Bone 163 (2022) 116487

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Relationship between sarcopenia classification and thigh muscle mass, fat area, muscle CT value and osteoporosis in middle-aged and older Japanese adults

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ARTICLE INFO

Keywords:
Sarcopenia
Mid-thigh computed tomography
Muscle cross-sectional area
Fat cross-sectional area
Bone mineral density

ABSTRACT

Sarcopenia is one of the most important health issues in today's ageing society. As an evaluation method, computed tomography (CT) is an effective means of assessing not only the quantity but also the quality of skeletal muscle. We aimed to examine the relationship between sarcopenia severity and muscle/fat area, and osteoporosis.

321 patients (116 men and 205 women, mean age 77.2 ± 7.1 years, age range 53–96 years) who visited the Integrated Healthy Aging Clinic from 2016 to 2017 were included in this study.

Based on the Asia Working Group for Sarcopenia2019 criteria, patients were divided into four groups: normal group, low-functional group (with normal skeletal muscle mass, but reduced muscle strength or physical function), sarcopenia group, and severe sarcopenia group.

We measured the skeletal muscle (SM), intermuscular adipose tissue (IMAT), and subcutaneous adipose tissue (SAT) areas and the CT attenuation values (CTV) using cross sections of the mid-thigh CT. We also measured bone mineral density. Then, we compared each result among the four groups.

We found a significant decrease in SM area in both men and women with sarcopenia ($p < 0.001$ for both sexes). In women, a decrease in SAT area was observed in the sarcopenia group ($p < 0.001$), and an increase in IMAT was observed in the low functional group ($p < 0.001$). The CTV decreased in men with sarcopenia and severe sarcopenia; similarly, women in the low functional and severe sarcopenia groups had decreased CTV ($p < 0.001$ for both sexes). An association between sarcopenia and osteoporosis in men was detected ($p = 0.004$).

By using not only muscle mass but also fat mass and CTV, we were able to better examine the pathogenesis of sarcopenia and differences between men and women in Japanese middle-aged and older adults.

1. Introduction

Sarcopenia is one of the most important health issues in today's ageing society. Sarcopenia, a term proposed by Rosenberg in 1989, refers to the loss of muscle mass and strength [1,2]. In 1998, Baumgartner et al. defined sarcopenia as a mean skeletal muscle mass index (SMI) of −2 standard deviations or less measured using dual-energy X-ray absorptiometry (DXA) in young people. Since then, SMI has been

Abbreviations: AWGS, Asia Working Group for Sarcopenia; BIA, bioimpedance analysis; CT, computed tomography; CTV, CT attenuation value; DXA, dual-energy X-ray absorptiometry; EWGSOP, European Working Group on Sarcopenia in Older People; IMAT, intermuscular adipose tissue; group LF, low functional group; group N, normal group; group S, sarcopenia group; group SS, severe sarcopenia group; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; SM, skeletal muscle; SMI, skeletal muscle mass index; SPPB, short physical performance battery; YAM, young adult mean; CRP, C-reactive protein.

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https://doi.org/10.1016/j.bone.2022.116487
Received 27 March 2022; Received in revised form 10 July 2022; Accepted 10 July 2022
Available online 14 July 2022
8756-3282/

used to assess sarcopenia [3]. However, it has been reported that muscle strength is more strongly associated with falls and limitation of movement than muscle mass [4,5]. Consequently, there have been arguments for including muscle strength and physical ability in the determination of sarcopenia. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as decreased muscle function and muscle mass [6]. The establishment of this definition enabled a universal standard for diagnosis. In 2014, the Asia Working Group for Sarcopenia (AWGS) presented the diagnostic criteria for sarcopenia based on data obtained from Asian countries [7]. Furthermore, the revision of EWGSOP2 in 2018 highlighted the importance of assessing muscle mass and quality for the definitive diagnosis of sarcopenia [8]. In the AWGS 2019 guidelines, diagnostic algorithms, protocols, and several criteria have been revised. In particular, the term "possible of sarcopenia," defined as having low muscle strength or reduced physical performance, has been introduced [7,9], and there have been various studies on its pathogenesis and associated diseases.

Considering that the amount of muscle loss in the lower limbs is twice that in the upper limbs due to ageing, the lower limbs are thought to be more susceptible to the effects of ageing [10]. The thigh is an area of significant muscle loss due to ageing and is often used in studies on muscles and ageing. Methods to evaluate muscle loss include DXA, bioimpedance analysis (BIA), computed tomography (CT) [11–14], and magnetic resonance imaging (MRI) [15–17]. DXA and BIA are most commonly used in clinical practice, as they are simple and economical. However, CT and MRI are more precise imaging systems that can separately evaluate muscle, fat, and soft tissue and are considered the gold standard for estimating muscle mass in research [6]. In particular, CT has a high spatial resolution and has proven to be an effective means of assessing skeletal muscle (SM) mass [18]. Measurements obtained from single cross-sectional slices have also correlated strongly with the distribution of adipose tissue and SM throughout the body, making them highly accurate for estimating body composition [19]. Regarding the CT attenuation value (CTV), research suggests utilizing the quadriceps muscle density and intermuscular adipose tissue (IMAT) area on CT because they can provide an accurate clinical diagnosis, assessment, and degree of disease progression in sarcopenia [20]. However, there are few reports on changes in muscle area according to sarcopenia severity in the older population.

In recent years, we have previously measured the quadriceps mass and quadriceps CTV using mid-thigh CT in older Japanese individuals, and illustrated age-related differences in community residents [20] and associations with sarcopenia in hospital cohorts [21]. However, there is a lack of research on changes in muscle area according to sarcopenia severity in the older population. Although there are many reports on the association between ageing, sarcopenia, and visceral fat [22], only a few studies have examined the relationship between sarcopenia and IMAT using CT. There were no reports of changes in sarcopenia by measuring the areas of muscle and fat, as far as we could find.

Osteoporosis is one of the most common musculoskeletal diseases among older people. It shares common pathophysiological factors with sarcopenia, and both influence the other negatively [23]. There are many overlapping treatment methods for both conditions, such as exercise and vitamin D supplementation [24]. However, there are few reports on the rate of osteoporosis based on sarcopenia classification using the AWGS criteria.

The aim of this study was to clarify the relationship between sarcopenia severity and skeletal muscle, IMAT, and subcutaneous adipose tissue (SAT) areas and CTV of the entire thigh, as well as osteoporosis in Japanese middle-aged and older adults.

2. Materials and methods

2.1. Participants

A total of 321 patients (116 men and 205 women, mean age 77.2 ± 7.1 years, age range 53–96 years) out of the 500 who visited the Integrated Healthy Aging Clinic at the National Center for Geriatrics and Gerontology, Japan, from 2016 to 2017 were included in this study. Patients with gait impairment caused by severe osteoarthritis of the knee and hip, progressive motor disease such as Parkinson's disease, and missing data were excluded. This study was approved by the Institutional Ethics Committee (approval no. 881) and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from all the participants.

2.2. Physical measurement

Participants were tasked with walking 6 m at a comfortable walking speed (Walkway MW-1000, ANIMA, Tokyo Japan). Grip strength on both the right and left hands was measured using a new grip dynamometer developed at our institution that uses a ZP-500N force gauge (Imada Corporation, Toyohashi, Aichi) [25], and the maximum value was adopted. A series of tests, known as the short physical performance battery (SPPB) were performed, including the five-times sit-to-stand test.

2.3. Measurement of total body muscle mass

Using DXA (Lunar iDXA, GE Healthcare, Chicago, IL), limb muscle mass was measured with whole-body scanning. SMI was calculated as appendicular skeletal muscle mass (ASM) divided by the square of height [3,22].

2.4. Diagnosis and classification of sarcopenia

We used the AWGS 2019 diagnostic criteria for sarcopenia [9]. Muscle strength (grip strength), physical function (walking speed, five-times sit-to-stand time, and SPPB total score), and skeletal muscle mass (SMI by DXA) were measured. The cutoff values were set at 28 kg for men and 18 kg for women for grip strength, and at 1.0 m/s for walking speed. Those for SMI were 7.0 kg/m^2 for men and 5.4 kg/m^2 for women [9]. If any one of the three parameters (SPPB total score, five-times sit-to-stand time, and 6 m walking speed) was worse than the cutoff value, the patient was considered to have decreased physical function.

Based on these results, we defined the "normal (N)" group as those with normal muscle strength and physical function with or without reduced SM; the "low functional (LF)" group as those with normal SM mass but reduced muscle strength or physical function; the "sarcopenia (S)" group as those with reduced SM mass and a decrease in either muscle strength or physical function, and the "severe sarcopenia (SS)" group as those with reduced muscle strength, physical function, and SM mass.

2.5. CT imaging and measurement

Participants underwent a single-slice CT examination of the right mid-thigh (location: midpoint of the superior pole of the patella and inguinal crease). We used SOMATOM Sensation 64 (Siemens, Munich, Germany) (settings: 120 kV, 120 mA; rotation time, 1 s; and field of view, 233 mm) and Aquilion CXL (CANON, Tochigi, Japan) (settings: 135 kV, 150 mA; rotation time, 1 s; and field of view, 4.0 mm). The total thigh area, muscle, fat area, and CTV from this single slice were analyzed using SliceOmatic software (version 5.0; TomoVision, Montreal, QC, Canada). The method used was as follows: First, we measured the areas of muscle and fat in the entire thigh cross-sectional area (Fig. 1a). Using the method by Goodpaster et al. as a reference, the CTV was set to between −190 and −30 Hounsfield unit (HU) for fat (red area in Fig. 1), between −30 and 0 HU for fascia and fibrous tissue (green), between 0 and 100 HU for SM (blue), and >100 HU for cortical bone (purple) [26]. We measured each area and CTV of muscle. The tangent lines

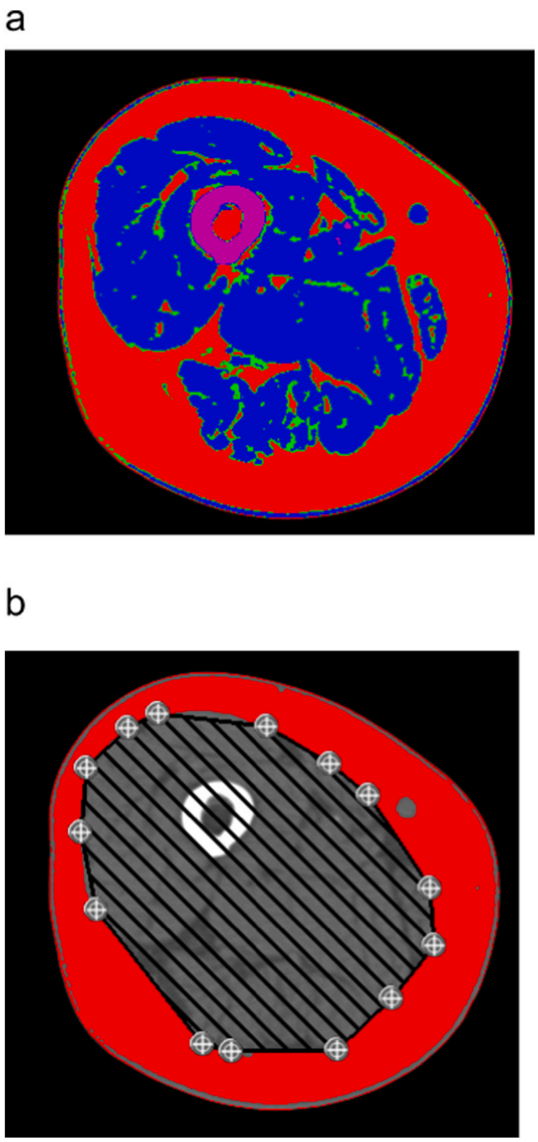

Fig. 1. a. The area was divided by CT attenuation value (CTV) into i) blue for muscle, ii) red for fat, and iii) green for fascia and fibrous tissue.
b. The tangent lines around the periphery of the muscle were connected, and subcutaneous fat was measured outside of the tangent lines. The area obtained by subtracting subcutaneous fat from the total thigh area was defined as the inter-muscular fat area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

around the periphery of the muscle were manually connected, the fat area contained within the tangent lines was defined as IMAT, and the fat outside the tangent lines was defined as SAT (Fig. 1b).

We examined intra-rater and inter-rater reliability by three researchers for the total thigh area, SM, SAT, and IMAT. The intra-rater reliability coefficients for each area were 1.000 for all three for total thigh area and SM area, 0.995, 0.997, and 1.000 for SAT area, and 0.901, 0.966, and 0.988 for IMAT area, while the inter-rater reliability coefficients were 1.000 for total thigh area, 1.000 for SM area, 0.996 for SAT area, and 0.990 for IMAT area. The intra-rater reliability and inter-rater reliability of the CTV were judged to be sufficiently high because they are automatically measured by SliceOmatic software.

Confirming this good validity, all measurements were performed once by the authors, who were blinded to the participants' data. Questionable measurements were discussed by Y.M., Y.S., and T.M. and a final decision was made. Based on these results, we compared the area and area ratio of muscle, SAT, and IMAT, as well as the CTV of muscle and the product of muscle area and CTV, to examine changes in both the quantity and quality of muscle in the four sarcopenia groups.

2.6. Assessment of osteoporosis

Young adult mean (YAM) values of the second to fourth lumbar vertebrae (L2–4) and femoral neck were measured using DXA (Lunar iDXA, GE Healthcare). The lower of the two values was adopted and subsequently classified according to the diagnostic criteria for primary osteoporosis of the Japanese Osteoporosis Society as follows: a bone mineral density (BMD) of ≥80 % of YAM values was considered normal, ≥70 % but <80 % as osteopenia, and <70 % as osteoporosis [27]. The number of people classified as having osteoporosis and the YAM values of the lumbar spine and femoral neck were compared among the four groups of sarcopenia classification.

2.7. Statistical analysis

Statistical analysis was performed using SPSS Statistics version 28.0.0 (IBM, Chicago, Illinois, USA). Statistical significance was set at $p < 0.05$. Analysis of variance was used to analyze demographic data. Total thigh area, SM area, IMAT area, SAT area, ratio of SAT area to IMAT area, ratio of muscle area to IMAT area, CTV, product of CTV and muscle area were stratified by sex and sarcopenia classification into four groups. Analysis of covariance was performed to confirm the association with age, and the Kruskal-Wallis test was performed to examine differences in distribution between the groups. Comparisons between the groups were adjusted for multiplicity using the Bonferroni correction. Fisher's exact probability test was used for osteoporosis classification. The patients were also stratified according to YAM values at the lumbar spine and femoral neck were also stratified, and comparisons were made using Pearson's chi-square test.

3. Results

There were 56 men in group N, 23 in group LF, 18 in group N, and 19 in group SS, while there were 103 women in group N, 66 in group LF, 16 in group S, and 20 in group SS. Age, height, weight, and BMI were significantly different among the four groups except height in men

Table 1
Patient demographic data.

		Group N	Group LF	Group S	Group SS	p value
Number of persons	Men	56	23	18	19	
	Women	103	66	16	20	
Age	Men	76.7 ± 6.1	75.9 ± 5.4	81.8 ± 4.3	80.5 ± 8.5	**
	Women	74.0 ± 7.2	78.5 ± 6.8	81.5 ± 3.9	81.7 ± 6.1	**
Body height (cm)	Men	164.2 ± 5.2	160.6 ± 7.4	162.0 ± 3.9	162.5 ± 6.5	0.07
	Women	151.6 ± 5.5	147.2 ± 7.3	149.0 ± 4.5	146.5 ± 6.1	**
Body weight (kg)	Men	62.2 ± 9.0	68.8 ± 9.2	56.8 ± 7.1	57.9 ± 10.1	**
	Women	52.4 ± 8.6	55.6 ± 10.6	44.4 ± 6.2	41.1 ± 6.1	**
Body mass index (kg/m^2)	Men	23.0 ± 2.9	26.6 ± 2.9	21.6 ± 2.4	21.6 ± 3.6	**
	Women	22.8 ± 3.6	25.6 ± 4.1	20.0 ± 2.6	19.1 ± 2.2	**

Mean values ± standard error for each group are shown. Analysis of variance was used for comparisons.
Group N, normal group; group LF, low functional group; group S, sarcopenia group; group SS, severe sarcopenia group.
** $p < 0.01$.

(Table 1). BMI ≧ 25 was found in 20 men (17.86 %) in group N, 15 (65.22 %) in group LF, 1 (5.56 %) in group S, and 3 (15.79 %) in group SS: and among women, none was found in group N, 34 (51.52 %) in group LF, 1 (6.25 %) in group S, and 3 (15 %) in group SS.

Table 2 shows the mean values of the items related to the CT area and the results of the comparison between the four groups. The total thigh areas for men in groups N, LF, S, and SS were 163.1 ± 28.3, 190.4 ± 30.4, 140.9 ± 27.6, and 142.3 ± 33.8 cm^2, respectively. For women, these were 168.7 ± 34.3, 176.2 ± 45.0, 133.9 ± 20.0, and 123.7 ± 32.8 cm^2, respectively. The SM areas of men were 109.5 ± 18.4, 116.7 ± 15.5, 89.6 ± 16.2, and 85.9 ± 16.3 cm^2, and those of women were 83.2 ± 14.8, 79.1 ± 12.5, 69.1 ± 6.4, and 59.6 ± 18.8 cm^2, respectively. The IMAT areas were 7.72 ± 3.69, 9.80 ± 3.77, 7.76 ± 3.64, and 9.11 ± 5.59 cm^2 for men and 8.99 ± 5.02, 10.65 ± 3.75, 7.87 ± 2.93, and 8.79 ± 5.78 cm^2 for women, respectively. The SAT areas were 27.3 ± 12.6, 41.8 ± 21.8, 26.1 ± 12.8, and 28.0 ± 18.9 cm^2 for men and 59.4 ± 25.6, 67.7 ± 35.9, 41.9 ± 17.2, and 39.6 ± 18.8 cm^2 for women, respectively. The ratios of SAT area to IMAT area were 3.65 ± 1.51, 4.44 ± 2.03, 3.48 ± 1.87, and 3.27 ± 1.28 for men and 7.36 ± 3.42, 6.54 ± 3.11, 5.54 ± 1.82, and 4.94 ± 2.37 for women, respectively. The ratios of IMAT area to SM area were 0.07 ± 0.04, 0.09 ± 0.04, 0.09 ± 0.04, and 0.11 ± 0.07 for men and 0.13 ± 0.29, 0.14 ± 0.05, 0.12 ± 0.05, and 0.16 ± 0.15 for women, respectively. Age-adjusted results for the IMAT and SAT areas showed that the IMAT area was associated with sarcopenia classification only in women ($p = 0.004$), and the SAT area was associated with sarcopenia classification in both men ($p < 0.001$) and women ($p = 0.003$).

The total thigh, SM, IMAT, SAT areas, ratio of SAT area to IMAT area, and ratio of IMAT area to SM area were compared among four groups. The total thigh, SM, and SAT areas were significantly different ($p < 0.001$ for both sexes for total thigh area and SM area, $p = 0.039$ for men and $p < 0.001$ for women for SAT area), whereas the IMAT area, ratio of SAT area to IMAT area, and ratio of IMAT area to SM area were significantly different only in women ($p < 0.001$, $p = 0.004$, and $p < 0.001$, respectively).

In men, the total thigh area was significantly larger in group LF than in groups N, S, and SS ($p = 0.006$, $p < 0.001$, and $p < 0.001$, respectively). In women, groups S and SS were significantly smaller than group N ($p = 0.001$ and $p < 0.001$, respectively) and group LF ($p = 0.001$ and $p < 0.001$, respectively). In particular, women showed a significant decrease in the area of sarcopenia. The SM area was significantly smaller in groups S and SS than in groups N and LF in both men and women (men: groups N and S, $p = 0.002$; groups N and SS, $p < 0.001$; groups LF and S, $p < 0.001$; groups LF and SS, $p < 0.001$; women: groups N and S, $p < 0.001$, groups N and SS $p < 0.001$, groups LF and S $p = 0.029$, and groups LF and SS $p < 0.001$). The IMAT area in women was significantly larger in group LF than in group N ($p = 0.004$) and significantly smaller in groups S and SS than in group LF ($p = 0.024$ and $p = 0.015$, respectively). Although there was a significant difference in the SAT area in men overall ($p = 0.039$), there was no significant difference between the groups using the Bonferroni correction. The SAT area in women was significantly larger in group LF than groups S and SS ($p = 0.018$ and $p = 0.003$, respectively), and group SS was significantly smaller than group N ($p = 0.017$). The ratio of SAT area to IMAT area in women was significantly smaller in group SS than in group N ($p = 0.005$). The ratio of IMAT area to SM area in women was significantly higher in group LF than in group N ($p < 0.001$).

Table 3 shows the results of the comparison between the items related to the CTV and the four sarcopenia groups. The mean CTV of each group was 48.9 ± 3.1 HU in group N, 47.4 ± 3.9 HU in group LF, 45.6 ± 2.6 HU in group S, and 45.2 ± 4.1 HU in group SS in men, and 46.6 ± 3.9 HU in group N, 44.1 ± 3.6 HU in group LF, 45.5 ± 2.3 HU in group S, and 42.4 ± 2.9 HU in group SS in women. In men, groups S and SS had significantly lower CTV than group N ($p = 0.001$ and $p = 0.001$, respectively). In women, groups LF and SS had significantly lower CTV than group N ($p < 0.001$ and $p < 0.001$, respectively), and group SS was lower than group S near the significance level, although the difference was not statistically significant ($p = 0.068$). The product of SM and CTV was 5384.5 ± 1112.7 in group N, 5565.9 ± 1035.8 in group LF, 4103.8 ± 883.3 in group S, 3909.6 ± 928.7 in group SS in men, and 3899.1 ± 851.4 in group N, 3500.8 ± 687.1 in group LF, 3149.5 ± 391.4 in group S, and 2550.0 ± 910.6 in group SS in women. Significant differences were observed in both men and women ($p < 0.001$ for both), and between-group comparisons showed that in men, groups S and SS were significantly lower the product of SM and CTV than group N ($p < 0.001$ for both), whereas groups S and SS were significantly lower than groups LF ($p < 0.001$ for both). In women, groups LF, S, and SS were significantly lower the product of SM and CTV than group N ($p = 0.019$, $p = 0.002$, and $p < 0.001$, respectively), and group SS was significantly lower than group LF ($p < 0.001$).

The osteoporosis classification for each group is shown in Table 4. In men, sarcopenia was associated with a significantly higher incidence of osteoporosis ($p = 0.019$); however, no association was found in women.

Table 5 shows the mean YAM values of the lumbar spine and femoral neck and a comparison among the four groups. The mean YAM values of the lumbar spine for men in groups N, LF, S, and SS were 107.2 ± 23.2, 113.2 ± 18.6, 96.4 ± 22.6, and 92.3 ± 22.2, respectively. For women, these were 88.0 ± 17.1, 89.9 ± 18.1, 74.3 ± 12.8, and 77.5 ± 14.1, respectively. The mean YAM values of femoral neck of men were 89.6 ±

Table 2

Comparison of area parameters across the four groups.

		Group N	Group LF	Group S	Group SS	p value	N vs			LF vs		S vs
							LF	S	SS	S	SS	SS
Total thigh area (cm^2)	Men	163.1	190.4	140.9	142.3	**	**	0.091	0.148	**	**	1.000
	Women	168.7	176.2	133.9	123.7	**	1.000	**	**	**	**	1.000
SM (cm^2)	Men	109.5	116.7	89.6	85.9	**	0.367	**	**	**	**	1.000
	Women	83.2	79.1	69.1	59.6	**	0.326	**	**	*	**	0.904
IMAT (cm^2)	Men	7.72	9.80	7.76	9.11	0.168						
	Women	8.99	10.65	7.87	8.79	**	**	1.000	1.000	*	*	1.000
SAT (cm^2)	Men	27.3	41.8	26.1	28.0	*	0.059	1.000	1.000	0.167	0.114	1.000
	Women	59.4	67.7	41.9	39.6	**	1.000	0.086	*	*	**	1.000
SAT/IMAT	Men	3.65	4.44	3.48	3.27	0.238						
	Women	7.36	6.54	5.54	4.94	**	0.616	0.363	**	1.000	0.166	1.000
IMAT/SM	Men	0.07	0.04	0.09	0.11	0.093						
	Women	0.13	0.14	0.12	0.16	**	**	1.000	0.075	0.428	1.000	1.000

The Kruskal-Wallis test was performed to examine differences in distribution between the groups.

Group N, normal group; Group LF, low functional group; Group S, sarcopenia group; Group SS, severe sarcopenia group.

SM, skeletal muscle; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; SAT/MAT, the ratio of SAT area to IMAT area; IMAT/SM, the ratio of IMAT area to SM area.

* $p < 0.05$.

** $p < 0.01$.

Table 3
Comparison of parameters related to CTV across the four groups.

		Group N	Group LF	Group S	Group SS	P Value	N vs			LF vs		S vs
							LF	S	SS	S	SS	SS
CTV (HU)	Men	48.9	47.4	45.6	45.2	**	0.502	**	**	0.394	0.463	1.000
	Women	46.6	44.1	45.5	42.4	**	**	1.000	**	0.955	0.441	0.068
SM × CTV	Men	5384.5	5565.9	4103.8	3909.6	**	1.000	**	**	**	**	1.000
	Women	3899.1	3500.8	3149.5	2550.0	**	*	**	**	0.436	**	0.373

The Kruskal-Wallis test was performed to examine differences in distribution between the groups.
Group N, normal group; Group LF, low functional group; Group S, sarcopenia group; Group SS, severe sarcopenia group.
CTV, CT value; SM, skeletal muscle.
* $p < 0.05$.
** $p < 0.01$.

Table 4
Number of osteopenia and osteoporosis across the four groups.

		Normal	Osteopenia	Osteoporosis	p value
Men	Group N	42	7	7	*
	Group LF	19	3	1	
	Group S	8	7	3	
	Group SS	9	4	6	
Women	Group N	35	37	31	0.202
	Group LF	22	20	24	
	Group S	2	5	9	
	Group SS	4	5	11	

Patients were classified as being normal, having osteopenia, or having osteoporosis according to the primary osteoporosis criteria, based on their young adult mean (YAM) values, and compared among the four groups.
Fisher's exact probability test was used for comparisons.
Group N, normal group; Group LF, low functional group; Group S, sarcopenia group; Group SS, severe sarcopenia group.
* $p < 0.05$.

13.8, 95.0 ± 17.8, 81.5 ± 14.7, and 80.3 ± 14.9; and those of women were 78.0 ± 11.0, 77.2 ± 15.6, 73.3 ± 10.0, 69.5 ± 13.8, respectively.

Only the femoral neck in group SS had a mean value of <70 %, but there were significant differences in YAM values among the four groups for both men and women in both the lumbar spine and femoral neck (men: lumbar spine, $p = 0.008$; femoral neck, $p = 0.008$; women: lumbar spine, $p < 0.001$; femoral neck, $p = 0.016$). In men, the YAM values of both the lumbar spine and femoral neck were significantly lower in group SS than in group LF ($p = 0.012$ and $p = 0.027$, respectively). In women, the YAM values of the lumbar spine were significantly lower in group S than in groups N and LF ($p = 0.021$ and $p = 0.008$, respectively), and that of the femoral neck was significantly lower in group SS than in group N ($p = 0.018$).

4. Discussion

To our knowledge, this is the first study to show an association between sarcopenia severity and measurements of thigh muscle and fat mass. The SM area was significantly smaller in groups S and SS than in groups N and LF for both men and women. Although there was no significant difference between groups S and SS, the SM area decreased as the severity of sarcopenia increased from group N to LF to S. Our results were similar to the previous report that individuals with sarcopenia have a significantly smaller muscle area than healthy individuals [20].

In this study, the results for total thigh and fat areas differed between men and women. In men, the total thigh area was significantly larger in group LF than in any other groups, and there was no difference in the IMAT or SAT area among the four groups. In women, the total thigh area and SAT area were significantly smaller in groups SS and were significantly or near significantly smaller in groups S than in the other two groups. These findings suggest that sarcopenia in men is caused by a decrease in SM mass, whereas that in women is affected by a decrease in SAT and SM. Kasai et al. reported that the age-related decrease in total thigh area was due to a decrease in muscle area in men and a decrease in fat area in women [14], which is consistent with our findings. However, we also observed a report of no difference in thigh area between sarcopenia and non-sarcopenia groups [28], which may be due to differences in the diagnostic criteria used for sarcopenia. In addition, we found that the SAT area and ratio of SAT area to IMAT area were significantly lower in group SS than in group N, but no significant difference was observed in group S, suggesting that SAT was reduced to a greater extent in severe sarcopenia than in sarcopenia. A report on patients with sarcopenia having cirrhosis suggested that fat utilization increases under the conditions of high energy demand before the onset of severe sarcopenia [29], which also supports our results. We speculated that the differences in findings were fewer in men than in women because men have larger total thigh and muscle areas and less fat mass.

A significant decrease in the CTV was observed in men in groups S and SS, and in women in groups LF and SS. In our previous study, we had reported on the CTV according to sarcopenia classification based on the quadriceps muscle alone [21]. While it showed a significant decrease in CTV in men in group SS, the results for women were similar to those of the present study. Therefore, it is suggested that the CTV of the entire thigh muscle, not just that of the quadriceps, may be a better indicator of sarcopenia. It has also been reported that the CTV was significantly

Table 5
Comparison of the YAM values parameters across the four groups.

		Group N	Group LF	Group S	Group SS	p value	N vs			LF vs		S vs
							LF	S	SS	S	SS	SS
Lumbar spine (%)	Men	107.2	113.2	96.4	92.3	**	1.000	0.776	0.080	0.144	*	1.000
	Women	88.0	89.9	74.3	77.5	**	1.000	*	0.061	**	0.061	1.000
Femoral neck (%)	Men	89.7	95.0	81.5	80.3	**	1.000	0.329	0.130	0.071	*	1.000
	Women	78.0	77.2	73.3	69.5	*	1.000	0.595	*	1.000	0.158	1.000

Pearson's chi-square test was used for comparisons.
Group N, normal group; Group LF, low functional group; Group S, sarcopenia group; Group SS, severe sarcopenia group.
YAM, young adult mean.
* $p < 0.05$.
** $p < 0.01$.

lower in patients with severe sarcopenia than in those without sarcopenia [29], suggesting that the CTV may show specificity in severe sarcopenia. In this study, group SS showed a near-significant level of decline compared with group S in women ($p = 0.068$).

Infiltration of the intramuscular adipose tissue is widely accepted as the cause of attenuation of the CTV in muscle tissues [30]. In addition, the IMAT area measures the fat between the muscles. Since these two represent different things, it seems necessary to consider both of them together. Women in group LF had a significantly larger IMAT area than those in the other three groups. The CTV was significantly lower in group LF than in group N, and the SM area was larger in group LF than in groups S and SS. The product of SM area and CTV was also higher in group LF than in group SS, in other words, the percentage of intramuscular fat was higher. Patients in group LF also had larger SAT areas than those in group N, although the difference was not statistically significant. These results suggest that patients in group LF had obesity, with a large amount of fat, although their muscle mass did not decrease. In this study, we were able to capture the characteristics of group LF by examining the distributions of muscle and fat in detail.

Ageing is accompanied by low levels of chronic systemic inflammation. Systemic inflammation and cytokine release cause muscle mass loss, whereas tumor necrosis factor alpha induces sarcopenia [31]. Khoja et al. suggested that a decrease in the SAT storage capacity of adipocytes and the conversion of non-adipocyte progenitors, such as muscle satellite cells, into triglyceride-storing mesenchymal adipocytes, may lead to an increase in intermuscular fat [32]. Goodpaster et al. reported that the distribution of IMAT correlates with insulin resistance and metabolic syndrome, even though it accounts for only 8 % of the total thigh fat [12]. In this study, the number of patients in group LF with a BMI of ≧ 25 was considerably higher than that in the other three groups, indicating that group LF may be a preliminary group for sarcopenic obesity. There are some reports that systemic inflammatory parameters, such as C-reactive protein (CRP) and Interleukin-6, are increased in sarcopenic obesity [33,34]. This study found correlations between IMAT area, CTV, and CRP. Moreover, it has been reported that that inflammation in sarcopenic obesity may reflect muscle quality because CRP is associated with muscle weakness and not muscle mass [35]. Thus, interventions to improve physical function and nutrition in this group, especially in obese patients, may help prevent deterioration and sarcopenia.

Regarding the association between osteoporosis and sarcopenia severity, the number of patients with osteoporosis increased with increasing sarcopenia severity in men; however, no association was found in women.

In this study, the YAM values of the lumber spine and femoral neck were significantly lower in groups S and SS than in groups N and LF. Particularly, there was a significant difference in YAM values between groups LF and SS for both lumbar spine and femoral neck in men. In women, there were also significant differences in YAM values for the lumber spine between groups N and S and between groups LF and S, and for the femoral neck between groups N and SS. These results indicate that there was an association between sarcopenia and osteoporosis in both the evaluation by osteoporosis classification and the evaluation by YAM value. These results agree with several reports of a lower BMD and a higher prevalence of osteoporosis in sarcopenia [28,36], suggesting that the loss of muscle mass has an important impact on bone health.

This study has some limitations. First, because it was not a longitudinal study, we could not assess whether muscle and fat areas changed as sarcopenia progressed over time, or assess changes due to treatment intervention. Second, although we were able to show the characteristics of group LF, we were not able to clearly show the difference between sarcopenia and severe sarcopenia due to the small number of cases. We would like to increase the number of cases in the future to continue our research. Third, osteoporosis was determined on the basis of bone density alone, without the addition of a fracture history. Lastly, because the participants were those who visited the Integrated Healthy Aging Clinic, the data did not include the general population, and the information on healthy controls was weak. Therefore, large-scale epidemiological studies are required in the future.

Despite these limitations, a strength of this study is that we were able to examine the pathogenesis of sarcopenia by using not only muscle mass but also fat mass and CTV as indicators, and we were able to show differences between men and women. Furthermore, we clarified the pathogenesis of LF.

5. Conclusions

We examined the relationship between sarcopenia classification and thigh muscle mass, fat area, muscle CTV, and osteoporosis in Japanese middle-aged and older adults. Both men and women showed a decrease in muscle area with sarcopenia. In women, sarcopenia was associated with a decrease in SAT, and in the group with low physical function, there was an increase in IMAT while SM mass remained unchanged. The CTV was decreased in men with sarcopenia and severe sarcopenia and in women with low physical function and severe sarcopenia. By combining the measurements of IMAT area and CTV, we were able to identify the characteristics of the group with low physical function. We also found an association between sarcopenia and osteoporosis in men.

Funding

This work was supported by a grant from Expenses for Research and Development of Geriatrics and Gerontology (29-12, 20-12); the funding agency played no role in this study.

CRediT authorship contribution statement

Kanae Kuriyama: Writing – original draft, Writing – review & editing, Visualization, Validation. **Yasumoto Matsui:** Conceptualization, Writing – review & editing. **Yasuo Suzuki:** Software, Validation. **Takafumi Mizuno:** Formal analysis. **Tsuyoshi Watanabe:** Resources, Data curation. **Marie Takemura:** Resources, Data curation. **Shinya Ishizuka:** Supervision. **Satoshi Yamashita:** Formal analysis. **Shiro Imagama:** Supervision. **Hidenori Arai:** Supervision, Project administration.

Acknowledgments

We would like to express our gratitude to everyone who cooperated in the study at the National Center for Geriatrics and Gerontology, particularly at the Center for Frailty and Locomotive Syndrome.

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