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Long-chain fatty acids in sarcopenia patients with cardiovascular diseases: importance of n-9 monounsaturated fatty acids

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Abstract

Background Long-chain fatty acids are essential components of the cellular energy supply, cellular membranes, and autacoid synthesis. It has been suggested that long-chain fatty acids might be involved in the pathophysiology underlying sarcopenia. We investigated the association between sarcopenia and serum long-chain fatty acid profile in patients with cardiovascular diseases.

Methods We retrospectively investigated 308 cardiovascular patients [age: 72 ± 12 (mean ± SD), 174 male patients] admitted to our hospital. All patients were evaluated by sarcopenia diagnostic tests and serum free fatty acid analyses.

Results Seventy-seven patients (25%) were diagnosed with sarcopenia. Serum fatty acid weight percentages of nervonic acid and erucic acid were elevated in patients with sarcopenia compared with those without. Nervonic acid, which was an independent factor for sarcopenia in binary logistics regression analysis (B = 2.559, $p < 0.001$), correlated negatively with skeletal muscle index ($r = -0.331$, $p < 0.001$), gait speed ($r = -0.387$, $p < 0.001$), and handgrip strength ($r = -0.372$, $p < 0.001$). These significant relationships were confirmed in subgroup analyses stratified by age and gender. In receiver operating characteristic curve analysis, the cut-off of nervonic acid weight percentage for diagnosis of sarcopenia was 1.37% with a sensitivity and specificity of 76.6% and 65.1%, respectively.

Conclusions Nervonic acid, an n-9 monounsaturated fatty acid, might serve as a new marker for sarcopenia in patients with cardiovascular diseases. Further studies with larger patient numbers will be needed to determine the roles of long-chain fatty acids in sarcopenia.

Keywords Sarcopenia; Fatty acids; Nervonic acid; Cardiovascular diseases

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Introduction

Decreases in muscle strength and loss of muscle mass with ageing and uncommunicable disease is termed sarcopenia; this condition has become an important issue because affected patients exhibit deterioration of physical function followed by decreases in physical activity and quality of life. Ultimately, sarcopenia results in frailty and premature death.[1–4] The condition can be classified as primary (age related) or secondary (disease related) sarcopenia. Cardiovascular disease is a risk factor for secondary sarcopenia, and the coexistence of sarcopenia worsens the disease process of cardiovascular diseases.[5] Thus, sarcopenia is an important pathophysiological condition in cardiovascular diseases.

Fatty acids are monovalent carboxylic acids that contain long-chain hydrocarbons (Supporting Information, *Figure*

S1). Fatty acids are the major component of cellular and intracellular membranes. Additionally, fatty acids are broken down to acetyl-CoA by beta oxidation in the mitochondria, thereby serving as a source of ATP generation.[6] Furthermore, fatty acids play pivotal roles in many pathophysiological conditions, such as gene expression followed by transcription factor activation and production of lipid mediators controlling inflammation.[7] Among fatty acids, long-chain polyunsaturated fatty acids are of great interest in contemporary medicine.[8,9]

In patients with sarcopenia, the n-3 long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been the targets of multiple basic and clinical studies.[8–10] One cross-sectional study showed a significant relationship between fatty fish intake and grip strength in the elderly.[11] Another study demonstrated that EPA and DHA supplementation was associated with skeletal muscle synthesis in older adults.[12] On the other hand, little is known about pathophysiological roles of other long-chain fatty acids, for example, n-9 long-chain fatty acids, in sarcopenia, especially sarcopenia in patients with cardiovascular disease.

Therefore, we investigated the association between sarcopenia and serum levels of long-chain fatty acids in patients with cardiovascular disease.

Materials and methods

Study subjects

This study enrolled 308 consecutive patients [age: 72 ± 12 (mean ± SD), 174 male patients] with cardiovascular disease who were admitted to our hospital and received serum fatty acid analysis between April 2013 and December 2015. Patients with pacemakers or implantable defibrillators were excluded because of our inability to adapt the bioelectrical impedance assay to these patients. Written informed consent was obtained from all subjects. The study was designed and conducted according to the ethical principles for medical research stated in the Declaration of Helsinki and was approved by the Ethics Committee of Kurume University.

Diagnosis of sarcopenia

Diagnosis of sarcopenia was performed by the diagnosis algorithm recommended by the guidelines of the Asian Working Group for Sarcopenia, as described previously.[13–15] Briefly, sarcopenia was diagnosed based on measurements of muscle mass, muscle strength, and physical performance. In practice, sarcopenia was diagnosed on the basis of low skeletal mass index (SMI; <7.0 kg/m^2 in male patients and <5.7 kg/m^2 in female patients) associated with either low handgrip strength (<26 kgf in male patients and <18 kgf in female patients) or low gait speed (<0.8 m/s). Non-sarcopenia was diagnosed when subjects had normal SMI (≥7.0 kg/m^2 in male patients and ≥5.7 kg/m^2 in female patients), normal handgrip strength (≥26 kgf in male patients and ≥18 kgf in female patients), or normal gait speed (≥0.8 m/s). The age criterion of more than 65 years old was not adopted for sarcopenia diagnosis in this study because age is a possible confounding factor of disease-related (secondary) sarcopenia; hence, patients with cardiovascular disease who were less than 65 years old were included in this study.

Muscle mass was measured by a bioelectrical impedance assay using the InBody S10 body composition analyser (Biospace, Tokyo, Japan). This system applies electricity at frequencies of 1, 5, 50, 250, 500, and 1000 kHz through the body. Whole-body impedance was measured using an ipsilateral foot hand electrical pathway. The recommended conditions for bioelectrical impedance assay measurements, as explained to the subjects, were (i) fasting for 4 h before measurements, (ii) bladder voided before measurements, and (iii) no exercise in the 8-h period prior to measurements.[16] When patients were in oedematous condition, the bioelectrical impedance assay was performed after an improvement in their oedema. Absolute appendicular muscle mass was calculated as the sum of the muscle of the arms and legs. Absolute appendicular muscle mass was converted to SMI by dividing the value by the square of the height in meters (kilograms per square metre).

Muscle strength was assessed as handgrip strength using a Smedley MY-2080 hand dynamometer (Matsumiya Ikaseiki Seisakusho Co. Ltd., Tokyo, Japan). One trial was performed for each hand, and the result from the stronger hand was used for sarcopenia diagnosis.

Physical performance was assessed as usual gait speed. We employed a modified version of a technique previously reported by Tanimoto *et al.*[17] Patients were asked to walk straight ahead for 12 m at their usual speed for measurement of 10-m walk time. The walking speed reached a steady speed within the first 2 m. Gait speed (metres per second) was calculated by dividing the distance covered (10 m) by the 10-m walk time (second).

Data collection

Data on admission were collected from hospital charts and database. Peripheral venous blood was drawn from the antecubital vein early in the morning after 8 h of fasting; the resulting specimens were subjected to biochemical and immunological measurements, including determination of serum fatty acid concentrations.

Fatty acid analysis

We determined the weight percentages of 24 major fatty acid metabolites (Supporting Information, *Figure S1*). Serum

concentrations of fatty acids were measured as described previously.[18] In brief, approximately 0.2 mL of serum sample and 2 mL of chloroform-methanol (2:1) solution (1-mL water, 666-μL methanol, and 333-μL chloroform) were placed in a Pyrex centrifuge tube, homogenized with a Polytron (PCU2-110, KINEMATICA GmbH, Switzerland), and then centrifuged at 900 × g for 10 min. An aliquot of the chloroform-methanol extract was transferred to another Pyrex tube and dried under a stream of nitrogen gas. Each dried specimen was dissolved in 100 μL of 0.4 M potassium methoxide-methanol/14% boron trifluoride-methanol solution, and the fatty acid concentrations in the solution were measured by gas chromatography (Shimazu GC 17A, Kyoto, Japan) at SRL, Inc. The fatty acid content for each fatty acid was expressed as a weight percentage of the total fatty acid, using the following equation: Fatty acid weight percentage = each fatty acid concentration (micrograms per millilitre)/total fatty acid concentration (micrograms per millilitre) × 100%.

Statistical analysis

Statistical analysis was performed using SPSS software (version 22; IBM Corp., Armonk, New York). Continuous variables are presented as mean ± SD, and categorical variables are expressed using numbers or frequencies. Data for two groups were compared using a non-paired Student's *t*-test or Welch's *t*-test, as appropriate; proportional data were analysed by chi-squared analysis or Fisher's exact test. Univariate and binary logistics regression analyses were performed to investigate the relationship between fatty acids and sarcopenia diagnosis. To examine the effects of gender and age on the relationships between nervonic acid levels and sarcopenia diagnostic components, univariate regression analysis was performed for data for the groups of men, women, younger individuals (<74 years old), and elder individuals (≥74 years old). The median age of 74 years old was set as the cut-off value for age. Receiver operating characteristics (ROC) curve analysis was performed to predict the cut-off values for sarcopenia diagnosis; area under the curve was measured to estimate the contribution to sarcopenia diagnosis. A two-sided probability value of ≦0.05 was considered significant.

Results

Patient characteristics and demographics

Patient characteristics and demographics in the sarcopenia and non-sarcopenia groups are shown in *Table* 1. Seventy-seven patients (25%) were diagnosed with sarcopenia. Patients with sarcopenia were older and had higher proportions of female patients (both $p < 0.001$). The prevalence of congestive heart failure was significantly higher in the sarcopenia group than in the non-sarcopenia group ($p = 0.003$). Background cardiovascular diseases did not differ between the sarcopenia and non-sarcopenia groups. As for comorbidities, the prevalence of chronic kidney disease was significantly higher ($p = 0.008$), and those of hypertension and dyslipidaemia were significantly lower, in the sarcopenia group ($p = 0.032$ and $p = 0.034$, respectively).

The frequencies of use of Ca antagonists and angiotensin receptor blockers were significantly higher in the non-sarcopenia group than in the sarcopenia group ($p = 0.012$ and $p = 0.025$, respectively) (*Table* 1). Conversely, diuretics were prescribed significantly more often in the sarcopenia group than in the non-sarcopenia group ($p = 0.003$). There was no difference in the frequency of EPA administration between the two groups.

The estimated glomerular filtration rate (eGFR), as well as the serum levels of triglycerides, glycated haemoglobin (HbA1C), and haemoglobin, were significantly higher in the non-sarcopenia group than in the sarcopenia group ($p = 0.008$, $p < 0.001$, $p = 0.039$, and $p < 0.001$, respectively) (*Table* 1). N-terminal pro-brain natriuretic peptide levels were significantly higher in the sarcopenia group than in the non-sarcopenia group ($p = 0.006$).

Diagnostic components for sarcopenia

Each of the three sarcopenia diagnostic components (i.e. skeletal muscle index, gait speed, and handgrip strength) were significantly lower in the sarcopenia group than in the non-sarcopenia group ($p < 0.001$ for each of the three parameters) (*Table* 1).

Physical measurements and nutritional markers

Body mass index (BMI) was significantly lower ($p < 0.001$), and heart rate was significantly higher ($p = 0.001$), in the sarcopenia group than in the non-sarcopenia group (*Table* 1). As for nutritional markers, the sarcopenia group had significantly lower serum albumin levels, significantly higher total lymphocyte counts, and significantly higher Controlling Nutritional Status scores ($p < 0.001$ for each).

Fatty acid analysis

Table 2 shows serum fatty acid weight percentages. Weight percentages of myristic acid, palmitic acid, oleic acid, and gamma-linoleic acid were lower in the sarcopenia group than in the non-sarcopenia group ($p = 0.025$, $p = 0.040$,

Table 1 Baseline characteristics and Characteristics

	Non-sarcopenia group	*n*	Sarcopenia group	n	*p*
Age, years old	69.6 ± 12.9	231	77.6 ± 8.5	77	<t>0.001
Male gender, *n* (%)	148(64.1)		26(33.8)		<t>0.001
Diagnostic components for sarcopenia					
Skeletal muscle index, kg/m^2	7.13 ± 1.21	231	5.12 ± 0.92	77	<t>0.001
Gait speed, m/s	1.14 ± 0.36	90	0.87 ± 0.29	38	<t>0.001
Handgrip strength, kgf	27.3 ± 12.1	175	13.3 ± 6.6	63	<t>0.001
Systolic blood pressure, mmHg	131.8 ± 20.8	217	129.9 ± 23.6	69	0.539
Diastolic blood pressure, mmHg	74.5 ± 11.7	217	73.4 ± 16.2	69	0.533
Heart rate, beats/min	69.0 ± 13.5	214	75.1 ± 13.4	70	0.001
eGFR, mL/min/1.73 m^2	63.9 ± 21.6	231	54.5 ± 27.6	76	0.008
Uric acid, mg/dL	6.1 ± 1.7	230	6.0 ± 2.1	77	0.903
Fasting blood glucose, mg/dL	112 ± 41	211	106 ± 36	70	0.224
HbA1C, %	6.3 ± 1.1	228	6.0 ± 0.9	74	0.039
NT-proBNP, pg/mL	926 ± 2,839	222	8,067 ± 20,847	70	0.006
Congestive heart failure (ACC/AHA CHF stage C, D)	52(22.5)	231	31(40.3)	77	0.003
Cardiovascular disease, *n* (%)					
Coronary heart disease	107(46.3)		34(44.2)		0.793
Hypertensive heart disease	18(7.8)		7(9.1)		0.810
Valvular heart disease	11(4.8)		5(6.4)		0.558
Idiopathic cardiomyopathy	13(5.6)		2(2.6)		0.372
Arrhythmia	38(16.0)		20(26.0)		0.062
Aortic disease	11(4.8)		4(5.2)		1.000
Comorbidity, *n* (%)					
Hypertension	145(62.8)		37(48.1)		0.032
Diabetes mellitus	84(36.4)		20(26.0)		0.126
Dyslipidaemia	82(35.5)		17(22.1)		0.034
Hyperuricaemia	19(8.2)		4(5.2)		0.462
Chronic kidney disease	26(11.3)		19(24.7)		0.008
Smoking	26(11.3)		5(6.5)		0.279
Medications, *n* (%)					
Ca channel blocker	132(57.1)		31(40.3)		0.012
Beta-blocker	80(34.6)		27(35.1)		1.000
Angiotensin receptor antagonist	125(54.1)		30(39.0)		0.025
Angiotensin converting enzyme inhibitor	24(10.4%)		13(16.9%)		0.156
Acetylsalicylic acid	103(44.6%)		32(41.6%)		0.692
P2Y12 inhibitor	70(30.3%)		23(29.9%)		1.000
Statins	96(41.6%)		26(33.8%)		0.282
EPA	39(16.9%)		12(15.6%)		0.861
Diuretics	53(22.9%)		32(41.6%)		0.003
DPP4 inhibitor	41(17.7%)		9(11.6%)		0.284
Biguanaide	20(8.7%)		3 (3.9%)		0.215
Sulfonylurea	27(11.6%)		1(1.3%)		0.005
Antiarrhythmic drugs	33(14.3%)		16(20.8%)		0.208
Anticoagulants	49(21.2%)		22(28.6%)		0.212
Nutrition markers					
Body mass index	25.3 ± 4.4	231	21.3 ± 3.2	77	<t>0.001
Triglyceride, mg/dL	133.0 ± 97.8	216	93.8 ± 38.7	69	<t>0.001
HDLC, mg/dL	47.7 ± 14.2	215	48.1 ± 13.4	70	0.839
LDLC, mg/dL	100.1 ± 30.4	216	94.5 ± 32.0	70	0.192
L/H	2.3 ± 0.9	215	2.1 ± 0.8	70	0.173
Haemoglobin, g/dL	13.0 ± 2.2	231	11.3 ± 1.8	76	<t>0.001
Albumin, mg/dL	4.14 ± 0.45	230	3.90 ± 0.43	76	<t>0.001
Total cholesterol, mg/dL	173 ± 39	217	167 ± 38	74	0.236
Total lymphocyte count, /μL	1,554 ± 670	231	1,186 ± 611	77	<t>0.001
CONUT score	1.89 ± 1.82	213	2.81 ± 1.77	72	<t>0.001

Numerical data are expressed as mean ± standard deviation. The numbers in parenthesis denote the percentage.
ACC/AHA CHF stage, American College of Cardiology/American Heart Association chronic heart failure stage; CONUT, Controlling Nutritional Status; eGFR, estimate glomerular filtration rate; EPA, eicosapentaenoic acid; HbA1C, glycated haemoglobin; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; L/H, low density lipoprotein cholesterol/high density lipoprotein cholesterol; NT-proBNP, N-terminal pro-brain natriuretic peptide.

p = 0.005, and p < 0.001, respectively). Nervonic acid, erucic acid, and DHA showed higher weight percentages in the sarcopenia group than in the non-sarcopenia group (p < 0.001, p < 0.001, and p = 0.015, respectively).

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Table 2 Fatty acids levels in non-sarcopenia and sarcopenia groups

Weight percentage (%)			
Fatty acids	Non-sarcopenia group (n = 231)	Sarcopenia group (n = 77)	p
Lauric acid (C12:0)	0.06 ± 0.09	0.05 ± 0.05	0.050
Myristic acid (C14:0)	0.74 ± 0.30	0.66 ± 0.24	0.025
Myristoleic acid (C14:1, n-5)	0.05 ± 0.03	0.04 ± 0.02	0.476
Palmitic acid (C16:0)	23.55 ± 1.67	23.11 ± 1.58	0.040
Palmitoleic acid (C16:1, n-7)	2.31 ± 0.80	2.39 ± 0.66	0.400
Stearic acid (C:18:0)	6.81 ± 0.69	6.82 ± 0.63	0.959
Oleic acid (C18:1, n-9)	22.49 ± 3.07	21.39 ± 2.57	0.005
Linoleic acid (C18:2, n-6)	24.56 ± 3.61	24.83 ± 3.31	0.573
Gamma-linolenic acid (C18:3, n-6)	0.26 ± 0.15	0.21 ± 0.11	<0.001
Alpha-linolenic acid (C18:3, n-3)	0.74 ± 0.21	0.71 ± 0.18	0.151
Arachidic acid (C20:0)	0.25 ± 0.05	0.25 ± 0.05	0.505
Gondoic acid (C20:1, n-9)	0.17 ± 0.05	0.18 ± 0.05	0.090
Eicosadienoic acid (C20:2, n-6)	0.21 ± 0.04	0.20 ± 0.04	0.611
Mead acid (C20:3, n-9)	0.07 ± 0.05	0.06 ± 0.04	0.175
Dihomo-gannma-linoeic acid (C20:3, n-6)	1.11 ± 0.30	1.03 ± 0.39	0.117
Arachidonic acid (C20:4, n-6)	6.01 ± 1.41	6.25 ± 1.28	0.204
Eicosapentaenoic acid (C20:5, n-3)	2.73 ± 1.96	3.23 ± 2.60	0.128
Behenic acid (C22:0)	0.53 ± 0.11	0.54 ± 0.12	0.426
Erucic acid (C22:1, n-9)	0.02 ± 0.02	0.03 ± 0.02	<0.001
Adrenic acid (C22:4, n-6)	0.14 ± 0.04	0.14 ± 0.04	0.468
Clupanodonic acid (C22:5, n-3)	0.74 ± 0.32	0.75 ± 0.36	0.946
Lignoceric acid (C24:0)	0.49 ± 0.10	0.52 ± 0.12	0.118
Docosahexaenoic acid (C22:6, n-3)	4.69 ± 1.27	5.10 ± 1.19	0.015
Nervonic acid (C24:1, n-9)	1.25 ± 0.34	1.55 ± 0.27	<0.001

Numerical data are expressed as mean ± standard deviation.

Simple and multi-variate regression analysis for sarcopenia diagnosis

The results of a simple regression analysis for the correlation of significant factors and fatty acids with sarcopenia diagnosis are summarized in Supporting Information, *Tables S1* and *2*. In brief, serum weight percentages of erucic acid, DHA, and nervonic acid (r = 0.206, p < 0.001; r = 0.138, p = 0.015; and r = 0.366, p < 0.001, respectively) exhibited positive correlations with sarcopenia diagnosis, whereas many other fatty acids exhibited inverse correlations with sarcopenia diagnosis.

Table 3 shows significant independent factors for sarcopenia diagnosis as assessed by binary logistics analysis. Female gender, BMI, and nervonic acid weight percentage were significant independent factors for sarcopenia diagnosis.

Correlations of nervonic acid with sarcopenia diagnostic components

As shown in *Figure* 1, significant correlations were found for nervonic acid weight percentage with SMI (r = −0.331, p < 0.001), gait speed (r = −0.387, p < 0.001), and handgrip strength (r = −0.372, p < 0.001). We further performed subgroup analyses stratified by gender and age for the relationships between nervonic acid weight percentage and SMI, gait speed, and handgrip strength (Supporting Information, *Figure S2*). Nervonic acid exhibited a significant correlation with SMI, gait speed, and handgrip strength in subjects stratified by men, women, younger age (<74 years old), and older age (≥74 years old).

Receiver operating characteristics curve analysis for detecting sarcopenia

As shown in *Figure* 2, ROC curve analysis revealed that nervonic acid weight percentage was a significant predictor for sarcopenia diagnosis (area under the curve = 0.751, p < 0.001). The cut-off value of nervonic acid weight percentage for detection of sarcopenia was 1.37% with a sensitivity and specificity of 76.6% and 64.9%, respectively.

Discussion

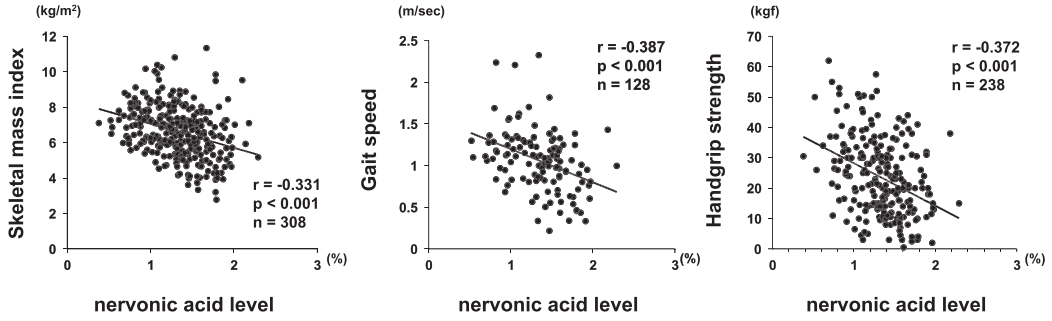
To the best of our knowledge, this investigation is the first to report the characteristic features of serum long-chain fatty acid profiles in cardiovascular patients with sarcopenia. We

Table 3 Independent predictors for sarcopenia diagnosis on binary logistics regression analysis

n = 262			
Independent variables	B	Standard error	p
Gender	1.626	0.380	<0.001
Body mass index	−0.305	0.060	<0.001
Nervonic acid weight percentage	2.559	0.607	<0.001

B, standardized partial regression coefficient.

Figure 1 Relationship between nervonic acid levels and sarcopenia diagnostic components.

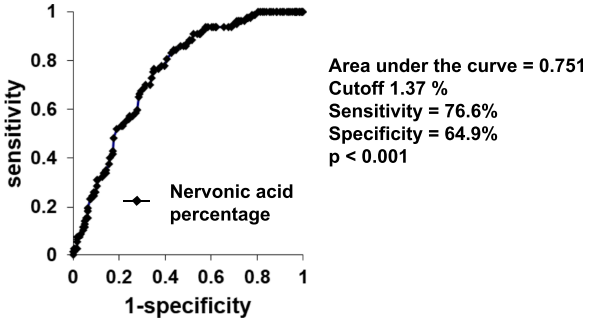


found that weight percentages of both nervonic acid and erucic acid (n-9 monounsaturated fatty acids) were significantly elevated in patients with sarcopenia compared with those without this disease; in contrast, the levels of many other fatty acids were decreased in patients with sarcopenia compared with those without this disease (*Table* 2). We believe that our most important finding is that nervonic acid weight percentage is a valuable marker for detecting sarcopenia with high diagnostic accuracy (*Figure* 2).

In the present study, patients with cardiovascular disease in combination with sarcopenia were older and had larger proportions of female patients, higher prevalences of congestive heart failure and chronic kidney disease, and lower prevalences of hypertension and dyslipidaemia (*Table* 1). Nutritional status evaluated by Controlling Nutritional Status score, BMI, and serum haemoglobin levels were significantly worse in the sarcopenia group than in the non-sarcopenia group. Levels of triglycerides and of glycated haemoglobin were significantly lower, and estimated glomerular filtration rate and N-terminal pro-brain natriuretic peptide levels were significantly higher, in the sarcopenia group than in the non-sarcopenia group (*Table* 1), results that were consistent with those of previous reports.[19,20]

Long-chain fatty acids are biosynthesized through two different mechanisms, namely, *de novo* formation and elongation.[21–23] *De novo* formation of long-chain fatty acids consisting of 14–18 carbon chains (C14 C18) is mediated by a cytosolic complex enzyme system. Very-long-chain fatty acids (C20 or more) then are synthesized by the addition of two-carbon units to precursor fatty acids via reactions catalysed by microsomal elongation enzymes. We examined the 24 major fatty acids metabolites in human (Supporting Information, *Figure S1*). As shown in *Table* 2, serum levels of lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1, n-9), and gamma-linoleic acid (C18:2, n-9) were lower in patients with sarcopenia than in those without this disease. These changes may reflect decreased *de novo* fatty acid synthesis, likely due to malnutrition. The present investigation detected significant malnutrition in patients with sarcopenia, an observation that is consistent with the results of previous studies.[13,24] Notably, the present work demonstrated that the levels of nervonic acid (C24:1, n-9) and its precursor erucic acid (C22:1, n-9) were increased, whereas that of oleic acid (C18:1, n-9), the most upstream fatty acid of the n-9 monounsaturated fatty acid biosynthesis pathway, was decreased, in subjects in the sarcopenia group. This finding suggested the specific activation of fatty acid elongation in the n-9 monounsaturated biosynthesis pathway in patients with sarcopenia. It is possible that the increase in nervonic acid might be a compensatory response to a metabolic disorder in patients with sarcopenia. The findings of the present study are in line with those of previous reports suggesting that nervonic acid synthesis is regulated by nutritional status in individuals with diseases. For instance, serum nervonic acid levels are significantly elevated in patients with continuous ambulatory peritoneal dialysis.[25] On the other hand, serum nervonic acid levels have been shown to be inversely correlated with BMI, triglyceride levels, total cholesterol levels, and fasting blood glucose levels in subjects without atherosclerotic cardiovascular diseases or diabetes mellitus.[26] Additionally, serum nervonic acid are decreased in subjects with metabolic syndrome (Mets) compared with non-Mets subjects.[27] However, the mechanisms underlying these correlations are unknown at present. Future studies will need to investigate the mechanisms and pathophysiological significance of the selective activation of the fatty acid elongation system in the n-9 monounsaturated

Figure 2 Receiver operating characteristics curve analysis of nervonic acid levels for the detection of sarcopenia.



fatty acid biosynthesis pathway in patients with sarcopenia in combination with cardiovascular disease.

We propose that the most important finding of this study is that increased nervonic acid is an independent marker for the detection of sarcopenia, as were female gender and smaller BMI (*Table* 3). Moreover, serum nervonic acid levels correlated significantly with sarcopenia diagnostic components, namely, SMI, gait speed, and handgrip strength (*Figure* 1). These significant relationships also were observed in each subgroup stratified by gender and age (Supporting Information, *Figure S2*). These findings suggest that nervonic acid may have a metabolic or pathophysiological role in patients with sarcopenia in combination with cardiovascular disease. Given that a nervonic acid weight percentage of 1.37% detected sarcopenia with moderate accuracy on ROC curve analysis (*Figure* 2), nervonic acid could be a new metabolic marker for sarcopenia.

Nervonic acid is known to be an essential component of nerve myelin synthesis, a process that proceeds via glycerophospholipid and sphingomyelin formation.[28] Age-related neural damage has been suggested to induce muscle dysfunction, thereby leading to sarcopenia.[29–31] Thus, it is possible that increased accumulation of nervonic acid may be a compensatory response to the nerve damage that occurs in sarcopenia. Recently, it has been reported that the red cell level of nervonic acid is a significant predictor for all-cause and cardiovascular death in patients with coronary artery disease[32] and that the plasma level of nervonic acid is a significant predictor for all-cause mortality in patients with chronic kidney disease.[33] In those studies, the prevalence of patients with sarcopenia and the possible effect of sarcopenia on survival outcomes were not investigated. These issues should be addressed in future studies.

Study limitations

This investigation was a retrospective cross-sectional study. Thus, the serum fatty acid analysis and examination of sarcopenia diagnostic components were performed only at time of registration. Thus, the effect of long-chain fatty acids on the progression of sarcopenia was not determined as part of this study. A future large-scale prospective study is warranted to determine whether long-chain fatty acids, such as nervonic acid, have pathophysiological significance in sarcopenia and are of use for the diagnosis and/or treatment of sarcopenia in patients with cardiovascular diseases.

Conclusions

The present study demonstrated that the levels of both nervonic acid and erucic acid (n-9 monounsaturated fatty acids) were increased in patients with sarcopenia in combination with cardiovascular disease. Nervonic acid was an independent marker for detection of sarcopenia and had moderate diagnostic accuracy for sarcopenia in patients with cardiovascular disease.

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Online supplementary material

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

Supplementary Figure S1. Metabolism of fatty acids analysed in this study.Supplementary Figure S2. Relationship between nervonic acid levels and sarcopenia diagnostic components in subgroups of men (a), women (b), younger subjects (< 74 years old) (c), and elder subjects (≥ 74 years old) (d).
Supplementary Table S1. Significant factors for sarcopenia diagnosis on simple regression analysis.Supplementary Table S2. Significant fatty acids for sarcopenia diagnosis on simple regression analysis.

Conflict of interest

The authors declare no conflict of interest.

Ethical guidelines

The authors certify that they comply with the ethical guidelines for authorship and publishing of the *Journal of Cachexia, Sarcopenia and Muscle*.[34]

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