ORIGINAL ARTICLE

Association of accumulated advanced glycation end-products with a high prevalence of sarcopenia and dynapenia in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: Advanced glycation end-products (AGEs), which are a major cause of diabetic vascular complications, accumulate in various tissues under chronic hyperglycemic conditions, as well as with aging in patients with diabetes. The loss of muscle mass and strength, so-called sarcopenia and dynapenia, has recently been recognized as a diabetic complication. However, the influence of accumulated AGEs on muscle mass and strength remains unclear. The present study aimed to evaluate the association of sarcopenia and dynapenia with accumulated AGEs in patients with type 2 diabetes.

Materials and Methods: We recruited 166 patients with type 2 diabetes aged ≥30 years (mean age 63.2 ± 12.3 years; body mass index 26.3 ± 4.9 kg/m^2; glycated hemoglobin 7.1 ± 1.1%). Skin autofluorescence as a marker of AGEs, limb skeletal muscle mass index, grip strength, knee extension strength and gait speed were assessed.

Results: Sarcopenia and dynapenia were observed in 7.2 and 13.9% of participants, respectively. Skin autofluorescence was significantly higher in patients with sarcopenia and dynapenia. Skin autofluorescence was the independent determinant for skeletal muscle mass index, grip strength, knee extension strength, sarcopenia and dynapenia.

Conclusions: Accumulated AGEs could contribute to reduced muscle mass and strength, leading to sarcopenia and dynapenia in patients with type 2 diabetes.

INTRODUCTION

The increase of elderly patients with type 2 diabetes is a major problem all over the world, especially in Japan. Sarcopenia, an age-related decrease in the strength of skeletal muscle accompanied with the loss of muscular mass, is known to impair physical ability[1,2] and increase the risk of incidental falls[3]. Patients with type 2 diabetes are reportedly candidates for sarcopenia[4,5]. These patients have low muscle mass and strength in the lower extremities despite having identical grip strength to healthy individuals, which might cause mobility limitations[6]. In contrast, an age-related decline in muscle strength before the reduction of muscle mass is proposed to be "dynapenia"[7,8]. However, the clinical impact of dynapenia on physical activity and the risk of incidental falls in patients with type 2 diabetes has not been determined.

Advanced glycation end-products (AGEs), produced by nonenzymatic binding of glucose and proteins, accumulate in various tissues with aging[9–12]. Chronic hyperglycemia also accelerates AGEs accumulation, which causes diabetic vascular complications, such as macro- and micro-angiopathy, through oxidative stress and chronic inflammation[9]. A longer duration of diabetes or sustained hyperglycemia is suggested to be relevant to muscle weakness in patients with diabetes[13]. However, the relationship among AGEs accumulation in patients with

type 2 diabetes and reduced muscle mass and strength, and physical performance is unclear. In addition, the association between AGEs accumulation and dynapenia in patients with type 2 diabetes has not been determined. Therefore, the aim of the present cross-sectional study was to investigate the clinical impact of sarcopenia and dynapenia, and clarify the influence of AGEs accumulation on muscle mass and strength in patients with type 2 diabetes.

MATERIAL AND METHODS

Study design and participants
The present study was approved by the ethics committee of Tokushima University Hospital (approval #2281). The inclusion criteria were outpatients with type 2 diabetes aged ≥30 years at Tokushima University Hospital. Written informed consent was obtained from all participants. Patients who were taking steroids, and those with severe peripheral neuropathy, end-stage renal failure, history of stroke, myopathy and motor functional disorders were excluded. A cross-sectional analysis was carried out of each set of data obtained from July until December 2016.

Assessment of body composition and muscle strength
The body mass index (BMI) was determined by the formula of bodyweight (kg) divided by height squared (m[2]). Percentage body fat (%body fat) and skeletal muscle mass were measured as previously reported using the bioelectrical impedance analysis-based measurements[14], which was shown to have a strong positive correlation with dual-energy X-ray absorptiometry in elderly individuals[3,15]. Skeletal muscle mass index (SMI) was calculated by dividing the upper- and lower-limb skeletal muscle mass by height squared[1,2]. Visceral fat area was measured with a medical visceral fat measuring device using multifrequency bioelectrical impedance analysis (HDS-2000 DUALSCAN; OMRON, Kyoto, Japan).

Grip strength, knee extension strength and gait speed were determined to evaluate muscle strength and physical performance. Upper extremity muscle strength was measured with each hand using maximum isometric grip strength (GRIP-D TKK5401; Takei, Niigata, Japan) in a standing position[1–3]. The highest grip strength value was used as the muscle strength in the present study. A handheld dynamometer (μTas F-1; ANIMA, Tokyo, Japan) was utilized to evaluate maximum isometric knee extension strength, which represents lower extremity muscle strength. The participants sat on a bench and the force sensor was fixed firmly by a belt on the distal end of the tibia to a rigid bar. We defined the maximal isometric knee extension strength as the highest value of three trials[16]. We multiplied the maximal isometric knee extension strength and the lever arm strength to calculate knee extension torque (Nm). The knee extension torque divided by bodyweight (Nm/kg) was calculated to estimate the knee extension strength. Measured body height was used to estimate lever arm strength[17,18]. The total 5-m walk time was recorded as gait speed[1–3].

Definition of sarcopenia and dynapenia
We used the definition of sarcopenia proposed by the Asian Working Group for Sarcopenia[2], which involves low handgrip strength, slow gait speed and low SMI. The cut-off values of low muscle mass, strength and physical performance according to the definition of sarcopenia and dynapenia are shown in Appendix S1. Dynapenia was defined according to the proposed criteria by Clark and Manini as low handgrip strength, low knee extension strength and a normal SMI[7,8,18]. Patients who were not diagnosed with sarcopenia or dynapenia were defined as the control group.

Skin autofluorescence measurement
Skin autofluorescence (AF) has been proposed as a marker of AGEs accumulation in the skin[19]. Skin AF was strongly correlated with the specific AGEs content in skin biopsy samples as reported previously[20,21]. Skin AF was measured by an AGE Reader™ (DiagnOptics Technologies BV, Groningen, the Netherlands), which estimates skin accumulation of AGEs according to the fluorescence properties of AGEs. The measurement protocol was reported in detail previously[19–21]. Skin AF was measured on the ventral side of the forearm three times in series, and the averaged value was utilized in the present study. Repeated skin AF measurements within a day showed an overall Altman error rate of 5.03%[20].

Clinical laboratory data
Glycated hemoglobin (HbA1c), serum high-density lipoprotein cholesterol, serum triglyceride and serum creatinine were examined in the hospital using standard laboratory methods. The duration of diabetes, history of hypertension and dyslipidemia, and use of antidiabetic medications were collected from the medical records of each individual participant. The estimated glomerular filtration rate (eGFR) was calculated using the following equation (eGFR = 194 × serum creatinine [mg/dL] − 1.094 × age − 0.287 [×0.739 for females]), and eGFR <60 mL/min/1.73 m[3] was defined as chronic kidney disease according to the criteria of Japanese Society of Nephrology[22]. Serum pentosidine, one of AGEs in the circulation[10], was measured using a competitive enzyme-linked immunosorbent assay kit. Blood pressure was measured with patients in the supine position.

Diabetic neuropathy was diagnosed in patients who fulfilled at least two of the following criteria: complaint of bilateral sensory symptoms in the toes and soles of the feet (specifically, at least two of the following: numbness, pain and dysesthesia), a bilaterally diminished or absent Achilles tendon reflex, and a bilaterally decreased vibratory sensation in the inner malleolus[23].

Assessment of physical activity and history of falls
The diagnostic survey method with a short version of the International Physical Activity Questionnaire[24,25] was used to estimate physical activity in each patient. The time spent within a

week (7 days) on various activities which, depending on their intensity, were ascribed a specified metabolic equivalent of 1 kcal/kg/h (MET). The International Physical Activity Questionnaire questionnaires collected MET data for each participant. The short version of the International Physical Activity Questionnaire details moderate (four MET) and intensive (eight MET) physical activity, as well as walking (3.3 MET). The data collected from the questionnaires were used to calculate the weekly energy consumption (EC) expressed as kilocalories per week[25]. An interview was carried out about the history of falls within the past year for each participant. A history of falls was defined as one or more falls. A "fall event" was identified for each participant who suddenly lost his/her balance and collapsed[3,26].

Statistical analysis

SPSS Statistics 22 (IBM Japan, Tokyo, Japan) was used for the statistical processing. All data are presented as mean ± standard deviation. Intergroup comparisons (control of non-sarcopenia and dynapenia, sarcopenia, dynapenia) were assessed using an unpaired one-way analysis of variance (continuous variables) or χ^2-test (categorical variables). Uni- and multivariate logistic regression analyses were used to calculate the cross-sectional association of a low SMI, low grip strength, slow gait speed, low knee extension strength, sarcopenia and dynapenia (input of covariates: age, female sex, %body fat, HbA1c, skin AF, serum pentosidine, eGFR, diabetic neuropathy and EC). Using the logistic regression models, the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Using a stepwise procedure, covariates in the multivariate logistic regression analyses were selected. The correlation analysis between skin AF/pentosidine and other clinical parameters used Pearson's correlation coefficient and multiple regression analyses. Statistical significance was defined as a P-value <0.05.

RESULTS

Clinical characteristics of sarcopenia and dynapenia

The total of 166 Japanese participants with type 2 diabetes (99 men, 67 women) aged ≥30 years was enrolled in the present study. The prevalence of sarcopenia, dynapenia and those components are shown in Figure 1. Of all participants with type 2 diabetes, 7.2% were diagnosed with sarcopenia and 13.9% with dynapenia. Patients with type 2 diabetes aged ≥65 years had a significantly higher rate of sarcopenia and dynapenia than those aged <65 years (sarcopenia: $P = 0.033$, dynapenia: $P < 0.001$). The prevalence of low SMI, low grip strength, slow gait speed and low knee extension strength were 11.5, 22.3, 6.6 and 59.0%, respectively. The patients aged ≥65 years had a significantly higher rate of low SMI, low grip strength and low gait speed values than those aged <65 years (low SMI: $P = 0.014$, low grip strength: $P < 0.001$, slow gait speed: $P = 0.001$). In contrast, there was no significant difference in the prevalence of low knee extension strength between patients aged ≥65 and <65 years.

The clinical characteristics of the study patients with sarcopenia and dynapenia are shown in Table 1, and Appendices S2 and S3. Sarcopenic patients were older with longer duration of diabetes, and had a higher rate of incidental falls, skin AF and serum pentosidine, and lower BMI and %body fat than those of control patients. In contrast, dynapenic patients with type 2 diabetes were also older with longer duration of diabetes, and had higher percentage incidence of falls and skin AF, but had higher BMI and % body fat than those of control patients (Table 1). Dynapenic patients had higher BMI, %body fat and visceral fat area, and had a lower skin AF than those of sarcopenic patients (Table 1).

ORs for low muscle mass, strength and physical performance

The ORs of clinical parameters for low muscle mass and strength, and physical performance in patients with type 2 diabetes are shown in Table 2. Skin AF and serum pentosidine were inversely associated with SMI in the univariate model. After the adjustment for all covariates on stepwise regression, skin AF and serum pentosidine remained to be inversely associated with SMI (skin AF: OR 6.38, 95% CI 1.93–21.08; serum pentosidine: OR 1.02, 95% CI 1.00–1.03).

Skin AF and serum pentosidine were also inversely associated with grip strength and knee extension strength, respectively, in the univariate model. Skin AF remained to be inversely associated with grip strength and knee extension strength (grip strength: OR 3.55, 95% CI 1.57–8.00; knee extension strength: OR 3.68, 95% CI 1.87–7.23) after adjustment for all covariates by stepwise regression. The results of the present study showed that skin AF was an independent determinant of low SMI, low grip strength and knee extension strength.

ORs for the risk of sarcopenia and dynapenia

The ORs of the risk for sarcopenia and dynapenia in type 2 diabetes patients are shown in Table 3. Age, skin AF, serum pentosidine, and a decrease in eGFR and %body fat were significantly associated with the prevalence of sarcopenia in a univariate model (Table 3). The association between skin AF and the prevalence of sarcopenia remained significant (skin AF: OR 7.73, 95% CI 2.13–28.02) after the adjustment for all covariates by stepwise regression.

Age, %body fat and skin AF were significantly associated with the prevalence of dynapenia in a univariate analysis. Percentage body fat and skin AF are significantly associated with the prevalence of dynapenia (%body fat: OR 1.11, 95% CI 1.03–1.19; skin AF: OR 3.03, 95% CI 1.07–8.54) after the adjustment for all covariates by stepwise regression. Elevation of skin AF was the independent risk factor for both sarcopenia and dynapenia.

In contrast, the use of antidiabetic agents was not significantly associated with sarcopenia nor dynapenia on the univariate and multivariate analyses (Appendix S3).
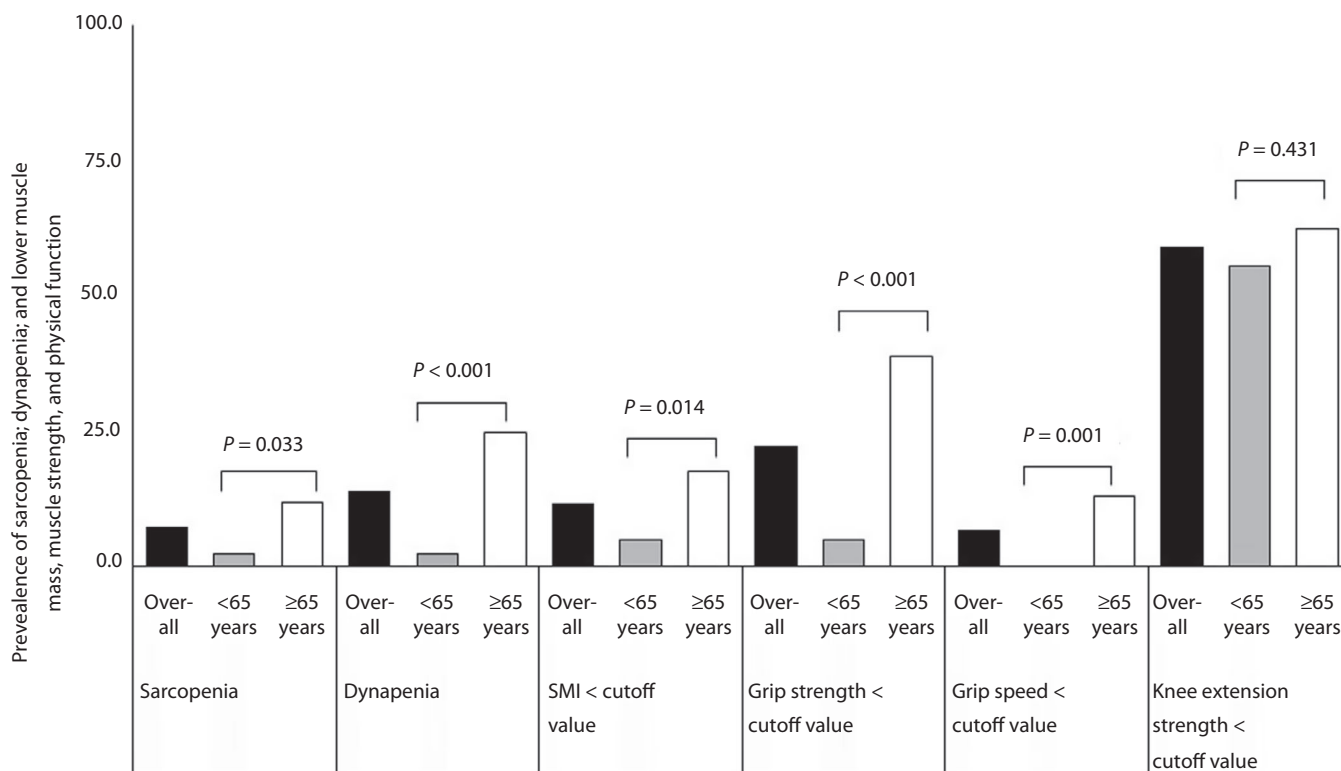
Sarcopenia: Overall; 12/166 (7.2%) <65 years; 2/81 (2.5%), ≥65 years; 10/85 (11.8%)

Dynapenia: Overall; 23/166 (13.9%) <65 years; 2/81 (2.5%), ≥65 years; 21/85 (24.7%),

SMI < cutoff value: Overall; 19/166 (11.5%) <65 years; 4/81 (4.9%), ≥65 years; 15/85 (17.7%)

Grip strength < cutoff value: Overall; 37/166 (22.3%) <65 years; 4/81 (4.9%), ≥65 years; 33/85 (38.8%)

Grip speed < cutoff value: Overall; 11/166 (6.6%) <65 years; 0/81 (0.0%), ≥65 years; 11/85 (12.9%)

Knee extension strength < cutoff value: Overall; 98/166 (59.0%) <65 years; 45/81 (55.6%), ≥65 years; 53/85 (62.4%)

Figure 1 | Prevalence of sarcopenia, dynapenia and those components.

Correlation analysis between skin AF/pentosidine and other clinical parameters

The correlation analysis between skin AF/pentosidine and other clinical parameters is shown in Appendix S4. Skin AF was significantly correlated with circulating serum pentosidine, and these markers of AGEs were correlated with age, duration of diabetes and decrease in eGFR. In addition, skin AF was inversely correlated with BMI and EC. Multiple regression analyses showed that the age, duration of diabetes and EC were independent determinants of skin AF (age: $\beta = 0.189$, $P = 0.025$, duration of diabetes: $\beta = 0.165$, $P = 0.049$, EC: $\beta = -0.208$, $P = 0.010$), and the duration of diabetes and eGFR were significantly associated with serum pentosidine level (duration of diabetes: $\beta = 0.195$, $P = 0.018$, eGFR: $\beta = -0.279$, $P = 0.001$).

DISCUSSION

Here, we showed for the first time that dynapenia is observed approximately twofold more frequently than sarcopenia, and these complications are associated with a comparable incidence of incidental falls in patients with type 2 diabetes. The typical clinical characteristics of dynapenia are high %body fat, as well as aging. In addition, accumulated AGEs were significantly associated with low muscle strength, as well as low muscle mass, and related to the prevalence of sarcopenia and dynapenia in patients with type 2 diabetes.

The previous studies showed that the prevalence of sarcopenia was 4.1–11.5% in the general elderly population[27] and 14.8% in type 2 diabetespatients[28] according to the Asian Working Group for Sarcopenia criteria. In contrast, the prevalence rate of dynapenia, defined by low handgrip strength and normal SMI, was 35.0% in men (mean age 83.8 ± 6.8 years) and 27.1% in women (mean age 82.5 ± 6.4 years) of general elderly Japanese aged ≥65 years, and those were twofold higher than those of sarcopenia (men 25.0%, women 22.7%)[29]. In the present study, we confirmed an equivalent prevalence rate of sarcopenia in patients with type 2 diabetes, and that the

Table 1 | Clinical characteristics of the study patients with sarcopenia and dynapenia

	Control (*n* = 131)	Sarcopenia (*n* = 12)	*P*-value (control vs sarcopenia)	Dynapenia (*n* = 23)	*P*-value (control vs dynapenia)	*P*-value (sarcopenia vs dynapenia)
Age (years)	60.7 ± 11.8	70.8 ± 12.6	0.006	73.0 ± 7.6	<0.001	0.523
Female (%)	37.4	33.3	1.000	60.9	0.041	0.164
Incident of fall (%)	16.0	58.3	0.002	47.8	0.001	0.725
BMI (kg/m^2)	26.6 ± 4.7	20.3 ± 2.5	<0.001	27.3 ± 5.2	0.550	<0.001
%Body fat (%)	30.2 ± 8.0	22.5 ± 8.0	0.002	34.6 ± 8.6	0.016	<0.001
Visceral fat area (cm^2)	109 ± 50	52 ± 38	0.002	121 ± 56	0.371	0.004
Duration of diabetes (years)	11.0 ± 7.6	18.3 ± 11.9	0.003	17.0 ± 10.1	0.012	0.657
HbA1c (%)	7.1 ± 1.1	7.1 ± 0.9	0.812	7.2 ± 0.8	0.943	0.711
Skin AF (AU)	2.57 ± 0.52	3.34 ± 0.70	<0.001	2.91 ± 0.54	0.005	0.049
Pentosidine (μg/mL)	0.0653 ± 0.0312	0.1025 ± 0.0609	<0.001	0.0766 ± 0.0499	0.257	0.326
TG (mg/dL)	168 ± 184	127 ± 47	0.462	182 ± 17	0.562	0.327
HDL (mg/dL)	55 ± 16	62 ± 28	0.394	51 ± 13	0.314	0.103
Dyslipidemia (%)	50.4	16.7	0.033	47.8	1.000	0.139
SBP (mmHg)	139 ± 20	137 ± 13	0.687	129 ± 30	0.051	0.389
DBP (mmHg)	84 ± 15	74 ± 5	<0.001	77 ± 8	0.001	0.348
Hypertension (%)	55.7	41.7	0.38	56.5	1.000	0.489
Diabetic neuropathy (%)	55.0	58.3	1.000	52.2	0.824	1.000
eGFR (mL/min/1.73 m^3)	73.7 ± 23.4	58.4 ± 19.2	0.030	53.3 ± 20.5	<0.001	0.446
EC (kcal/day)	123 ± 186	24 ± 43	<0.001	73 ± 88	0.048	0.104

Data are shown as the mean value ± standard deviation. %Body fat, percentage of body fat; AF, autofluorescence; BMI, body mass index; DBP, diastolic blood pressure; EC, energy consumption of three or more metabolic equivalents; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride.

prevalence rate of dynapenia was also approximately twofold higher than that of sarcopenia. However, the average age of patients with type 2 diabetes in the present study (73.9 ± 6.6 years) was approximately 10 years younger than the previous reports[29]. In addition, 38.8 and 62.4% of patients with type 2 diabetes aged ≥65 years showed low handgrip and knee extension strength in the present study, respectively, and this prevalence was higher than that of the Japanese general elderly population[30]. It is possible that type 2 diabetes might complicate muscle weakness at a younger age than the general population. Therefore, the typical characteristics of muscle dysfunction in elderly patients with type 2 diabetes could be lower muscle strength in the extremities, but not a decrease in muscle volume compared with individuals without type 2 diabetes. Furthermore, impaired knee extension strength was observed frequently, even at the age of <65 years (Figure 1), suggesting that the muscle strength of lower limbs could be a better indicator of muscle dysfunction than the grip strength in patients with type 2 diabetes.

Sarcopenia is known to be a risk factor for incidental falls in general elderly people[31]. In the present study, the complication of dynapenia increased the incidence of falls, as well as sarcopenia (47.8% vs 58.3%) in patients with type 2 diabetes. Therefore, we should be aware of dynapenia, as well as sarcopenia, to prevent incidental falls and fractures among elderly patients with type 2 diabetes.

Sarcopenia and dynapenia were observed in elderly patients with longer duration of diabetes, and the difference of clinical characteristics between these comorbidities was adiposity, such as BMI, %body fat and visceral fat area. Sarcopenic patients showed low BMI, whereas dynapenic patients showed comparable BMI with patients without sarcopenia and dynapenia. A previous study reported that obese patients with type 2 diabetes had lower muscle strength than healthy normal bodyweight participants[5,6]. The accumulation of intramuscular fat is inversely associated with lower limb muscle function in elderly individuals[32]. In contrast, increased bodyweight could be an overload to maintain muscle volume. Indeed, high %body fat was significantly and independently associated with the risk of dynapenia in patients with type 2 diabetes in the present study. Elderly obese patients with type 2 diabetes, therefore, might have a higher prevalence of dynapenia, but not sarcopenia. However, because the intramuscular fat, body fat mass by site and adipokine levels were not evaluated, the detailed mechanism for developing dynapenia could not be clarified in the present study.

AGEs, which are produced through non-enzymatic glycation, are accumulated in various tissues under the diabetic condition; that is, chronic hyperglycemia[9–12]. Skin AF, representing the accumulation of AGEs, was inversely associated with SMI, grip strength and knee extension strength in an adjusted multivariate logistic regression model in the present study. As reported previously, reduced muscle mass, regarded as the primary phenotype of sarcopenia, is associated with accumulated AGEs in humans as they age[33]. Oxidative stress and inflammatory

Table 2 | Odds ratios of lower muscle mass, strength and physical function in patients with type 2 diabetes

	SMI < cut-off value						Grip strength < cut-off value					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.05	1.01–1.10	0.028	NS			1.11	1.06–1.16	<0.001	1.09	1.04–1.15	<0.001
Female	NS			NS			NS			NS		
%Body fat	0.85	0.78–0.91	<0.001	0.85	0.77–0.92	<0.001	NS			NS		
HbA1c	NS			NS			NS			NS		
Skin AF	7.4	2.82–19.45	<0.001	6.38	1.93–21.08	0.002	4.65	2.22–9.73	<0.001	3.55	1.57–8.00	0.02
Pentosidine	1.02	1.01–1.03	<0.001	1.02	1.00–1.03	0.013	1.01	1.00–1.02	0.009	NS		
eGFR	NS			NS			0.96	0.94–0.98	<0.001	0.97	0.95–0.99	0.023
Diabetic neuropathy	NS			NS			NS			NS		
EC	NS			NS			NS			NS		

	Gait speed < cutoff value						Knee extension strength < cutoff value					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Age	1.15	1.05–1.25	0.002	1.16	1.06–1.27	0.002	NS			NS		
Female	NS			NS			2.78	1.42–5.42	0.003	NS		
%Body fat	NS			NS			1.08	1.04–1.13	<0.001	1.10	1.05–1.15	<0.001
HbA1c	NS			NS			NS			NS		
Skin AF	NS			NS			2.68	1.46–4.95	0.002	3.68	1.87–7.23	<0.001
Pentosidine	NS			NS			1.01	1.00–1.02	0.039	NS		
eGFR	0.96	0.93–0.99	0.012	NS			NS			NS		
Diabetic Neuropathy	NS			NS			2.09	1.11–3.92	0.022	NS		
EC	NS			NS			NS			NS		

%Body fat, percentage of body fat; AF, autofluorescence; EC, energy consumption of three or more metabolic equivalents; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS, not significant.

Table 3 | Odds ratios of sarcopenia and dynapenia complications in type 2 diabetes patients

	Sarcopenia						Dynapenia					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.09	1.02–1.17	0.01	NS			1.13	1.07–1.21	<0.001	1.14	1.07–1.21	<0.001
Female	NS			NS			2.6	1.05–6.46	0.039	NS		
%Body fat	0.89	0.82–0.96	0.003	0.91	0.83–0.99	0.045	1.07	1.01–1.14	0.018	1.11	1.03–1.19	0.004
HbA1c	NS			NS			NS			NS		
Skin AF	10.08	3.08–32.95	<0.001	7.73	2.13–28.02	0.002	3.41	1.42–8.21	0.006	3.03	1.07–8.54	0.036
Pentosidine	1.02	1.01–1.04	0.002	1.02	1.00–1.03	0.017	NS			NS		
eGFR	0.97	0.94–0.99	0.031	NS			NS			NS		
Diabetic Neuropathy	NS			NS			NS			NS		
EC	NS			NS			NS			NS		

%Body fat, percentage of body fat; AF, autofluorescence; EC, energy consumption of three or more metabolic equivalents; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS, not significant.

cytokines are increased by AGEs[34]. In addition, AGEs induce cross-link and breakdown of muscular protein in elderly humans and a rodent aging model[35]. The expression of AGEs receptors in muscle tissue is increased with aging[36,37], suggesting that the intracellular signaling of AGEs–AGEs receptors is accelerated in the muscle of elderly patients with diabetes. AGEs levels in the circulation and in the various tissues are increased under the diabetic condition[9–12]. AGEs accumulation in the hind limb muscles of diabetic mice is associated with the decline in muscle mass, muscle endurance and regenerative capacity[38]. Furthermore, it was reported in a human study that AGEs accumulation in the fast twitch muscle fibers cross-links with muscle collagen, increases muscle stiffness and reduces the tonic force of the muscle contraction[14,39,40]. Thus, it is suggested that accumulated AGEs levels are associated with muscle mass reduction and sarcopenia in patients with type 2 diabetes.

HbA1c was not associated with low muscle mass, grip strength and knee extension strength in the present study. We recently showed that lower limb muscle strength was inversely associated with skin AF, but not associated with HbA1c in patients with type 1 diabetes[14]. It was also reported recently that skin AF reflects integration over the past 15 years' long-term glycemic control, but not present glycemic control in patients with type 1 diabetes[41]. These results suggest that muscle weakness reflects long-term glycemic control in both patients with type 1 and type 2 diabetes. The serum pentosidine was not inversely associated with muscle strength on an adjusted multivariate logistic regression model (including covariate of skin AF) in the present study. The duration of type 2 diabetes was positively correlated with skin AF and serum pentosidine, but not HbA1c significantly (Appendix S4). Also, serum pentosidine was positively correlated with skin AF. Serum pentosidine was inversely associated with eGFR. Taken together, it is suggested that chronic hyperglycemia, aging and a long duration of disease increase the accumulation of AGEs, which could contribute to impaired muscle strength in patients with type 2 diabetes according to the present study. Improvements of glycemic control could attenuate the accumulation of AGEs. Skin AF was inversely associated with EC. Physical inactivity is a related factor for obesity and type 2 diabetes, both of which can accelerate the formation and accumulation of AGEs. In the present study, patients with type 2 diabetes were associated with a high prevalence of sarcopenia and dynapenia, even for those aged <65 years. Therefore, the early diagnosis of muscle weakness is important, and good glycemic control and weight management with exercise and dietary intervention might be beneficial to prevent the progression of muscle weakness in these patients.

Several studies have showed that the use of statins is associated with muscle weakness[42], but such a finding was not consistent in the other studies, including the present study. In addition, the participants in the present study had type 2 diabetes, which could be a strong inducer of muscle weakness. Therefore, we could not find any relationship between the use of statins and the prevalence of sarcopenia or dynapenia in the present study.

The present study had several limitations. First, it is impossible to infer causality because of the cross-sectional design, although we showed a significant association between AGEs accumulation and sarcopenia and dynapenia in patients with type 2 diabetes. Second, there was no information about what the participants ate, which could affect both muscle mass and strength. Insufficient protein intake might result in the progression of sarcopenia or decline in muscle mass[1,2]. Third, we could not evaluate biomarkers of inflammation (such as tumor necrosis factor-α, interleukin-6), growth factors (such as insulin-like growth factor) or adipokines (such as leptin, adiponectin)[43]. Finally, the small sample size of participants from a single hospital in Japan was a major limitation to generalizing this result. Therefore, further study is necessary to clarify the prevalence rate of dynapenia, and the relationship between AGEs and muscle weakness in a multi-institutional cohort study.

In conclusion, the prevalence rate of dynapenia was higher than sarcopenia. Dynapenia tended to be observed in obese patients with type 2 diabetes. Finally, accumulated AGEs was an independent factor for sarcopenia and dynapenia in patients with type 2 diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, *et al*. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010; 39: 412–423.
2. Chen LK, Liu LK, Woo J, *et al*. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc* 2014; 15: 95–101.
3. Tanimoto Y, Watanabe M, Sun W, *et al*. Sarcopenia and falls in community-dwelling elderly subjects in Japan: defining sarcopenia according to criteria of the European Working Group on Sarcopenia in Older People. *Arch Gerontol Geriatr* 2014; 59: 295–299.
4. Park SW, Goodpaster BH, Lee JS, *et al*. Health, aging, and body composition study. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care* 2009; 32: 1993–1997.
5. Park SW, Goodpaster BH, Strotmeyer ES, *et al*. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. *Diabetes* 2006; 55: 1813–1818.

6. Park SW, Goodpaster BH, Strotmeyer ES, et al. Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes Care 2007; 30: 1507–1512.

7. Clark BC, Manini TM. Sarcopenia ≠ dynapenia. J Gerontol A Biol Sci Med Sci 2008; 63: 829–834.

8. Manini TM, Clark BC. Dynapenia and aging: an update. J Gerontol A Biol Sci Med Sci 2012; 67: 28–40.

9. Singh R, Barden A, Mori T, et al. Advanced glycation end-products: a review. Diabetologia 2001; 44: 129–146.

10. Beisswenger PJ, Makita Z, Curphey TJ, et al. Formation of immunochemical advanced glycosylation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. Diabetes 1995; 44: 824–829.

11. Monnier VM, Vishwanath V, Frank KE, et al. Relation between complications of type 1 diabetes mellitus and collagen-linked fluorescence. N Engl J Med 1986; 314: 403–408.

12. Genuth S, Sun W, Cleary P, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. Diabetes 2005; 54: 3103–3111.

13. Kalyani RR, Metter EJ, Egan J, et al. Hyperglycemia predicts persistently lower muscle strength with aging. Diabetes Care 2015; 38: 82–90.

14. Mori H, Kuroda A, Araki M, et al. Advanced glycation end-products are a risk for muscle weakness in Japanese patients with type 1 diabetes. J Diabetes Investig 2017; 8: 377–382.

15. Ling CH, de Craen AJ, Slagboom PE, et al. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. Clin Nutr 2011; 30: 610–615.

16. Nomura T, Ishiguro T, Ohira M, et al. Diabetic polyneuropathy is a risk factor for decline of lower extremity strength in patients with type 2 diabetes. J Diabetes Investig 2018; 9: 186–192.

17. Hayakawa M, Sakurai Y, Kato E. Estimation of basal energy expenditure predicted by the tibial length in Japanese elderly patients. Jap J Surg Metab Nutr 2003; 37: 297–304 (Japanese).

18. Manini TM, Visser M, Won-Park S, et al. Knee extension strength cutpoints for maintaining mobility. J Am Geriatr Soc 2007; 55: 451–457.

19. Meerwaldt R, Graaff R, Oomen PHN, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia 2004; 47: 1324–1330.

20. Meerwaldt R, Links T, Graaff R, et al. Simple noninvasive measurement of skin autofluorescence. Ann N Y Acad Sci 2005; 1043: 290–298.

21. Lutgers HL, Graaff R, Links TP, et al. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. Diabetes Care 2006; 29: 2654–2659.

22. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009; 53: 982–992.

23. Yasuda H, Sanada M, Kitada K, et al. Rationale and usefulness of newly devised abbreviated diagnostic criteria and staging for diabetic polyneuropathy. Diabetes Res Clin Pract 2007; 77: 178–183.

24. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003; 35: 1381–1395.

25. IPAQ Research Committee. International physical activity questionnaire. Available from: https://sites.google.com/site/theipaq/scoring-protocol. Accessed July 30, 2016.

26. Morris JN, Fries BE, Bernabei R, et al. RAI – Home Care Assessment Manual. Washington, DC: InterRAI Coporation, 1996.

27. Chen LK, Lee WJ, Peng LN, et al. Asian Working Group for Sarcopenia. Recent advances in sarcopenia research in Asia: 2016 update from the Asian Working Group for Sarcopenia. J Am Med Dir Assoc 2016; 17: 767, e1–7.

28. Wang T, Feng X, Zhou J, et al. Type 2 diabetes mellitus is associated with increased risks of sarcopenia and pre-sarcopenia in Chinese elderly. Sci Rep 2016; 6: 38937.

29. Yamada M, Kimura Y, Ishiyama D, et al. Differential characteristics of skeletal muscle in community-dwelling older adults. J Am Med Dir Assoc. 2017; 18: 807, e9–807.

30. Yuki A, Ando F, Otsuka R, et al. Epidemiology of sarcopenia in elderly Japanese. J Phys Fit Sports Med 2015; 4: 111–115.

31. Niino N, Tsuzuku S, Ando F, et al. Frequencies and circumstances of falls in the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 2000; 10: S90–S94.

32. Akima H, Yoshiko A, Tomita A, et al. Relationship between quadriceps echo intensity and functional and morphological characteristics in older men and women. Arch Gerontol Geriatr 2017; 70: 105–111.

33. Drenth H, Zuidema S, Bunt S, et al. The Contribution of Advanced Glycation End product (AGE) accumulation to the decline in motor function. Eur Rev Aging Phys Act 2016; 13: 3.

34. Haus JM, Carrithers JA, Trappe SW, et al. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. J Appl Physiol 2007; 103: 2068–2076.

35. Snow LM, Fugere NA, Thompson LV. Advanced glycation end-product accumulation and associated protein modification in type II skeletal muscle with aging. J Gerontol A Biol Sci Med Sci 2007; 62: 1204–1210.

36. de la Maza MP, Uribarri J, Olivares D, et al. Weight increase is associated with skeletal muscle immunostaining for advanced glycation end products, receptor for advanced

glycation end products, and oxidation injury. *Rejuvenation Res* 2008; 11: 1041–1048.

37. Payne GW. Effect of inflammation on the aging microcirculation: impact on skeletal muscle blood flow control. *Microcirculation* 2006; 13: 343–352.

38. Chiu CY, Yang RS, Sheu ML, *et al.* Advanced glycation end-products induce skeletal muscle atrophy and dysfunction in diabetic mice via a RAGE-mediated, AMPK-down-regulated, Akt pathway. *J Pathol* 2016; 238: 470–482.

39. Ramamurthy B, Hook P, Jones AD, *et al.* Changes in myosin structure and function in response to glycation. *FASEB J* 2001; 15: 2415–2422.

40. Momma H, Niu K, Kobayashi Y, *et al.* Skin advanced glycation end product accumulation and muscle strength among adult men. *Eur J Appl Physiol* 2011; 111: 1545–1552.

41. Sugisawa E, Miura J, Iwamoto Y, *et al.* Skin autofluorescence reflects integration of past long-term glycemic control in patients with type 1 diabetes. *Diabetes Care* 2013; 36: 2339–2345.

42. Kawai H, Ihara K, Kera T, *et al.* Association between statin use and physical function among community-dwelling older Japanese adults. *Geriatr Gerontol Int* 2018; 18: 623–630.

43. McKee A, Morley JE, Matsumoto AM, *et al.* Sarcopenia: an endocrine disorder? *Endocr Pract* 2017; 23: 1140–1149.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1 | Cut-off value of low muscle mass, strength and physical performance according the sarcopenia and dynapenia definitions.

Appendix S2 | Muscle mass, strength and physical performance of the study patients by the presence of sarcopenia and dynapenia.

Appendix S3 | Medication for diabetes of the study patients by the presence of sarcopenia and dynapenia.

Appendix S4 | The correlation analysis between skin autofluorescence/pentosidine and other clinical parameters.