ORIGINAL ARTICLE

Association between sarcopenia and the severity of diabetic polyneuropathy assessed by nerve conduction studies in Japanese patients with type 2 diabetes mellitus

Kentaro Mikura* [ID], Eriko Kodama, Tatsuya Iida, Hideyuki Imai, Mai Hashizume, Yasuyoshi Kigawa, Rie Tadokoro, Chiho Sugisawa, Kei Endo, Toru Iizaka, Fumiko Otsuka, Shoichiro Nagasaka

Division of Diabetes, Metabolism, and Endocrinology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan

*Correspondence
Kentaro Mikura
Tel.: +81-45-971-1151
Fax: +81-45-973-1019
E-mail address:
fresh_brash_new_man@yahoo.co.jp

ABSTRACT

Aims/Introduction: This study examined the association between the severity of diabetic polyneuropathy (DPN) based on the Baba classification, and sarcopenia and its related factors.

Materials and Methods: The participants were 261 patients with type 2 diabetes mellitus. DPN was classified as stages 0–4 according to the Baba classification. Sarcopenia was diagnosed based on measurements of the skeletal mass index, grip strength and walking speed, using the Asia Working Group for Sarcopenia 2019 diagnostic criteria.

Results: The median age of the participants was 67 years, the proportion of men was 58.6%, the median estimated duration of diabetes was 10 years and the median values for glycated hemoglobin were 10.3%. With regard to DPN, the prevalence of Baba classification stages 0–2 was 90.8% ($n = 237$), and that of stage 3 or 4 was 9.2% ($n = 24$). The prevalence of sarcopenia was 19.9%. A trend toward an increase in the frequency of slow walking speed was seen as the stage of DPN progressed. The frequencies of sarcopenia and slow walking speed were higher in the group with the Baba classification stages 3 and 4 than in the group with stages 0–2. On multiple logistic regression analyses, however, DPN was not significantly related to sarcopenia and walking speed.

Conclusions: Although severe DPN might be related to sarcopenia, the frequency of severe DPN is low in the clinical setting, indicating that its contribution to sarcopenia is modest.

INTRODUCTION

Diabetic polyneuropathy (DPN) is the earliest complication of microangiopathy to occur in diabetes mellitus[1]. In Japan, the diagnostic criteria for DPN based on subjective symptoms, the Achilles tendon reflex and vibratory sensation are widely used as convenient methods of diagnosing DPN in the clinical setting[2]. Pathological evaluations involving the comparison of electrophysiological findings based on nerve conduction studies (NCS) and neurohistological findings have been described previously[3,4], and NCS are useful for early diagnosis, determining severity and differentiating from other pathological conditions

that can cause neurological disorders[5]. In Japan, the Baba classification was proposed as a means of diagnosing DPN using NCS, and it is useful for determining severity[2].

The number of elderly patients with diabetes mellitus is increasing throughout the world[6], and analyzing the pathophysiology based on loss of skeletal muscle mass, referred to as sarcopenia or frailty, is therefore important. Rosenberg defined sarcopenia as age-related loss of muscle mass[7]. Sarcopenia was defined by the European Working Group on Sarcopenia in Older People in 2010 as being a progressive and systemic loss of muscle mass and muscle strength that is associated with a risk of low physical function, poor quality of life and death[8]. In Asia, the Asian Working Group for Sarcopenia (AWGS)

developed diagnostic criteria for sarcopenia based on evidence in Asian people, and these criteria were revised in 2019[9].

Both sarcopenia and type 2 diabetes mellitus are progressive disorders, and the factors common to both include aging, microangiopathy, macroangiopathy and an increase in advanced glycation end-products resulting from insulin resistance[10]. Several previous studies have examined the associations between DPN and muscle strength, as well as physical function. The Health ABC Study found that impaired peripheral nerve function assessed by NCS is involved in low physical performance (assessed by chair stands, the standing balance ratio and walking speed) of elderly patients with diabetes mellitus[11]. Nomura et al.[12] evaluated the association between lower extremity strength as evaluated by knee extension force and DPN in Japanese patients with type 2 diabetes mellitus, and showed that DPN contributed to decreased knee extension force in patients aged ≥50 years.

Although these studies examined the associations between DPN and muscle strength, as well as physical performance, there have been few reports of the association between DPN progression and sarcopenia[13]. To test the hypothesis that the severity of DPN is related to sarcopenia, the present study examined the association between severe DPN based on the Baba classification and sarcopenia and its related factors.

MATERIALS AND METHODS
Participants
The participants were 261 Japanese patients with type 2 diabetes mellitus who were admitted to Showa University Fujigaoka Hospital, Yokohama, Japan, for treatment of diabetes mellitus, and underwent NCS between January 2017 and December 2019. Excluded from the study were patients with a past history of cerebrovascular diseases or orthopedic disorders (e.g., cerebral infarction, peripheral arterial disease, carpal tunnel syndrome, lumbar and sacral neuropathy, lumbar spinal stenosis, cervical spondylosis), patients who had been admitted for treatment of diabetic ketoacidosis or infection and patients who had received drug therapy to improve a neurological disorder. In particular, the ankle-brachial index was measured to assess peripheral arterial disease, and ankle-brachial index >1.0 was confirmed in all participants. Patients were also excluded if conduction impairment of the median nerve (measured in all participants) and/or numbness of fingers, suggesting carpal tunnel syndrome, were present.

Whether the participant consumed alcohol or smoked was determined according to whether the response was "yes" or "no" during history taking. Patients who were receiving drug therapy for hypertension or dyslipidemia at admission were considered to have these conditions, and those who were not receiving such therapy were considered not to have these conditions.

Diabetic nephropathy was classified as prenephropathy, incipient and overt nephropathy or kidney failure using the criteria of the Joint Committee on Diabetic Nephropathy[14], and nephropathy was considered present if it was incipient nephropathy or higher. Dialysis patients were excluded. Diabetic retinopathy was classified as none, simple, preproliferative or proliferative retinopathy using the modified Davis classification[15], and retinopathy considered present if it was simple diabetic retinopathy or higher.

The study was carried out as a retrospective analysis of data obtained in treatment, and the informed consent of the individual patients was not obtained. The patients were given an opportunity to refuse having their treatment data used in the study. The study was carried out after being approved by the local ethics committee of Showa University Fujigaoka Hospital (approval no. 2016-63).

Diagnosis of DPN
The Baba classification of NCS was used to diagnose DPN and classify its severity[2]. NCS was carried out using an electromyography/evoked potential measurement system (Neuropack X1®; MEB-2300, Nihon Kohden, Tokyo, Japan) in an examination room maintained at a temperature of 26°C. To eliminate the methodological error between measurements, all patients were tested by the same physician (KM). For all of the nerves measured, the contact resistance of each electrode was sufficiently decreased, and the electrical stimulus was applied at a supramaximal level. The measurement method was implemented as described by Yanagida et al.[16] The nerves measured were the tibial nerve, where the compound muscle action potential, motor conduction velocity, F wave latency and A wave were measured, and the sural nerve, where the sensory conduction velocity and sensory nerve action potential were measured. These measurement parameters were used to classify DPN according to the Baba classification stages 0–4. The severity categories of the Baba classification are shown in Figure S1.

Diagnosis of autonomic neuropathy
Autonomic neuropathy was assessed by the coefficient of variation of the R-R interval (CV R-R) using electrocardiograph (Cardiofax ECG-1550; Nihon Kohden, Tokyo, Japan). After a 15-min rest, the mean (mean R-R) and standard deviation (SD R-R) of R-R intervals were recorded from 100 consecutive resting heart rates in the supine position. The CV R-R was calculated using the following equation; CV R-R = SD R-R / mean R-R × 100. Autonomic neuropathy was considered present if CV R-R was lower than the reference values based on age and sex, as described previously[17,18].

Diagnosis of sarcopenia
Sarcopenia was diagnosed based on the AWGS 2019 diagnostic criteria[9]. The evaluation parameters were grip strength as a measure of muscle strength, 10-m walking speed as a measure of physical performance and skeletal muscle mass (skeletal mass index [SMI]). Grip strength was measured using a digital grip strength meter (T.K.K 5401; Takei Scientific Instruments, Co., Ltd., Niigata, Japan). The measurement was carried out twice

for each hand with participants standing, and the maximum value was used. For the measurement of walking speed, the time required to transverse a 10-m measurement area with 3 m added on either end was measured, while participants were asked to walk at their usual speed. SMI was measured by bioelectrical impedance analysis using a body composition analysis system (InBody 770; InBody Japan, Tokyo, Japan). Of the measured muscle mass, the SMI was defined as the total muscle mass of the upper and lower extremities divided by the square of the height. Sarcopenia was defined as low SMI and either low grip strength or slow walking speed. Based on the AWGS 2019 diagnostic criteria[9], low SMI was defined as <7.0 kg/m^2 for men and <5.7 kg/m^2 for women, low grip strength was defined as <28 kg for men and <18 kg for women, and slow walking speed was defined as <1.0 m/s.

International physical activity questionnaire

The physical activity level was evaluated using the International Physical Activity Questionnaire (IPAQ). The IPAQ is a questionnaire developed by a World Health Organization working group to evaluate physical activity levels using an internationally uniform set of standards[19]. The IPAQ asks how many days and for how much time the respondent engages in physical activity in an average week. There are long and short versions of this questionnaire, and Murase *et al.* have prepared Japanese-language versions of them[20]. The short version of the Japanese-language questionnaire was used in the present study. The IPAQ short version asks how many days and for how much time the respondent engages in three categories of physical activity: vigorous exercise, moderate exercise, and walking. The exercise level is then calculated using the total time and metabolic equivalents for each category.

Statistical analysis

The data are expressed as medians and interquartile ranges for continuous variables, and as numbers or percentages for categorical variables. First, the baseline characteristics, sarcopenia, and its related factors were compared for each stage of the Baba classification stages 0–4. The methods used in the comparison were the Kruskal–Wallis test and the Cochran–Armitage trend test. Examination of each Baba classification stage showed that the frequencies of microangiopathy, sarcopenia and its related factors increased in stages 3 and 4. Baba *et al.*[21] also reported an increased incidence of diabetic foot and macroangiopathic events in patients with the Baba classification stages 3 and 4. Consequently, the baseline characteristics, sarcopenia and its related factors were compared for two groups: one with the Baba classification stages 0–2; and one with stages 3 and 4. The methods used in the comparison were the Wilcoxon test and Fisher's exact test. Next, risk factors for sarcopenia and slow walking speed were identified by univariate logistic regression analysis. Based on theoretical considerations, age, sex, estimated duration of diabetes, glycated hemoglobin, body mass index, alcohol consumption, smoking, IPAQ score,

and the presence of autonomic neuropathy, DPN (stages 3 and 4), retinopathy and nephropathy were included in these analyses. By using the factors identified in the univariate logistic regression analysis ($P < 0.25$ in these analyses), a multivariate logistic regression analysis was carried out. The Wald test was used to test the odds ratios and 95% confidence intervals of the logistic regression analysis. Additional analyses were similarly carried out in patients aged ≥65 years. A significance level of P-value <0.05 was used. The statistical analysis was carried out using JMP Pro version 15.0.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Participants' characteristics

The median age of the participants was 67 years (Table 1). The proportion of men was 58.6%, the median estimated duration of diabetes was 10 years, the median glycated hemoglobin level at admission was 10.3% and the median body mass index (BMI) was 24.3 kg/m^2. The median IPAQ was 792 metabolic equivalents·min/week. The prevalence of autonomic neuropathy was 32.2%. The prevalence of DPN was 18.8% for the Baba classification stage 0 ($n = 49$), 34.5% for stage 1 ($n = 90$), 37.5% for stage 2 ($n = 98$), 6.5% for stage 3 ($n = 17$) and 2.7% for stage 4 ($n = 7$). The prevalence of retinopathy was 31.8%, and that of nephropathy was 31.4%. The prevalence of low SMI was 31.0%, and that of low grip strength was 30.3%. Slow walking speed was seen in 21.1% of the participants, and the prevalence of sarcopenia was 19.9%. The NCS results are shown in Table 1.

Participants' characteristics by the severity categories of the Baba classification are shown in Table 2. Age and the estimated duration of diabetes increased as the Baba classification severity categories progressed. The frequencies of hypertension, retinopathy and nephropathy also increased. With regard to sarcopenia and its related factors, the frequency of slow walking speed showed a tendency to increase with the severity categories. Although the frequencies of low SMI, low grip strength and sarcopenia were high in the severity categories of stages 3 and 4, no significant differences in their frequencies were seen between stages 0–4.

Participants' characteristics by the severity categories of the Baba classification stages 0–2 versus stages 3–4 are shown in Table 3. The group with the severity categories of stages 0–2 consisted of 237 participants (90.8%), and the group with stages 3 and 4 consisted of 24 participants (9.2%). Significant differences were seen between these two groups in age, estimated duration of diabetes, IPAQ score, and frequency of autonomic neuropathy and retinopathy. With regard to sarcopenia and its related factors, significantly higher frequencies of slow walking speed and sarcopenia were seen in stages 3 and 4.

Univariate and multivariate logistic regression analyses for sarcopenia are shown in Table 4 and walking speed are shown in Table 5. On both the univariate and multivariate analyses, significant relationships were seen between sarcopenia and

Table 1 | Participants' characteristics

n	261
Age (years)	67 (52–75)
Male, *n* (%)	153 (58.6)
Estimated duration (years)	10 (2–18)
HbA1c (%)	10.3 (8.9–11.7)
BMI (kg/m^2)	24.3 (21.7–27.5)
Alcohol, *n* (%)	127 (48.7)
Smoking, *n* (%)	58 (22.2)
Hypertension, *n* (%)	145 (55.6)
Dyslipidemia, *n* (%)	171 (65.5)
IPAQ (METs•min/week)	792 (330–1535)
Autonomic neuropathy (%)	84 (32.2)
Diabetic polyneuropathy (severity categories of the Baba classification)	Stage 0: 49 (18.8)
	Stage 1: 90 (34.5)
Stage, *n* (%)	Stage 2: 98 (37.5)
	Stage 3: 17 (6.5)
	Stage 4: 7 (2.7)
Diabetic retinopathy, *n* (%)	83 (31.8)
Categories, *n* (%)	None: 178 (68.2)
	SDR: 72 (27.6)
	PPDR: 8 (3.1)
	PDR: 3 (1.1)
Diabetic nephropathy, *n* (%)	82 (31.4)
Categories, *n* (%)	Prenephropathy: 179 (68.6)
	Incipient nephropathy: 52 (19.9)
	Overt nephropathy: 24 (9.2)
	Kidney failure: 6 (2.3)
Sarcopenia and related-factors	
Low skeletal mass index, *n* (%)	81 (31.0)
Low grip strength, *n* (%)	79 (30.3)
Slow walking speed, *n* (%)	55 (21.1)
Sarcopenia, *n* (%)	52 (19.9)
Nerve conduction studies	
Tibial nerve F-lat (ms/m^2)	49.8 (47.1–54.2)
Tibial nerve MCV (m/s)	41.0 (38.5–44.1)
A wave (%)	22.1
Tibial nerve CMAP (mV)	15.3 (10.1–19.9)
Sural nerve SCV (m/s)	48.7 (43.7–53.8)
Sural nerve SNAP (μV)	5.4 (3.0–8.1)

Categorical variables are shown as numbers or percentages. Continuous variables are shown as medians (interquartile range). BMI, body mass index; CMAP, compound muscle action potential; F-lat, F-wave latency; IPAQ, International Physical Activity Questionnaire; MCV, motor conduction velocity; METS, metabolic equivalents; PDR, proliferative diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; SCV, sensory conduction velocity; SDR, simple diabetic retinopathy; SNAP, sensory nerve action potential.

advanced age, low BMI, absence of a history of alcohol consumption, and low IPAQ score. No significant involvement of DPN was seen.

Only age was significantly related to walking speed on univariate analysis, whereas advanced age and female sex were significantly related to walking speed on multivariate analysis. No

significant involvement of DPN was seen. Examination of the factors in slow walking speed stratified by sex showed that, in men, advanced age and the presence of retinopathy were significantly related to slow walking speed (Table S1). Only advanced age was significantly related to slow walking speed in women (Table S2).

Frequencies of slow walking speed were 15.7% for participants without retinopathy, 33.3% for simple, 25.0% for preproliferative and 33.3% for proliferative retinopathy ($P = 0.020$). This trend was also significant in men ($P < 0.001$), but not in women ($P = 0.702$).

Additional analyses in patients aged ≥65 years (*n* = 146)

As the Baba classification severity categories progressed, frequencies of sarcopenia and its related factors increased, but no significant differences were seen between stages 0 to 4. A significantly higher frequency of slow walking speed, but not that of sarcopenia, was seen between severity categories of stages 0–2 (*n* = 129) and stages 3–4 (*n* = 17). In the univariate and multivariate logistic regression analyses for sarcopenia and walking speed, age was still a main significant factor. No significant involvement of DPN was seen (data not shown).

DISCUSSION

In the present study, the frequency of slow walking speed showed a tendency to increase with the severity of DPN based on the Baba classification. Of sarcopenia and its related factors, the frequency of slow walking speed and sarcopenia was particularly high in the Baba classification stages 3 and 4. Comparison of the group with the severity categories of stages 0–2 and the group with stages 3 and 4 showed significant differences in the frequencies of slow walking speed and sarcopenia. On multiple logistic regression analysis, however, DPN (stages 3 and 4) was not a significant variable, whereas age, low BMI, the absence of a history of alcohol consumption and a low IPAQ score were significantly related to sarcopenia. Age and female sex were significantly related to slow walking speed. These findings showed that, although severe DPN appears to be associated with sarcopenia and its related factors, variables, such as age, play a larger role. Because the frequency of severe DPN is low in the clinical setting, the contribution of severe DPN to the occurrence of sarcopenia in type 2 diabetes mellitus is thought to be modest.

Approximately 20% of the patients in the present study were diagnosed with sarcopenia. This is comparable to previous studies in Japan and South Korea, which have reported sarcopenia in approximately 15% of patients with type 2 diabetes mellitus[22,23]. The frequencies of the Baba classification DPN stages (Table 1) were 19% for stage 0, just over 70% for stages 1 and 2, and <10% for stages 3 and 4. As in the present study, Kamiya *et al.*[24] used the Baba classification to evaluate 357 patients hospitalized for diabetes treatment and reported a distribution similar to that of the present study: 66% with the severity categories of stages 1 or 2 and <10% with stages 3 or

Table 2 | Participants' characteristics by the severity categories of the Baba classification

Severity categories of the Baba classification	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	P-values
n	49	90	98	17	7	
Age (years)[†]	57 (42–70)	65 (49–74)	70 (55–76)	72 (68–78)	64 (64–82)	0.002
Male (%)[‡]	40.8	63.3	63.3	52.9	71.4	0.066
Estimated duration (years)[†]	5 (1–14)	10 (3–18)	10 (3–19)	18 (6–25)	17 (10–28)	0.012
HbA1c (%)[†]	10.4 (9.2–12.4)	10.2 (8.9–11.7)	10.0 (8.7–11.4)	10.9 (9.6–12.4)	10.6 (9.5–13.5)	0.216
BMI (kg/m^2)[†]	23.4 (21.1–27.2)	24.4 (22.7–27.3)	24.3 (21.1–27.5)	24.3 (21.0–30.8)	24.2 (20.9–30.0)	0.813
Alcohol (%)[‡]	30.6	52.2	55.1	47.1	42.9	0.066
Smoking (%)[‡]	16.3	26.7	23.5	11.8	14.3	0.554
Hypertension (%)[‡]	38.8	53.3	65.3	58.8	57.1	0.044
Dyslipidemia (%)[‡]	57.1	70.0	65.3	64.7	71.4	0.655
IPAQ (METS•min/week)[†]	792 (330–1752)	924 (570–1799)	840 (396–1483)	297 (0–1708)	462 (0–751)	0.203
Autonomic neuropathy (%)[‡]	22.2	28.2	35.6	58.8	42.8	0.083
Diabetic retinopathy (%)[‡]	12.3	28.9	31.2	81.3	85.7	<0.0001
None (%)	87.8	71.1	68.4	17.7	14.3	
SDR (%)	12.2	26.7	24.5	70.6	85.7	
PPDR (%)	0	1.1	5.1	11.8	0	
PDR (%)	0	1.1	2.0	0	0	
Diabetic nephropathy (%)[‡]	10.2	35.6	33.7	41.2	71.4	0.0007
Prenephropathy (%)	89.8	64.4	63.3	58.8	28.6	
Incipient nephropathy (%)	10.2	24.4	20.4	17.7	28.6	
Overt nephropathy (%)	0	8.9	10.2	23.5	28.6	
Kidney failure (%)	0	2.2	3.1	0	14.2	
Sarcopenia and related-factors						
Low skeletal mass index (%)[‡]	30.6	27.8	31.6	41.2	42.9	0.345
Low grip strength (%)[‡]	30.6	23.3	32.7	47.1	42.9	0.137
Slow walking speed (%)[‡]	10.2	18.9	23.5	47.1	28.6	0.004
Sarcopenia (%)[‡]	22.5	15.6	17.4	41.2	42.9	0.178

Categorical variables are shown as percentages. Continuous variables are shown as medians (interquartile range). P-values were calculated by the Kruskall–Wallis test ([†]) or the Cochran-Armitage trend test ([‡]). BMI, body mass index; IPAQ, International Physical Activity Questionnaire; METS, metabolic equivalents; PDR, Proliferative diabetic retinopathy; PPDR, Preproliferative diabetic retinopathy; SDR, Simple diabetic retinopathy.

4. Thus, the results obtained in the present study for sarcopenia morbidity and the Baba classification were comparable to those obtained in previous studies in East Asian patients.

The frequencies of sarcopenia and its related factors by the Baba classification DPN stage showed large increases in stages 3 and 4 (Table 2). An analysis comparing the Baba classification stages 0–2 with stages 3 and 4 showed significant differences in the frequencies of sarcopenia and slow walking speed. As possible causes of slow walking speed in patients with DPN, Kwon et al.[25] suggested a decrease in the moment and decreased stability of the ankle during walking. Severinsen et al.[26] reported associations between the size of the extensor digitorum brevis muscle and the compound muscle action potential of the tibial and peroneal nerves. These findings indicate that progression of DPN severity is a factor that gives rise to an abnormal gait due to instability during walking, leading to slow walking speed.

In contrast, the results of the multiple logistic regression analysis showed no significant association between DPN and sarcopenia or slow walking speed. Several factors might be involved in this finding. First is the effect of aging. The analysis results for both sarcopenia and slow walking speed have shown that age is significantly involved in both, even in the analyses restricted to patients aged ≥65 years. The main pathological change in the peripheral nerves that occurs with aging is axonal degeneration, and NCS shows gradual decreases in amplitude and conduction velocity with aging[27]. In fact, prevalence of polyneuropathy based on NCS was as high as 9% in the Dutch elderly general population[28]. Furthermore, a characteristic pathological change that occurs with DPN is axonal degeneration that begins from the peripheral distal region[29]. Consequently, from a pathological and electrophysiological perspective, the changes that occur with aging and DPN are similar. In this light, the use of NCS to differentiate between those changes is considered difficult[2]. Mori et al.[30] used multivariate analysis to evaluate the factors related to sarcopenia in Japanese patients with type 2 diabetes mellitus. The results showed that, whereas age had a strong effect (odds ratio [OR] 13.067), DPN was not a related factor (OR 0.972). Second, factors other than aging need to be considered. Factors other than aging in sarcopenia that were identified on multivariate logistic regression analysis were BMI, alcohol consumption and IPAQ score. A low BMI is known to be associated with decreased

Table 3 | Participants' characteristics by the severity categories of the Baba classification stages 0–2 versus stages 3–4

Severity categories of the Baba classification	Stages 0–2	Stages 3–4	*P*-values
n	237	24	
Age (years)[†]	66 (51–75)	70 (64–79)	0.037
Male (%)[‡]	58.7	58.3	1.000
Estimated duration (years)[†]	9 (2–17)	18 (8–25)	0.003
HbA1c (%)[†]	10.2 (8.9–11.6)	10.8 (9.6–12.9)	0.085
BMI (kg/m^2)[†]	24.3 (21.9–27.4)	24.2 (21.0–29.9)	0.852
Alcohol (%)[‡]	49.0	45.8	0.832
Smoking (%)[‡]	23.2	12.5	0.307
Hypertension (%)[‡]	55.3	58.3	0.832
Dyslipidemia (%)[‡]	65.4	66.7	1.000
IPAQ (METs•min/week)[†]	882 (462–1581)	314 (0–1271)	0.032
Autonomic neuropathy (%)[‡]	30.0	54.6	0.029
Diabetic retinopathy (%)[‡]	26.6	82.6	<0.0001
None (%)	73.4	16.7	
SDR (%)	22.8	75.0	
PPDR (%)	2.5	8.3	
PDR (%)	1.3	0	
Diabetic nephropathy (%)[‡]	29.5	50.0	0.062
Prenephropathy (%)	70.5	50.0	
Incipient nephropathy (%)	19.8	20.8	
Overt nephropathy (%)	7.6	25.0	
Kidney failure (%)	2.1	4.2	
Sarcopenia and related-factors			
Low skeletal mass index (%)[‡]	30.0	41.7	0.252
Low grip strength (%)[‡]	28.7	45.8	0.102
Slow walking speed (%)[‡]	19.0	41.7	0.016
Sarcopenia (%)[‡]	17.7	41.7	0.012

Categorical variables are shown as percentages. Continuous variables are shown as medians (interquartile range). *P*-values were calculated by the Wilcoxon test ([†]) and Fisher's exact test ([‡]). BMI, body mass index; HbA1c, glycated hemoglobin; IPAQ, International Physical Activity Questionnaire; PDR, proliferative diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; SDR, simple diabetic retinopathy.

Table 4 | Univariate and multivariate logistic regression analyses for sarcopenia

	Univariate			Multivariate		
	OR	95% CI	*P*-values	OR	95% CI	*P*-values
Age	1.062	1.018, 1.115	0.004	1.079	1.038, 1.130	<0.0001
Sex (male)	1.633	0.634, 4.351	0.311			
Estimated duration	1.018	0.970, 1.067	0.464			
HbA1c	1.191	0.958, 1.494	0.116	1.134	0.923, 1.399	0.231
BMI	0.713	0.597, 0.828	<0.0001	0.712	0.606, 0.819	<0.0001
Alcohol	0.449	0.171, 1.120	0.087	0.364	0.150, 0.839	0.017
Smoking	0.570	0.146, 1.984	0.384			
IPAQ	0.9990	0.998, 0.9997	0.002	0.9992	0.999, 0.9997	0.002
Autonomic neuropathy	1.168	0.455, 2.999	0.624			
Diabetic polyneuropathy[†]	3.696	0.760, 20.939	0.107	2.844	0.720, 11.851	0.136
Diabetic retinopathy	1.034	0.357, 2.994	0.786			
Diabetic nephropathy	0.803	0.280, 3.414	0.676			

[†]Diabetic polyneuropathy: defined by the presence of stages 3 and 4 by the Baba classification. BMI, body mass index; CI, confidence interval; IPAQ, International Physical Activity Questionnaire; OR, odds ratio.

Table 5 | Univariate and multivariate logistic regression analyses for walking speed

	Univariate			Multivariate		
	OR	95% CI	P-values	OR	95% CI	P-values
Age	1.082	1.036, 1.139	0.0009	1.079	1.042, 1.124	<0.0001
Sex (male)	0.490	0.205, 1.132	0.095	0.378	0.181, 0.776	0.008
Estimated duration	0.994	0.951, 1.037	0.765			
HbA1c	1.064	0.868, 1.298	0.546			
BMI	1.088	0.984, 1.205	0.100	1.058	0.966, 1.160	0.222
Alcohol	0.749	0.325, 1.712	0.492			
Smoking	1.163	0.327, 3.694	0.805			
IPAQ	0.99994	0.9995, 1.0003	0.774			
Autonomic neuropathy	2.270	0.982, 5.063	0.055	2.037	0.956, 4.197	0.064
Diabetic polyneuropathy[†]	2.246	0.658, 7.789	0.194	1.800	0.608, 5.200	0.283
Diabetic retinopathy	1.989	0.789, 5.032	0.144	2.074	0.969, 4.441	0.060
Diabetic nephropathy	1.013	0.408, 2.446	0.977			

[†]Diabetic polyneuropathy: defined by the presence of stages 3 and 4 by the Baba classification. BMI, body mass index; CI, confidence interval; IPAQ, International Physical Activity Questionnaire; OR, odds ratio.

skeletal muscle and poor nutrition[31]. Alcohol consumption, together with smoking, is a known risk factor for sarcopenia[32]. Although the absence of a history of alcohol consumption was associated with sarcopenia in the present study, the reason is unknown. Ko et al.[33] showed that a group with high IPAQ scores was less likely to develop sarcopenia.

In the examination of walking speed as a factor other than age, a sex difference was detected. Phillip et al.[34] found a greater loss of muscle strength in postmenopausal women than in men of the same age. Approximately 40% of the participants in the present study were women, and the median age of the participants was 67 years, which suggests that many were post-menopausal women. AWGS 2019 defines slow walking speed as ≤1.0 m/s for both men and women[9], and a difference between men and women in physical performance therefore might also have played a role in the results obtained in the present study. Factors that were significantly related to slow walking speed in men in the present study were advanced age and the presence of retinopathy. In women, only advanced age was significantly related to slow walking speed. Marked differences between men and women were seen with respect to the factors related to slow walking speed.

The present study had several limitations. First, it was carried out at a single center. In addition, because the study participants were patients with type 2 diabetes mellitus and worse glucose control, selection bias might have been present. Specifically, the participants were older, and their glycated hemoglobin levels were high. Consequently, it is unclear whether the results are applicable to patients with type 2 diabetes mellitus as a whole, including those of other races. However, the frequencies of sarcopenia and DPN severity among the participants in this study were comparable to those reported elsewhere for East Asia or Japan[22–24]. Second, vitamin B_1 and B_{12} deficiency was not evaluated in the present study, both of which can lead to

neurological damage[35]. However, significant deficiencies of those vitamins could be excluded by routine clinical evaluation. Third, the number of patients in the study was small, particularly the number of patients with the severity categories of the Baba classification stages 3 and 4. If the participants had included many patients with severe DPN, the results might have been different. However, the frequency of severe DPN among participants in this study was comparable to the frequencies reported in other studies in Japan[24]. Furthermore, the frequency of DPN has been decreased due to the overall improvement in glucose control in recent years[36]. In the clinical setting, sarcopenia progression resulting from aging and low physical performance is considered a higher priority than sarcopenia progression resulting from severe DPN. A strong point of this study is that it evaluated the association between sarcopenia and DPN severity determined by NCS in detail.

In conclusion, although the frequencies of sarcopenia and slow walking speed increased with the severity of DPN based on the Baba classification, multivariate logistic regression analysis showed that aging plays a major role in the development of sarcopenia and its related factors. Although severe DPN might be a factor related to sarcopenia, the frequency of severe DPN is low in the clinical setting, indicating that the contribution of severe DPN to sarcopenia in type 2 diabetes mellitus patients is modest.

Approval of the research protocol: The study was carried out after being approved by the local ethics committee of Showa University Fujigaoka Hospital.

Informed consent: Informed consent was not obtained from all individuals.

Registry and the registration no. of the study: N/A.

Animal studies: N/A.

REFERENCES

1. Bushi R, Boulton A, Feldman E, *et al*. Diabetic neuropathy: a position statement by the American diabetes association. *Diabetes Care* 2017; 40: 136–154.

2. Himeno T, Kamiya H, Nakamura J, *et al*. Lumos for the long trail: strategies for clinical diagnosis and severity staging for diabetic polyneuropathy and future directions. *J Diabetes Investig* 2020; 11: 5–16.

3. Gilliatt RW, Willison RG. Peripheral nerve conduction in diabetic neuropathy. *J Neurol Neurosurg Psychiatry* 1962; 25: 11–18.

4. Behse F, Buchthal F, Carlsen F. Nerve biopsy and conduction studies in diabetic neuropathy. *J Neurosurg Psychiatry* 1977; 40: 1072–1082.

5. Dyck PJ, Albers JW, Andersen H, *et al*. Toronto expert panel on diabetic neuropathy. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev* 2011; 27: 620–628.

6. American Diabetes Association. 12. older adults: standards of medical care in diabetes-2021. *Diabetes Care* 2021; 44(Suppl 1): S168–S179.

7. Rosenberg IH. Summary comments. *Am J Clin Nutr* 1989; 50: 1231–1233.

8. 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.

9. Chen LK, Woo J, Assantachai P, *et al*. Asian working group for sarcopenia: 2019 Consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc* 2020; 21: 300–307.

10. Mesinovic J, Zengin A, De Courten B, *et al*. Sarcopenia and type 2 diabetes mellitus: a bidirectional relationship. *Diabetes Metab Syndr Obes: Targets Ther* 2019; 12: 1057–1072.

11. Strotmeyer ES, Rekeneire ND, Schwartz AV, *et al*. The relationship of reduced peripheral nerve function and diabetes with physical performance in older White and Black adults: the Health, Aging, and Body Composition (Health ABC) Study. *Diabetes Care* 2008; 31: 1767–1772.

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

13. Zhang Y, Shen X, He L, *et al*. Association of sarcopenia and muscle mass with both peripheral neuropathy and nerve function in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2020; 162: 1080–1096.

14. Haneda M, Utsunomiya K, Koya D, *et al*. A new classification of diabetic nephropathy 2014: a report from joint committee on diabetic nephropathy. *Clin Exp Nephrol* 2015; 19: 1–5.

15. Takahashi H, Tampo H, Arai Y, *et al*. Applying artificial intelligence to disease staging: deep learning for improved staging of diabetic retinopathy. *PLoS One* 2017; 12: e0179790.

16. Yanagida H, Ando A, Kenta O, *et al*. Determination of reference ranges for nerve conduction studies: Influence of age, height and gender. *Jichi Med Univ J* 2015; 38: 27–39.

17. Agelink MW, Malessa R, Baumann B, *et al*. Standardized tests of heart rate variability: normal ranges obtained from 309 healthy humans, and effects of age, gender, and heart rate. *Clin Auton Res* 2001; 11: 99–108.

18. Muramatsu T, Takahashi M, Kakinuma R, *et al*. Decline in renal function associated with cardiovascular autonomic neuropathy positively coordinated with proteinuria in patients with type 2 diabetes. *J Diabetes Investig* 2022; 13: 102–111.

19. 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.

20. Murase N, Katsumura T, Ueda C, *et al*. International standardization of physical activity. *Kosei no Shihyo* 2002; 49: 1–9. (in Japanese).

21. Baba M, Suzuki C, Ogawa Y. Severity grading system of diabetic neuropathy in type-2 diabetes by nerve conduction study: Five-year prospective study on occurrence of diabetic foot, macroangiopathic events, and eventual death. *Japn J Clin Neurophysiol* 2018; 46: 71–77. (in Japanese).

22. Kim TN, Park MS, Yang SJ, *et al*. Prevalence and determinant factors of sarcopenia in patients with type 2 diabetes: the Korean sarcopenic obesity study (KSOS). *Diabetes Care* 2010; 33: 1497–1499.

23. Murata Y, Kadoya Y, Yamada S, *et al*. Sarcopenia in elderly patients with type 2 diabetes mellitus: prevalence and related clinical factors. *Diabetol Int* 2018; 9: 136–142.

24. Kamiya H, Shibata Y, Himeno T, *et al*. Point-of-care nerve conduction device predicts the severity of diabetic polyneuropathy: a quantitative, but easy-to-use, prediction model. *J Diabetes Investig* 2021; 12: 583–591.

25. Kwon OY, Minor SD, Maluf KS, *et al*. Comparison of muscle activity during walking in subjects with and without diabetic neuropathy. *Gait Posture* 2003; 18: 105–113.

26. Severinsen K, Andersen H. Evaluation of atrophy of foot muscles in diabetic neuropathy - A comparative study of nerve conduction studies and ultrasonography. *Clin Neurophysiol* 2007; 118: 2172–2175.

27. Kimura J. Part IX: Interpretation of study results. In: Electrodiagnosis in diseases of nerve and muscle: principles

and practice, 4th ed, Oxford University Press, Oxford, 2013, 933–954.

28. Hanewinckel R, Drenthen J, Ojien MV, et al. Prevalence of polyneuropathy in the middle-aged and elder population. *Neurology* 2016; 87:1892–1898.

29. Boulton AJ, Malik RA. Diabetic neuropathy. *Med Clin North Am* 1998; 82: 909–929.

30. Mori H, Kuroda A, Yoshida S, et al. High prevalence and clinical impact of dynapenia and sarcopenia in Japanese patients with type 1 and type 2 diabetes: findings from the Impact of Diabetes Mellitus on Dynapenia study. *J Diabetes Investig* 2021; 12: 1050–1059.

31. Xu F, Zhao L, Su J, et al. The relationship between glycemic variability and diabetic peripheral neuropathy in type 2 diabetes with well-controlled HbA1c. *Diabetol Metab Syndr* 2014; 6: 139.

32. Prokopidis K, Witard OC. Understanding the role of smoking and chronic excess alcohol consumption on reduced caloric intake and the development of sarcopenia. *Nutri Res Rev* 2021: 1–10. https://doi.org/10.1017/S0954422421000135. Online ahead of print.

33. Ko YC, Chie WC, Wu TY, et al. A cross-sectional study about the relationship between physical activity and sarcopenia in Taiwanese older adults. *Sci Rep* 2021; 11: 11488.

34. Phillips SK, Rook KM, Siddle NC, et al. Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clin Sci* 1993; 84: 95–98.

35. Sun Y, Lai MS, Lu CJ. Effectiveness of vitamin B12 on diabetic neuropathy: systematic review of clinical controlled trials. *Acta Neurol Taiwan* 2005; 14: 48–54.

36. Yokoyama H, Araki S, Kawai K, et al. Declining trends of diabetic nephropathy, retinopathy and neuropathy with improving diabetes care indicators in Japanese patients with type 2 and type 1 diabetes (JDDM 46). *BMJ Open Diabetes Res Care* 2018; 6: e000521.

SUPPORTING INFORMATION

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

Figure S1 | Flowchart for classifying the severity of diabetic polyneuropathy (DPN) based on the Baba classification. DPN is classified as stages 0–4 based on the nerve conduction study results shown in the figure.

Table S1 | Univariate and multivariate logistic regression analyses for walking speed in men.

Table S2 | Univariate and multivariate logistic regression analyses for walking speed in women.