Methods

The present cross-sectional study examined the reduction of muscle mass of patients with or without hip fracture. Between June 2002 and January 2009, all patients with a fresh hip fracture who were at least 55 years-of-age and admitted to a single study institution in Japan were eligible and assigned to the hip fracture group (HF group). Exclusion criteria were the refusal to give informed consent or inability to carry out whole-body dual energy X-ray absorptiometry (DXA) within 48 h of admission. DXA evaluation, not only of the lumbar spine or hip regions, but also of the whole body, was routinely applied for the diagnosis of osteoporosis at the study institution. Patients who visited the outpatient clinic of the study institution and received DXA during the same period were assigned to the non-fracture group (NF group) if they had no previous history of hip fracture. The study protocol was approved by the Institutional Review Board of the National Center for Geriatrics and Gerontology, and all patients gave detailed written informed consent.

During the study period, 422 acute hip fracture patients aged 55 years and older were admitted to the study institution. Of these patients, 34 (8.1%) were excluded because there was no time to carry out DXA preoperatively due to the need for urgent surgical repair, and 31 (7.3%) were excluded because they or their family were unable to give informed consent. The final study population of 357 participants for the HF group (304 females, 82.7 ± 9.3 years and 53 males, 80.3 ± 9.4 years) did not differ significantly from the non-participants with regard to age, sex, height or bodyweight. During the same period, 2816 consecutive patients aged over 55 years without a history of hip fracture visited the outpatient clinic of the same study institution to check their bone mineral density (BMD) by DXA. Of these patients, 305 were excluded from the study because they received only lumbar or hip DXA and lacked the data of whole-body DXA. Finally, 2511 patients (1893 females, 70.5 ± 11.1 years and 618 males, 67.5 ± 11.6 years) received whole-body DXA and were assigned to the NF group.

Body composition was measured by whole-body DXA (DPX-NT; GE Medical Systems Lunar, Madison, WI, USA). Bone mineral content, fat mass and lean soft-tissue mass were measured separately for each part of the body, including the arms and legs. The lean soft-tissue masses of the arms and legs were nearly equal to the skeletal muscle mass. As absolute muscle mass correlates with height, the skeletal muscle mass index was calculated by the following formula: lean mass (kg) / height^2 (m^2), which is directly analogous to body mass index (BMI; weight [kg]/height^2 [m^2]). Arm skeletal muscle mass index (arm SMI) was defined as (arm lean mass [kg]/height^2 [m^2]). Leg skeletal muscle mass index (leg SMI) was defined as (leg lean mass [kg]/height^2 [m^2]). Appendicular skeletal muscle mass index (appendicular SMI) was defined as the sum of the arm SMI and the leg SMI.[16] Appendicular SMI is commonly used to assess muscle mass in various sarcopenic studies.[9] Sarcopenia was defined according to the criteria for the Japanese based on the report by Sanada *et al.* [7] The value of the Japanese criterion was calculated as below two standard deviations (SD) of the mean appendicular SMI of 569 Japanese healthy volunteers whose ages ranged from 18 to 40 years. The criterion value was an appendicular SMI below 5.46 kg/m^2 in women and below 6.87 kg/m^2 in men. We simultaneously measured the BMD of the whole body, including the lumbar spine, by DXA for all of the participants in the present study.

We used Mann–Whitney's *U*-test to estimate the patient characteristics between the study groups. To compare the prevalence of sarcopenia, we carried out χ^2-test and Fisher's exact test. To find a significant relationship between appendicular SMI and BMD, Pearson's correlation was carried out in each group. We evaluated the appendicular SMI value on continuous variables by using a general linear model to control with covariates as age and sex.[18] The general linear model is a generalization of multiple linear regression model to the case of more than one dependent variable.[19,20] Prevalence of sarcopenia was calculated according to four age groups (age less than 70 years, between 70 years to 74 years, between 75 years to 80 years, more than 80 years). The Mantel–Haenszel method was used for testing significance for age–sex adjusted prevalence of sarcopenia.

To determine the presence of the sarcopenia as an independent variable in predicting the occurrence of hip fracture selected as dependent variables, we used the stepwise multiple logistic regression model. The regression model also included the patient characteristics of age, sex, whole body BMD, weight and height, which were known to be the key predictors for skeletal muscle mass.[18] The strength of association of the chosen variables and the occurrence of hip fracture was reported as the odds ratio (OR) and 95% confidential intervals (CI) in relation to a reference group.

Statistical analyses were carried out using SPSS for Windows software (version 19.0; SPSS, Chicago, IL, USA). A *P*-value of <0.05 was considered significant.

Results

Table 1 shows the characteristics, body composition and skeletal muscle mass index of the patients included in the present study. All the patients (both men and women) in the HF group were older adults. The height, weight and BMI were significantly lower in the HF

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Table 1 Characteristics of participants, body composition and skeletal muscle mass index in both males and females

Characteristics	Females			Males		
	HF group ($n = 304$)	NF group ($n = 1893$)	P value	HF group ($n = 53$)	NF group ($n = 618$)	P-value
Age (years)	82.7 ± 9.3	70.5 ± 11.1	<0.001	80.3 ± 9.4	67.5 ± 12.9	<0.001
Height (cm)	146.2 ± 7.2	149.5 ± 6.9	<0.001	160 ± 8.7	163.1 ± 7	0.004
Weight (kg)	43.1 ± 9	50 ± 9.8	<0.001	51.4 ± 10.6	60.3 ± 12.2	<0.001
BMI (kg/m^2)	20.1 ± 3.6	22.3 ± 3.7	<0.001	20 ± 3.3	22.6 ± 3.7	<0.001
Whole body BMD (g/cm^2)	0.84 ± 0.09	0.93 ± 0.12	<0.001	0.86 ± 0.13	1.02 ± 0.2	<0.001
Whole-body T-score	−3.29 ± 1.29	−2.08 ± 1.51	<0.001	0.95 ± 0.11	1.09 ± 0.15	<0.001
Whole-body bone mineral content (kg)	1.25 ± 0.33	1.62 ± 0.40	<0.001	1.81 ± 0.36	2.33 ± 0.53	<0.001
Whole-body fat tissue mass (kg)	9.67 ± 6.68	15.11 ± 7.28	<0.001	8.55 ± 6.57	13.63 ± 7.18	<0.001
Whole-body lean mass (kg)	30.72 ± 3.77	32.61 ± 4.01	<0.001	39.35 ± 5.59	43.55 ± 6.52	<0.001
Arm bone mineral content (kg)	0.14 ± 0.05	0.18 ± 0.05	<0.001	0.25 ± 0.07	0.31 ± 0.08	<0.001
Arm fat tissue mass (kg)	0.95 ± 0.84	1.49 ± 0.88	<0.001	0.70 ± 0.66	1.11 ± 0.7	<0.001
Arm lean mass (kg)	2.98 ± 0.95	3.16 ± 0.67	<0.001	4.18 ± 1.16	4.67 ± 1.03	<0.001
Arm SMI (kg/m^2)	1.40 ± 0.45	1.41 ± 0.28	0.001	1.64 ± 0.48	1.75 ± 0.35	0.006
Leg bone mineral content (kg)	0.40 ± 0.13	0.55 ± 0.15	<0.001	0.66 ± 0.18	0.84 ± 0.19	<0.001
Leg fat tissue mass (kg)	3.08 ± 2.02	4.51 ± 2.19	<0.001	2.69 ± 1.92	3.56 ± 1.85	<0.001
Leg lean mass (kg)	9.1 ± 1.72	10.11 ± 1.72	<0.001	11.48 ± 2.12	13.39 ± 2.59	<0.001
Leg SMI, kg/m^2 (kg)	4.27 ± 0.77	4.51 ± 0.65	<0.001	4.49 ± 0.82	5.01 ± 0.76	<0.001
Appendicular SMI† (kg/m^2)	5.66 ± 1.04	5.92 ± 0.84	<0.001	6.13 ± 1.2	6.76 ± 1.01	<0.001

All data were expressed as mean ± SD. All P-values were from Mann–Whitney's U-test. †Appendicular skeletal muscle mass index (SMI) is defined as the sum of leg SMI and arm SMI. BMD, bone mineral density; BMI, body mass index; HF group, hip fracture group; NF group, non-fracture group; SD, standard deviation.

group for females and males. Whole-body lean mass and fat mass were significantly low in both men and women in the HF group.

For adjusting the differences of age and the ratio of females between the NF group and the HF group, general linear model analysis was used to compare the characteristics, body composition and skeletal muscle mass index of patients in both the study groups, after controlling for age and sex (Table 2). No differences were observed in height, after controlling for age and sex. The weight, BMI and whole-body BMD significantly decreased in patients in the HF group. No significant difference in the arm SMI was observed between the HF and NF groups. In contrast, the patients in the HF group had significantly lower leg SMI than those in the other groups. The appendicular SMI – which was the sum of the arm SMI and leg SMI – also decreased in patients in the HF group, even after controlling for age and sex. No difference was observed in the whole-body lean mass. The prevalence of sarcopenia was significantly higher in the HF group after adjusting by age and sex with the Mantel–Haenszel method.

A stepwise logistic regression analysis was carried out to identify predictive factors for the occurrence of a hip fracture. We found that the presence of sarcopenia, older age and lower whole-body BMD were significant factors for the occurrence of a hip fracture ($P = 0.002$, $P < 0001$ and $P < 0.001$, respectively; Table 3).

Table 4 shows the estimated prevalence of sarcopenia in each age group of the HF and NF group patients. The prevalence of sarcopenia was higher in every age group of the HF group, and there were significances in the females in the 70–74 years group and 74–80 years group ($P = 0.004$, $P = 0.001$, respectively).

Overall in the HF group, the prevalence of sarcopenia in women and men was 44.7% and 81.1%, respectively; overall in the NF group, it was 27.2% and 52.8%, respectively. Sarcopenia prevalence was significantly higher in both men and women overall in the HF group than in the NF group. For comparing the prevalence of sarcopenia between males and females, differences were not observed in the group aged less than 70 years ($P > 0.95$) and in the group aged between 70 and 74 years ($P = 0.598$) in the HF group. However, this prevalence was significantly higher in men than in women aged between 75 and 80 years ($P = 0.005$) and those aged more than 80 years ($P < 0.001$). In the NF group, sarcopenia prevalence was high in all patients from all age groups ($P < 0.001$).

The relationship between muscle volume and bone mineral density in each group is shown in Figure 1.

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Table 2 Characteristics of participants, body composition, skeletal muscle mass index, and prevalence of sarcopenia in the fracture and non-fracture group controlled by the general linear model procedure

Characteristics	HF group	NF group	*P*-value
Height (cm)	152.5 ± 0.34	152.3 ± 0.14	>0.95
Weight (kg)	48.8 ± 0.54	51.9 ± 0.19	<0.001
BMI (kg/m^2)	20.7 ± 0.21	22.3 ± 0.081	<0.001
Whole-body BMD (g/cm^2)	0.93 ± 0.0061	0.97 ± 0.0024	<0.001
Whole-body T-score	−2.47 ± 0.77	−1.88 ± 0.30	<0.001
Whole-body bone mineral content (kg)	1.57 ± 0.020	1.77 ± 0.0079	<0.001
Whole-body fat tissue mass (kg)	11.31 ± 0.39	14.54 ± 0.15	<0.001
Whole-body lean mass (kg)	34.55 ± 0.24	35.02 ± 0.96	0.45[illegible]
Arm bone mineral content (kg)	0.19 ± 0.003	0.19 ± 0.001	<0.001
Arm fat tissue mass (kg)	1.07 ± 0.046	1.39 ± 0.018	<0.001
Arm lean mass (kg)	3.48 ± 0.043	3.50 ± 0.017	>0.95
Arm SMI (kg/m^2)	1.48 ± 0.018	1.50 ± 0.070	>0.95
Leg bone mineral content (kg)	0.54 ± 0.007	0.61 ± 0.003	<0.001
Leg Fat tissue mass (kg)	3.50 ± 0.11	4.23 ± 0.045	<0.001
Leg lean mass (kg)	1.05 ± 0.099	1.08 ± 0.039	<0.005
Leg SMI (kg/m^2)	4.45 ± 0.038	4.64 ± 0.015	<0.001
Appendicular SMI (kg/m^2)	5.93 ± 0.020	6.13 ± 0.050	<0.001
Prevalence of sarcopenia (%)	47.4	31.9	<0.001†

All data were controlled with age and sex. All data except the prevalence of sarcopenia were expressed as mean ± SE. *P*-values were obtained using the general linear model procedure except for prevalence of sarcopenia. †A *P*-value was obtained using the Mantel–Haenszel method after adjusting for age and sex. BMD, bone mineral density; BMI, body mass index; HF group, hip fracture group; NF group, non-fracture group; SE, standard error; SMI, skeletal muscle mass index.

Table 3 Stepwise logistic regression analysis for a hip fracture

	B	OR	95% CI	*P*-value
Presence of sacopenia	0.389	1.476	1.154–1.888	0.002
Age	0.098	1.103	1.087–1.120	<0.001
Whole-body BMD	−3.587	0.082	0.009–0.087	<0.001

The dependent variable was the occurrence of a hip fracture. The presence of sarcopenia was conventionally attributed a value of 1, the absence of sarcopenia was attributed a value of 0. BMD, bone mineral density; CI, confidence interval; OR, odds ratio.

Table 4 Prevalence of sarcopenia in both males and females from each age group

Age (years)	Females			Males		
	HF group	NF group	*P*-value	HF group	NF group	*P*-value
<70	37.5% (12/32)	22.7% (196/864)	0.051*	42.9% (3/7)	37.6% (120/319)	>0.95**
70–74	50.0% (11/22)	22.7% (80/352)	0.004*	75.0% (3/4)	55.7% (64/115)	0.631**
75–80	51.1% (23/45)	26.8% (77/287)	0.001*	92.9% (13/14)	70.7% (65/92)	0.107**
80<	43.9% (90/205)	41.3% (161/390)	0.539*	85.7% (24/53)	83.7% (77/92)	>0.95**
All Age	44.7% (136/304)	27.2% (514/1893)	<0.001*	81.1% (43/53)	52.8% (326/618)	<0.001*

P-values were obtained using the *χ^2-test and **Fisher's exact test. HF group, hip fracture group; NF group, non-fracture group.

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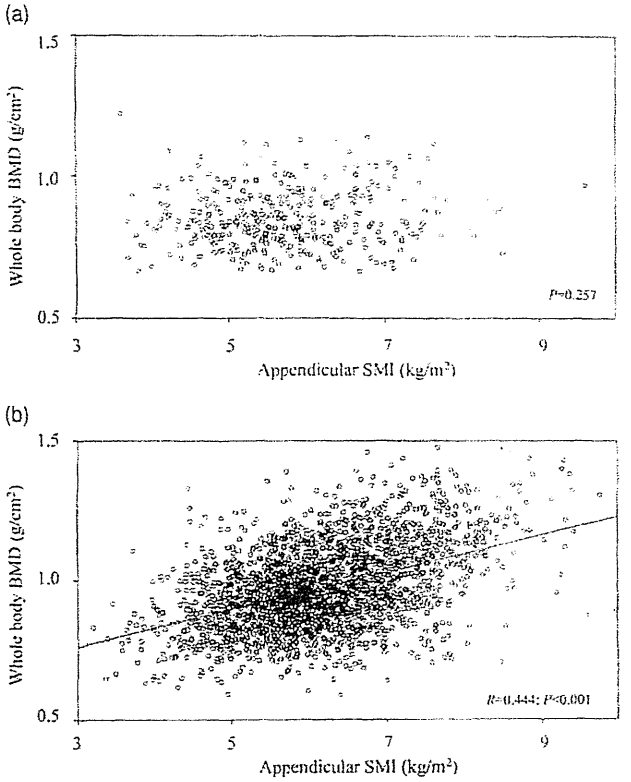

Figure 1 Correlation of appendicular skeletal muscle mass index (SMI) and whole-body bone mineral density (BMD) in (a) the hip fracture (HF) group and (b) the non-fracture (NF) group. *P*-values were from Pearson's test.

There was no significant difference in the HF group ($P = 0.257$). In contrast, there was a positive correlation between appendicular SMI and whole-body BMD in the NF group. ($R = 0.444$; $P < 0.001$).

Discussion

Sarcopenia, which is a barometer of disability and frailty, is one of the most crucial problems for the elderly. In contrast, hip fracture is already known to affect the morbidity and ADL of osteoporotic patients. The relationship between sarcopenia and hip fracture, however, has not been extensively examined. The present study examined the prevalence of sarcopenia in hip-fractured Japanese older adults diagnosed by DXA imaged within 48 h of their hip fracture occurrence, and hospital controls defined as appendicular muscle mass two standard deviations below the healthy normal young population.

The prevalence of sarcopenia in community-dwelling women aged more than 80 years in New Mexico, as reported by Baumgartner *et al.*, was 43.2% for Caucasians and 60% for Hispanics.[18] In the current study, the prevalence of sarcopenia in Japanese women aged more than 80 years without fracture was 41.3%. The prevalence of sarcopenia of women aged 70–80 years of age in New Mexico was 23.1–35.9% and that of Japanese women in the present study was 22.7–26.8%. These are consistent with Baumgartner's findings. An earlier study reported the prevalence of sarcopenia in 313 hip-fractured women whose average age was 79.7 years to be 58%.[21] These patients were diagnosed by DXA at an average of 21 days after occurrence. Estimation of muscle mass in the subacute phase of fracture was affected by surgical intervention and disuse atrophy, and had the possibility of overestimation of the prevalence of sarcopenia. Therefore, the prevalence of sarcopenia in their study might be higher than the real value. In contrast, the present results are regarded as more accurate, because we measured muscle mass in the short-term after the fracture.

In the present study, 44.7% of female and 81.1% of male patients with an acute phase of hip fracture were found to be sarcopenic; sarcopenia prevalence was significantly higher in patients with hip fractures than those without hip fractures, even when adjusted for age and sex. Sarcopenia prevalence was observed to be higher in men with hip fractures than in women with hip fractures. Men with hip fractures were found to have high mortality levels.[22,23] A high prevalence of sarcopenia might reflect the poor general health or frail condition of the patient. For example, the glucose tolerance was impaired, and elevated glycated hemoglobin was observed in sarcopenic patients.[17,24] The risk of nosocomial infection was doubled for patients with sarcopenia in geriatric wards.[25] Montano-Loza *et al.* recently mentioned in their paper that sarcopenia was an independent risk factor for mortality in patients with liver cirrhosis.[26] Szulc *et al.* reported that the loss of appendicular skeletal muscle mass was a predicting factor for mortality in older men.[27] Sarcopenia diagnosed using DXA should be considered as an important indicator of frailty in men.

Muscle volume and strength are the main factors to maintain the motor function of aged people. A number of studies have shown the relationship between sarcopenia and falls. Baumgartner *et al.* reported that people with lower appendicular SMI had a higher incidence of falls and lower body balance.[18] In a cohort study of 2148 English participants, Sayer *et al.* observed that patients with a history of falls presented with significantly lower muscle power.[28] According to a 5-year prospective study with 141 participants, the risk of deteriorated ADL doubled for those participants with decreased appendicular skeletal muscle.[29]

Furthermore, the muscle mass of the lower extremities was significantly decreased in patients with hip fracture in the present study. In contrast, the muscle mass of the arm did not differ regardless of hip fracture. This result supports previous reports about that the attenuation of leg muscle increasing the risk of falls and

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hence fractures.[30–32] The sarcopenic leg, or the muscle decrease and weakness of lower extremities, was already known to be associated with poor leg performance,[33,34] and to be a risk factor for recurrent falls and fracture.[35] Although handgrip strength instead of knee flexion/extension strength was recommended as a diagnosis tool in a European consensus on the definition and diagnosis of sarcopenia,[9] the measurement of muscle mass and strength of the lower extremities can be valuable for predicting the fracture risk of a hip.

In addition, there was a significant positive correlation between muscle mass and BMD in patients without a hip fracture in the present study, a finding that is compatible with past reports.[32,36,37] The mechanisms underlying disease, such as malnutrition, insufficiency of vitamin D and lack of physical activity, are common to sarcopenia and osteopenia.[38] In contrast, our study showed the BMD and appendicular SMI were not correlated significantly for the patients in the acute phase after occurrence of a hip fracture. Patients with a hip fracture had developed more severe sarcopenia and osteoporosis, as shown in Table 1. There was the speculation that the BMD and appendicular SMI were not correlated in patients who were especially frail. Furthermore, multivariate analysis showed that not only low BMD, but also the presence of sarcopenia, was a potential risk factor for an osteoporotic fracture. Simultaneous muscle and bone loss causes more severe instability in the frail elderly, which leads to falls and subsequent fracture.

Several ways to assess muscle volume have been established. Evaluation of the thigh muscle cross-sectional area by computed tomography or magnetic resonance imaging is the gold standard measurement for research,[9] but various limitations, such as high cost, the invasiveness of radiation and poor accessibility, have been reported. DXA is a precise and reproducible, as well as more accessible, less invasive and lower-costing alternative.[39] The technical errors of DXA compared with computed tomography were reported to be just 2.5%.[40] Anthropometric measurements, including calf circumference, are the traditional and convenient way to estimate skeletal muscle mass, but their accuracy is inadequate for the screening of sarcopenia.[41] Bioelectrical impedance analysis for sarcopenia is also a non-invasive and easy-to-use method. However, its validity has not been ascertained for those patients whose hydration status alters, such as the extremely elderly and fractured patients.[42] Currently, DXA is the preferred measurement method for clinical and research use.

The present study had several limitations. The participants in the NF group were neither randomly selected residents nor healthy volunteers. They were those patients who were suspected to be osteoporotic at our outpatient clinic. Because there is positive relationship between muscle mass and BMD, the patients with osteoporosis were estimated to have a higher prevalence of sarcopenia. We probably underestimated the difference of skeletal muscle mass between the hip fracture patients and the normal population. The second limitation is that we did not assess the function of the muscle, menopause status, comorbidities, the degree of pre-injury daily activity and energy expenditure in the present study. Recent reports mentioned that bed rest and low energy intake of inpatients might affect sarcopenia and hospitalization-associated disability.[43,44] However, the present study estimated the muscle mass within only 48 h from admission and the effect was limited. It is also well known that muscle strength declines much more rapidly than muscle mass, and sometimes the declines of muscle mass and strength were different between elderly individuals.[45] In future studies, we need to take into account a larger number of such covariates, which might confound the muscle mass–fracture relationship. There is another limitation about the sample size to evaluate the prevalence of sarcopenia in the each age group (Table 4). Our sample size analysis carried out with G*Power software (version 3.1.3, Faul *et al.*;[46] Heinrich-Heine-University, Dusseldorf, Germany) showed that statistical power for the female group aged <70 years, 70–74 years, 75–80 years, >80 years and all ages were 17.4%, 90.0%, 95.6%, 15.0% and 100%, respectively. Statistical power for the male age groups were <70 years, 70–74 years, 75–80 years, >80 years and all ages were 8.8%, 18.8%, 55.2%, 8.2% and 99.2%, respectively. Although adequate sample size was not mandatory, because the present study was an exploratory study, calculated statistical power over 80% was generally optimal for a significant result. A further study is required to validate the results in another dataset with a sufficient number of cases in the groups with inadequate statistical power, such as all age groups of the male and female group aged less than 70 years.

In conclusion, the present study showed that a higher prevalence of sarcopenia in Japanese patients in the acute phase of hip fracture than those patients from outclinics who did not have hip fractures, and that leg muscles of patients with a hip fracture were more sarcopenic. The diagnosis of sarcopenia and the evaluation of leg muscle by DXA can be the key to estimating patients at risk of a hip fracture.

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Disclosure statement

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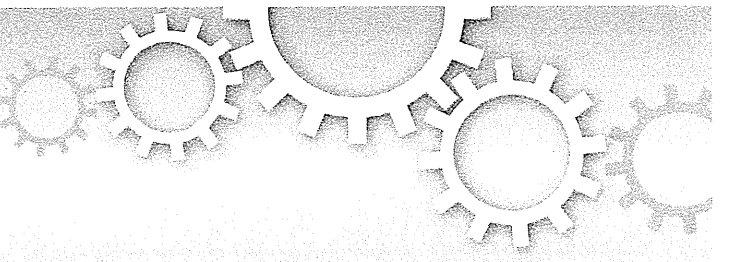

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Relationship between Low Free Testosterone Levels and Loss of Muscle Mass

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We assessed longitudinal relationships between baseline testosterone and muscle mass changes in Japanese men. Data were collected from community-dwelling 957 adult men who participated in a longitudinal study of ageing biennially from 1997–2010. Appendicular muscle mass (AMM) was measured with dual-energy X-ray absorptiometry at baseline and follow-up examinations. The cut-off point of sarcopenia was defined as a skeletal muscle index (AMM/height^2) < 6.87 kg/m^2. Total testosterone (TT) and free testosterone (FT) were measured with a radioimmunoassay. The calculated FT (cFT) was determined with a formula using albumin, TT, and sex hormone-binding globulin levels. We analyzed 4,187 or 2,010 cumulative data points using generalized estimating equations. Low TT was not associated with sarcopenia. Low cFT (odds ratio = 2.14, 95% confidence interval: 1.06–4.33) and FT (odds ratio = 1.83, 95% confidence interval: 1.04–3.22) were associated with sarcopenia. Low FT may be a predictor of risk for muscle loss in Japanese men.

Sarcopenia is the degenerative loss of skeletal muscle mass and strength associated with ageing[1]. Sarcopenia accelerates the frailty syndrome and leads to deterioration of activities of daily living and quality of life[2,3]. Development of preventative measures for sarcopenia is essential for extending a healthy life expectancy. The European Working Group on Sarcopenia in Older People assumed that muscle loss is a required component of sarcopenia diagnosis and suggested that muscle loss is a symptom of deterioration in muscle strength and physical performances[4]. Estimation of the risks for muscle loss appears to be necessary for developing steps to prevent sarcopenia.

Several cross-sectional studies have reported an association between serum levels of testosterone (T) and muscle mass in men[5,6]. Appendicular muscle mass was correlated with the serum level of free T (FT) in non-Hispanic white men aged 65–97 years[5]. Low appendicular muscle mass was observed in French men in the group with the lowest serum level of FT[6]. Additionally, androgen deprivation therapy for prostate cancer induces a decrease in muscle mass[7]. These studies suggest that the age-related decline in T is a trigger for muscle loss during ageing. Although T is associated with muscle mass, few longitudinal epidemiological studies have been published showing that circulating T levels are associated with prospective decreases in muscle mass[8]. In particular, muscle mass differs among races/ethnicities[9]. Circulating T levels also differ by race/ethnicity or the environment[10–12]. Demonstrating an association between muscle decrease and a decline in T appears to be necessary in each race/ethnicity.

The aim of this study was to determine whether circulating T levels predict muscle loss in middle-aged and elderly Japanese men. We assessed muscle loss with ageing using 10-year follow-up examinations and dual-energy X-ray absorptiometry (DXA) in middle-aged and elderly Japanese men. We also measured serum levels of T and evaluated the association between prospective muscle loss and the T levels in community-living middle-aged and elderly Japanese men using longitudinal analysis.

Results

Table 1 presents the elementary statistics of the participants at baseline according to sarcopenia status. Of the total of 957 men, 249 participants (26.0%) had a diagnosis of sarcopenia at baseline. Participants with sarcopenia were significantly older than those without sarcopenia (each, $p < 0.0001$). Body height ($p = 0.0018$), body weight

Table 1 | The characteristics of participants at the baseline examination. Means ± SE. The p values were obtained using the t-test for continuous data and the chi-square test (Fisher's exact test) for categorical data

	Normal (n = 708)	Sarcopenia (n = 249)	p
Age (years)	58.1 ± 0.4	63.1 ± 0.8	<0.0001
≥60 years (n)	314 (44.4%)	168 (67.5%)	<0.0001
Body height (cm)	164.9 ± 0.2	163.4 ± 0.4	0.0018
Body weight (kg)	65.0 ± 0.3	53.9 ± 0.4	<0.0001
Body mass index (kg/m^2)	23.9 ± 0.1	20.2 ± 0.1	<0.0001
% of body fat	21.8 ± 0.2	20.2 ± 0.3	<0.0001
Appendicular muscle mass (kg)	21.0 ± 0.1	17.2 ± 0.1	<0.0001
Skeletal muscle index (kg/m^2)	7.7 ± 0.02	6.4 ± 0.02	<0.0001
Albumin (mg/ml)	44.4 ± 0.2	44.3 ± 0.2	0.6894
Total testosterone (ng/ml)	5.0 ± 0.06	5.3 ± 0.1	0.0431
Sex hormone binding globulin (nmol/l)†	54.3 ± 1.3	71.4 ± 2.5	<0.0001
Calculated free testosterone (pg/ml)†	78.6 ± 1.1	68.9 ± 1.8	<0.0001
Free testosterone (pg/ml)	13.4 ± 0.1	12.3 ± 0.3	0.0002
Total energy intake (kcal/day)	2363.6 ± 14.2	2170.3 ± 22.7	<0.0001
Total protein intake (g/day)	88.9 ± 0.6	80.9 ± 1.0	<0.0001
Vitamin D intake (μg/day)	10.3 ± 0.2	9.6 ± 0.4	0.1502
Leisure-time physical activity (METs × hour/day)	2.2 ± 0.1	2.2 ± 0.2	0.9518
Current smoker (n)	236 (33.3%)	111 (44.6%)	0.0015
With medical history			
Stroke (n)	26 (3.7%)	10 (4.0%)	0.8063
Heart disease (n)	86 (12.2%)	33 (13.3%)	0.6491
Cancer (n)	17 (2.4%)	13 (5.2%)	0.0281
Diabetes (n)	59 (8.3%)	34 (14.0%)	0.0148
Osteoporosis (n)	4 (0.6%)	10 (4.0%)	<0.0001
Rheumatoid arthritis (n)	41 (5.8%)	18 (7.2%)	0.4171

†cFT and SHBG levels obtained from 327 normal men and 128 men with sarcopenia.

(p < 0.0001), Body mass index (p < 0.0001), and percent of body fat (p < 0.0001) were significantly lower in the sarcopenia group than in the normal group. Appendicular muscle mass (AMM) and skeletal muscle index (SMI) were also significantly lower in the sarcopenia group than in the normal group (each, p < 0.0001). Total T (TT; p = 0.0431) and sex hormone binding globulin (SHBG; p < 0.0001) were significantly higher in the sarcopenia group than in the normal group. cFT and FT were significantly lower in the sarcopenia group than in the normal group (p < 0.0001, p = 0.0002, respectively). Total energy and protein intake were significantly lower in the sarcopenia group than in the normal group (p < 0.0001). No significant differences in albumin, vitamin D intake, and leisure-time physical activity were noted between the normal and sarcopenia groups. The ratio of current smokers (p = 0.0015) in the sarcopenia group was significantly higher than in the normal group. No differences in the ratios of stroke, heart disease, and rheumatoid arthritis history were noted. The ratios of cancer (p = 0.0281), diabetes (p = 0.0148), and osteoporosis (p < 0.0001) history in the sarcopenia group were significantly higher than in the normal group.

Table 2 presents the frequencies according to sarcopenia and the T level status at baseline. No differences in the ratio of sarcopenia between the normal TT group and the low TT group were observed. The ratio of sarcopenia in the low cFT group was significantly higher than that in the normal cFT group (p = 0.0353). The ratio of sarcopenia in the low FT group was also significantly higher than that in the normal FT group (p = 0.0002).

Among the 4,187 cumulative samples, the numbers of samples in the normal and sarcopenia groups were 3,084 (73.7%) and 1,103 (26.3%), respectively. The numbers of participants with low TT in the normal muscle status group (n = 3,084) and the sarcopenia group (n = 1,103) were 141 (4.6%) and 67 (6.1%), respectively (p = 0.0487). The numbers of participants with low FT in the normal muscle status group (n = 3,084) and the sarcopenia group (n = 1,103) were 103 (3.3%) and 87 (7.9%), respectively (p < 0.0001). Among the 2,010 cumulative samples that were analyzed for cFT, the numbers of samples in the normal and sarcopenia groups were 1,460 (72.6%) and 550 (27.4%), respectively. The numbers of participants with low cFT in the normal muscle status group (n = 1,460) and the sarcopenia group (n = 550) were 56 (3.8%) and 40 (7.3%), respectively (p = 0.0013).

The results from the generalized estimating equations (GEE) analyses, controlling for the effects of repeated observations within

Table 2 | The testosterone levels and sarcopenia status at the baseline examination. The p values were obtained using the chi-square test

	Normal (Skeletal muscle index ≥ 6.87 kg/m^2)		Sarcopenia (Skeletal muscle index <6.87 kg/m^2)	
	n	%	n	%
Total testosterone				
Normal (≥2.9 ng/ml)	677	74.5	232	25.5
Low (<2.9 ng/ml)	31	64.6	17	35.4
Calculated free testosterone				
Normal (≥46.3 pg/ml)	313	73.0	116	27.0
Low (<46.3 pg/ml)	14	53.9	12	46.1
Free testosterone				
Normal (≥7.7 pg/ml)	681	75.3	224	24.7
Low (<7.7 pg/ml)	27	51.9	25	48.1

Table 3 | Longitudinal relationships between baseline testosterone levels and sarcopenia. The cumulative data were analyzed with generalized estimating equations. Moderator variables: Crude model: none; Model 1: baseline age; Model 2: age, leisure-time physical activity, nutrition intake (total energy, total protein, vitamin D), medical history (stroke, heart disease, cancer, diabetes, osteoporosis, rheumatoid arthritis), and smoking habit at baseline

		Odds ratio (95% confidence intervals)	
Total testosterone	Normal (≥2.9 ng/ml)	Low (<2.9 ng/ml)	p value
n	3979	208	
Crude model	1.00 (Reference)	1.6178 (0.9486 – 2.7592)	0.0774
Model 1	1.00 (Reference)	1.4790 (0.8606 – 2.5416)	0.1566
Model 2	1.00 (Reference)	1.5717 (0.9004 – 2.7434)	0.1116
Calculated free testosterone	Normal (≥46.3 pg/ml)	Low (<46.3 pg/ml)	p value
n	1914	96	
Crude model	1.00 (Reference)	2.6503 (1.3182 – 5.3285)	0.0062
Model 1	1.00 (Reference)	2.1396 (1.0555 – 4.3370)	0.0349
Model 2	1.00 (Reference)	2.1432 (1.0617 – 4.3262)	0.0334
Free testosterone	Normal (≥7.7 pg/ml)	Low (<7.7 pg/ml)	p value
n	3997	190	
Crude model	1.00 (Reference)	2.8915 (1.7116 – 4.8846)	<0.0001
Model 1	1.00 (Reference)	1.9416 (1.1046 – 3.4129)	0.0211
Model 2	1.00 (Reference)	1.8296 (1.0391 – 3.2215)	0.0364

participants and confounding factors, are presented in Table 3. No significant association of TT levels with sarcopenia was observed in any model. The association of the cFT and FT levels with sarcopenia was significant in all models. The odds ratios of sarcopenia in low cFT participants compared to that in normal cFT participants were 2.65 (95% confidence interval [CI], 1.32–5.33; $p = 0.0062$) in the crude model, 2.14 (95% CI, 1.06–4.34; $p = 0.0349$) in model 1, and 2.14 (95% CI, 1.06–4.33; $p = 0.0334$) in model 2. The odds ratios of sarcopenia in low FT participants compared to that in normal FT participants were 2.89 (95% CI, 1.71–4.88; $p < 0.0001$) in the crude model, 1.94 (95% CI, 1.10–3.41; $p = 0.0211$) in model 1, and 1.83 (95% CI, 1.04–3.22; $p = 0.0364$) in model 2.

Discussion

The etiology of sarcopenia is assumed to be multi-factorial, including factors such as ageing, diseases, nutritional deprivation, and inactivity[4]. Few epidemiologic studies have been published about sarcopenia in Japanese people, and the risk factors for sarcopenia are not understood[9]. In this study, significant associations between muscle loss and FT, regardless of whether FT was calculated or measured, remained after adjustment for age, medical history, nutrition intake, and physical activity. Low FT levels appeared to be independently associated with muscle loss in middle-aged and elderly Japanese men, regardless of these factors. Our result is in line with previous studies that reported a relationship between low FT and low muscle mass in men[5,6]. The observed association between muscle loss and FT in this study appears to have biological plausibility. T stimulates protein synthesis and inhibits protein degradation in muscle cells[13,14]. T also increases satellite cell replication and activation in older men[15]. In this study, no significant association between TT levels and muscle loss were observed. However, recent longitudinal cohort studies have reported that elderly American people with higher baseline TT levels have a low risk of decline in appendicular lean mass[8]. Although a progressive decrease in TT levels with ageing is observed in middle-aged and elderly American men[16,17], the TT levels do not change during ageing in Japanese men[21,22]. The decrease in TT may occur at a later stage when hypogonadism has advanced in Japanese men[21]. FT levels may be a good marker for the loss of muscle mass in Japanese men.

In Japanese men, preventing the decline in FT may prevent the loss of muscle mass during ageing. In this cohort, participants in the low cFT group (<46.3 pg/ml) had approximately a 2.1- to 2.7-fold risk of muscle loss compared to those in the normal cFT group (≥46.3 pg/ml) (Table 3). Participants in the low FT group (<7.7 pg/ml) also had approximately a 1.8- to 2.9-fold risk of muscle loss compared to those in the normal FT group (≥7.7 pg/ml) (Table 3). The serum levels of FT decrease by approximately 50%, from the 20 s through the 70 s in Japanese men[14]. The Japanese Urological Association defined the reference value for androgen replacement therapy as a serum level of 8.5 pg/ml FT as measured with radioimmunoassay (RIA)[21]. Thus, the FT level associated with the risk of muscle loss in this cohort was lower than the reference value for androgen replacement therapy for Japanese men. Improvement in circulating FT levels with appropriate therapies, such as androgen replacement therapy or lifestyle interventions, may reduce the risk of muscle loss during ageing.

The effect of ageing on sarcopenia in Japanese men appear to be large. The prevalence of sarcopenia increased significantly with age[1]. In this study, the baseline age of men in sarcopenia group was statistically older than men in normal group (Table 1). The odds ratio of sarcopenia calculated by the model 1 which were controlled for the baseline age were smaller than those by the crude model (Table 3). The muscle loss might have been affected by the age-related accumulation of the various factors, such as a muscle fiber apoptosis or a mitochondrial dysfunction[4].

Approximately 1% to 2% of T in the blood exists as FT[21]. However, the FT values using RIA are much lower than cFT values[22,23]. In fact, serum FT levels were one-fifth to one-sixth of those of cFT in this study (Table 1). The odds ratio of sarcopenia determined by GEE appeared to have been influenced by these results. The odds ratios of sarcopenia determined by cFT were higher than those of FT, except for in the unadjusted crude model (Table 3). The risk of sarcopenia may be underestimated when FT measured by RIA is an index.

Interestingly, appendicular muscle loss was significantly associated with low levels of FT. These results suggest that a threshold level of FT exists for muscle loss, rather than a dose-response relationship. In the previous cross-sectional and longitudinal studies of French and American men, no dose-response relationships were reported between T and muscle mass[6,8]. A minimal serum level of FT may be needed to preserve muscle mass in men, regardless of race/ethnicity.

This study has significant strengths. The longitudinal design of our analyses lends strength to our inferences. Our study that the same individuals were followed over time provided evidence of a causal association between low level of endogenous FT and the appendicular muscle loss. We adjusted our analyses for potential confounders, including age, physical activities, nutrition intake, medical history, and smoking habit. This is the first population study to evaluate the relationship between sarcopenia and circulating T levels.

This study has several limitations. The first limitation is that the odds ratios of the muscle loss were determined based on serum levels of T at baseline. Although T decreases during ageing, the rate of the decline in T varies depending on different environments and lifestyles among individuals[10,11]. Further studies with longitudinal measurements of T may clarify an association between the decrease in T and muscle loss during ageing. Second, women, who have little T compared with men[18], were not examined in this study. In women, serum FT levels also decrease during ageing[18]. Total lean mass is associated with bioavailable T in postmenopausal women[19]. Further studies are needed to determine the role of androgens in preserving muscle mass in women.

In summary, using the longitudinal design of the cohort, we evaluated the association between loss of muscle mass and decline in FT in community-living, middle-aged and elderly Japanese men with a 10-year follow-up duration. Our data confirm that a low FT level is a significant predictor of a risk for loss of appendicular muscle. The findings in this study may be beneficial for developing methods to prevent sarcopenia in Japanese men.

Methods

Participants. The participants in this study were from the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), which involves ongoing population-based biennial examinations of a cohort of approximately 2,300 persons. The participants in the NILS-LSA were randomly selected from resident registrations and stratified by both decade of age and sex. The NILS-LSA is a comprehensive and interdisciplinary study to observe age-related changes and consists of various gerontological and geriatric measurements, including medical examinations, blood chemical analysis, body composition, anthropometry, nutritional analysis, psychological tests, physical function, and physical activity[20]. Those who did not consent to have blood samples taken and those who did not complete the measurement of muscle mass with DXA were excluded. Participants with a current medical history of Parkinson's disease and androgen preparation users were also excluded. The baseline participants of this study were 957 men aged 40–79 years who completed the first-wave examinations of NILS-LSA between November 1997 and April 2000. Of these, 777 (81.2%) took part in the second-wave examination between April 2000 and May 2002, 689 (72.0%) took part in the third-wave examination between May 2002 and May 2004, 638 (66.7%) participated in the fourth-wave examination between May 2004 and July 2006, 590 (61.7%) took part in the fifth-wave examination between July 2006 and July 2008, and 536 (56.0%) participated in the sixth-wave examination between July 2008 and July 2010. The mean number of repeat visits was 3.2. The total number of visits, including repeat visits, was 4,187; participants from whom the data were derived were 40–88 years of age and took part in the NILS-LSA between November 1997 (the first wave) and July 2010 (the sixth wave).

The study protocol was approved by the Ethics Committee of the National Center for Geriatrics and Gerontology, and written informed consent was obtained from all participants.

Blood sampling and measurement of T. Blood samples were taken between 0800 and 0900 h, separated immediately by centrifugation at 2000 × *g* for 15 min, and sera were frozen and stored in a deep freezer (−80°C). Samples were transferred to the laboratory (SRL Inc., Tokyo, Japan) for TT, FT, SHBG, and albumin measurement.

The serum levels of TT (ng/ml) and FT (pg/ml) were also measured with a RIA using commercially available kits (Diagnostic Products Corporation, Los Angeles, CA, USA). The inter-assay coefficients of variation (CV) were less than 15% for both kits, according to the manufacturer's information. In 455 men who were randomly selected by decade of age at the time of the first-wave examinations, SHBG (nmol/l) was also measured with RIA using a commercially available kit (Diagnostic Products Corporation). The CV was less than 8.5% according to the manufacturer's information. Serum albumin (mg/ml) was measured with nephelometry.

For measuring FT, the detection precision of the equilibrium dialysis was better than that of RIA[21]. However, equilibrium dialysis is not used in Japan, because equilibrium dialysis is difficult to perform, not automated, and largely inaccessible to most clinicians[21]. Thus, the calculated FT (cFT) was derived from serum levels of albumin, TT, and SHBG in 455 male participants[22,23]. In this study, the coefficient of correlation between cFT and FT was 0.80438 ($n = 455$; $p < 0.0001$).

Definition of sarcopenia. Appendicular muscle mass (AMM, kg) and fat mass were assessed with DXA (QDR-4500; Hologic, Bedford, MA, USA). AMM is equal to the appendicular fat-free mass minus bone mineral contents, and is assumed to be an index of the amount of muscle mass.

We used the SMI to evaluate sarcopenia[1]. The SMI was calculated by AMM divided by height squared (kg/m^2). Sarcopenia was defined as muscle mass minus 2 standard deviations below the mean for young adult healthy people[1]. In this study, we set the cut-off point of sarcopenia as SMI < 6.87 kg/m^2. The SMI of 6.87 kg/m^2 was muscle mass minus 2 standard deviations below the mean for young adult healthy people in the Japanese men[9]. Sanada et al.[9] also measured appendicular muscle mass with DXA using the same model (QDR-4500; Hologic) we used in this study. The participants were divided into two groups based on DXA results at baseline and follow-up examinations: the sarcopenia group (SMI < 6.87 kg/m^2) and the normal group (SMI ≥ 6.87 kg/m^2).

Other parameters. Body height and weight were measured using a digital scale. Body mass index (kg/m^2) was calculated by weight divided by height squared. Medical history, smoking habit, and use of medications were assessed with questionnaires, which were confirmed by a physician at the medical examinations. All prescribed and non-prescribed medications used during the previous 2 weeks were documented and brought by the participants; the physicians confirmed and coded them. Trained interviewers used a questionnaire and asked the participants about the frequency and exercise intensity (metabolic equivalents: METs) of their physical activity habits during leisure time over the past 12 months[24]. The means per day for leisure-time physical activity (metabolic equivalents; METs × h/day) were calculated. Nutritional intake was assessed with a 3-day diet record[25]. Foods were weighed separately on a scale before cooking or portion sizes were estimated. Participants used a disposable camera to take photographs of meals before and after eating. Registered dietitians used the photographs to complete missing data and telephoned participants to resolve any discrepancies or to obtain further information when necessary. The average over the 3 days for 119 nutrient intake periods was calculated. The means per day for total energy intake (kcal/day), total protein intake (g/day), and vitamin D intake (μg/day) were calculated from the 3-day dietary record.

Statistical analysis. Statistical testing was performed using the Statistical Analysis System release 9.3 (SAS Institute Inc., Cary, NC, USA). A probability level less than 0.05 was considered significant. The results are shown as the means ± standard error (SE). Differences in continuous and class variables between the normal and sarcopenia groups were assessed with t-tests and chi-square tests, respectively. To assess differences in the medical history of osteoporosis between the normal and sarcopenia groups, Fisher's exact test was used because the minimum expected cell size was less than five.

Cumulative data were analyzed using GEE, which take into account the dependency of repeated observations within participants; this is an important feature that is necessary for longitudinal analyses. An additional advantage of GEE is that participants are included regardless of missing values. Thus, participants who were lost to follow-up after early wave examination were also included in the analyses. GEE models were fitted using the GENMOD procedure of SAS. The GENMOD procedure fits generalized linear models. The correlation structure was specified to be autoregressive.

The serum T levels were modeled as dichotomized variables in GEE analyses. In this study, the cut-off values of T were established based on the serum level of FT, because under the current circumstances in Japan, hypogonadism is diagnosed using the serum level of FT. The FT decreases during ageing, whereas the TT levels do not change during ageing in Japanese men[21,22]. In addition, measurement of SHBG cannot be performed for the diagnosis of hypogonadism in Japan, because SHBG measurement is not included in the gonadal function tests covered by health insurance. The participants were divided into two groups based on the serum level of FT in the baseline examination: the low level group (<7.7 pg/ml) and the normal level group (≥7.7 pg/ml). The FT of 7.7 pg/ml, which was minus 2 standard deviations below the mean for healthy Japanese men aged 40–49 years, was approximately equal to the 5th percentile of participants in this study[21]. Thus, the cut-off values of TT and cFT were defined as the 5th percentile of serum levels (TT 2.9 ng/ml; cFT 46.3 pg/ml) in participants.

Analyses were carried out with an unadjusted crude model and several adjusted models, controlling for different combinations of confounding variables: age was taken as a moderator variable in model 1; age, leisure-time physical activity, nutrition intake (total energy, total protein, vitamin D), medical history (stroke, heart disease, cancer, diabetes, osteoporosis, rheumatoid arthritis), and smoking habit were considered moderator values in model 2.

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Author contributions

A.Y. designed the study, carried out the statistical analyses and wrote the manuscript. R.O., R.K., I.K. and T.O. participated in data collection and analysis. F.A. and H.S. revised the manuscript and managed the overall project.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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