tr>
<tr>
<td>No medication</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>OHA</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td>insulin</td>
<td>10</td>
<td>13</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>OHA+insulin</td>
<td>9</td>
<td>15</td>
<td>24</td>
<td>6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD values.
BMI, body mass index; FPG, fasting plasma glucose; HOMA-R, homeostasis model assessment of insulin resistance; CPR, C-peptide im-
munoreactivity; AMM, appendicular muscle mass; SMI, skeletal muscle index (AMM/weight × 100); OHA, oral hypoglycemic agent
a Subjects with insulin administration were excluded. *, Statistically significant (p < 0.05) difference between all male and female pa-
tients; †, Statistically significant (p < 0.05) difference between men < 65 years and men ≥65 years; ‡, Statistically significant (p < 0.05)
difference between women < 65 years and women ≥ 65 years
**Table 2** Correlation between skeletal muscle index and clinical parameters of diabetes mellitus in male patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age &lt; 65 years</th>
<th>Age ≥ 65 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 37)</td>
<td>(n = 49)</td>
<td>(n = 86)</td>
</tr>
<tr>
<td>FPG</td>
<td>0.09</td>
<td>−0.16</td>
<td>−0.09</td>
</tr>
<tr>
<td>HbA1c</td>
<td>−0.10</td>
<td>0.02</td>
<td>−0.05</td>
</tr>
<tr>
<td>Log stimulated CPR</td>
<td>−0.40*</td>
<td>−0.24</td>
<td>−0.17</td>
</tr>
</tbody>
</table>

Data are expressed as values of correlation coefficients (r). Correlation coefficients (r) and p-values were calculated using Pearson’s correlation analysis. *p < 0.05

FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; CPR, C-peptide immunoreactivity

**Table 3** Correlations between skeletal muscle index and clinical parameters of diabetes mellitus in female patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age &lt; 65 years</th>
<th>Age ≥ 65 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 29)</td>
<td>(n = 23)</td>
<td>(n = 52)</td>
</tr>
<tr>
<td>FPG</td>
<td>−0.01</td>
<td>−0.09</td>
<td>−0.05</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.22</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>Log stimulated CPR</td>
<td>−0.40*</td>
<td>−0.19</td>
<td>−0.31*</td>
</tr>
</tbody>
</table>

Data are expressed as values of correlation coefficients (r). Correlation coefficients (r) and p-values were calculated using Pearson’s correlation analysis. *p < 0.05

FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; CPR, C-peptide immunoreactivity

**Fig. 1** Correlations between age and appendicular muscle mass (AMM) in the study patients. A, male patients (n = 86, $r = −0.61$, $p < 0.001$); B, female patients (n = 52, $r = −0.56$, $p < 0.001$).
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Immunoreactive insulin (IRI) levels, is a simple and useful method for evaluating insulin resistance. In the current study, no significant differences in HOMA-R values were found among the patients by age or sex. However, the values obtained using the HOMA-R could not be used in the present study because daily injection of insulin had affected the serum IRI levels of 67 of the 138 patients.

In the NHANES III, a study of 14,528 subjects aged over 20 years, sarcopenia, defined as an SMI at least 2 standard deviations below that of the mean young adult value as measured by BIA, was found to be associated with dysglycemia based on HbA1c levels only in obese (BMI >30 kg/m²) individuals [7]. In a study of 1,488 Japanese aged 18 to 85 years that defined sarcopenia as an SMI at least 1 standard deviation below that of the sex-specific mean young adult valued as measured by DXA, the HbA1c levels of men with sarcopenia were found to be significantly higher than those in men without sarcopenia [17]. However, neither FPG nor HbA1c levels were found to be correlated with SMI in the current study. The mean FPG and HbA1c levels of the patients with T2DM in this study were almost 180 mg/dL and 10%, respectively, values much higher than those reported in a study in the United States (US) (HbA1c <6.1%) and a Japanese study (mean HbA1c 5.6%). These findings indicate that poor glycemic control over a certain period may affect skeletal muscle mass, a hypothesis that can only be further examined by comparing the skeletal muscle mass of patients who have experienced varying levels of glycemic control over time.

T2DM is characterized by both insulin resistance and β-cell dysfunction. A community-based study in

<table>
<thead>
<tr>
<th>Partial regression coefficient (B)</th>
<th>Standardized partial regression coefficient (β)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-transformed stimulated CPR value</td>
<td>SMI (AMM/weight×100)</td>
<td>−0.021</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>−0.217</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>−0.007</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>FPG</td>
<td>−0.001</td>
</tr>
<tr>
<td></td>
<td>HbA1c</td>
<td>0.006</td>
</tr>
</tbody>
</table>

CPR, C-peptide immunoreactivity; SMI, skeletal muscle index; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin

Discussion

In this investigation of the factors associated with skeletal muscle mass in Japanese T2DM patients, SMI was found to be negatively correlated with stimulated CPR values in both male and female patients <65 years, suggesting that low skeletal muscle mass is associated with increased endogenous insulin secretion. In a previous anthropometric investigation of 4,003 Japanese community-dwelling subjects >18 years, the average height and weight of men (mean age, 63 years) were 167.0 ± 6.3 cm and 67.1 ± 9.2 kg, respectively, and those of women (mean age, 62 years) were 153.7 ± 5.3 cm and 52.3 ± 7.6 kg, respectively [15]. While similar mean values for height were obtained in the studies, the values obtained for weight were higher in the present study, indicating that many of the patients with T2DM in this study were overweight.

A notable aspect of this study was its evaluation of not only FPG and HbA1c levels but also stimulated CPR values as clinical parameters of T2DM. As stimulated CPR value is determined after intravenous injection of glucagon, a direct and potent stimulus to islet β-cells, it is also a useful indicator for evaluation of insulin secretion and β-cell function [16]. The homeostasis model assessment of insulin resistance (HOMA-IR), another parameter in diabetes calculated using FPG and serum immunoreactive insulin (IRI) levels, is a simple and useful method for evaluating insulin resistance. In the current study, no significant differences in HOMA-R values were found among the patients by age or sex. However, the values obtained using the HOMA-R could not be used in the present study because daily injection of insulin had affected the serum IRI levels of 67 of the 138 patients.
the US found that a 10% increase in SMI was associated with an 11% relative reduction in HOMA-IR [18], suggesting that increased muscle mass is associated with decreased insulin resistance. In the current study, which, to our knowledge, was the first study of the relationship between skeletal muscle mass and β-cell function, a significant negative relationship was found between skeletal muscle mass and stimulated CPR values in patients <65 years, suggesting that increase in skeletal muscle mass reduces insulin secretion while it improves insulin resistance. These data might indicate that increase in skeletal muscle ameliorates insulin resistance, resulting in the relief of insulin oversecretion.

The results of one-way analysis of variance (ANOVA) with the Kruskal–Wallis test revealed differences in SMI based on insulin/OHA use only in male patients aged ≥65 years. Games–Howell analysis subsequently performed to examine differences in SMI based on insulin/OHA use in male patients aged ≥65 years revealed that the mean SMI values of the insulin and the OHA + insulin groups were significantly higher than those of the OHA group (no medication group: 31.4 ± 2.4%, OHA group: 30.6 ± 2.4%, insulin group: 32.9 ± 1.8%, OHA + insulin group: 33.9 ± 3.8%; *p* < 0.05). These results suggest that the use of exogenous insulin affects muscle mass in elderly patients.

This study faced several limitations that must be considered when reviewing the results. First, the use of cross-sectional design did not allow for consideration of disease duration or determination of the change in skeletal muscle mass over time. To address these limitations, we are currently performing a longitudinal study to explore the changes in both skeletal muscle mass and T2DM parameters in Japanese patients over time. Second, only a small number of patients were examined. As skeletal muscle mass can vary greatly among individuals of different ages, a greater number of both male and female patients with T2DM should be examined in future studies.

In conclusion, the findings of the present study provide evidence that muscle mass is negatively associated with insulin secretion in Japanese patients with T2DM who are <65 years of age.

**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

**References**


