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ORIGINAL ARTICLE

Adverse plasma branched-chain amino acid profile mirrors fatty muscle degeneration in diabetic heart failure patients

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Abstract

Aims Elevated plasma branched-chain amino acids (BCAAs) are tightly linked to incident diabetes and its complications, while lower BCAAs are associated with adverse outcomes in the elderly and heart failure (HF) patients. The interplay between body compositions and plasma BCAAs, especially under the influence of co-morbid diabetes in HF patients, is not well understood. Here, we examined the impact of diabetes on the prognostic value of plasma BCAA and its association with body compositions in HF patients.

Methods and results We retrospectively examined 301 HF patients (70 ± 15 years old; 59% male), among which 36% had diabetes. Blood samples for plasma BCAA measurements were collected in a fasting state after stabilization of HF and analysed using ultraperformance liquid chromatography. A dual-energy X-ray absorptiometry scan assessed regional body compositions, and muscle wasting was defined as appendicular skeletal muscle mass index (ASMI) < 7.00 and <5.40 kg/m^2 for males and females, respectively, according to the criteria of the Asian Working Group for Sarcopenia. Although analyses of covariance revealed that plasma BCAAs were significantly higher in diabetic patients, low valine (<222.1 nmol/mL) similarly predicted adverse events defined by HF hospitalization, lethal arrhythmia, or all-cause death in both diabetic and non-diabetic patients independently of age, sex, and NT-proBNP (adjusted hazard ratio [HR] 3.1, 95% confidence interval [CI] of 1.1–8.6 and adjusted HR 2.67, 95% CI 1.1–6.5, respectively; *P* for interaction 0.88). In multivariate linear regression analyses comprising age, sex, and regional body compositions as explanatory variables, plasma BCAAs were positively correlated with visceral adipose tissue area in non-diabetic patients (standardized β coefficients [β] = 0.44, $P < 0.001$). In contrast, in diabetic patients, plasma BCAAs were correlated positively with ASMI (β = 0.49, *P* = 0.001) and negatively with appendicular fat mass index (AFMI; β = −0.42, *P* = 0.004). Co-morbid diabetes was independently associated with muscle wasting (adjusted odds ratio 2.1, 95% CI 1.1–4.0) and significantly higher plasma 3-methylhistidine level, a marker of myofibrillar degradation. In diabetic patients, ASMI uniquely showed a J-shaped relationship with AFMI, and in a subgroup of HF patients with muscle wasting, diabetic patients showed 12% higher AFMI than non-diabetic patients despite comparable ASMI reductions.

Conclusions Despite higher plasma BCAA levels in HF patients with diabetes, the prognostic value of low valine remained consistent regardless of diabetes status. However, low BCAAs were distinctly associated with fatty muscle degeneration in the extremities in diabetic patients, suggesting the importance of targeted interventions to prevent such tissue remodelling in this population.

Keywords Body composition; Branched-chain amino acid; Diabetes; Heart failure; Prognosis

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Introduction

Elevated plasma levels of branched-chain amino acids (BCAAs) are highly associated with obesity, insulin resistance, and diabetes.[1–3] Elevation of levels of BCAAs predicts not only the development of diabetes but also future cardiovascular diseases (CVD) in general populations[4–8] and the development of incident heart failure (HF) in patients with diabetes.[9] On the other hand, plasma BCAA levels decrease with aging[10] and are decreased in some, but not all, HF patients.[11,12] Notably, recent studies have demonstrated that lower BCAA levels are associated with elevated brain natriuretic peptide (BNP) level, impaired exercise tolerance, and adverse prognosis in HF patients.[13–16] Using plasma amino acid profiling, we have recently shown that a lower plasma level of valine, a BCAA, independently predicts adverse events in elderly HF patients.[16] However, it remains unclear whether the prognostic value of BCAAs differs in HF patients complicated with diabetes, which is rapidly increasing worldwide,[17] because each condition could drive plasma BCAAs in opposing directions.

Changes in plasma BCAA levels are associated with body compositional changes, likely due to the significant contribution of skeletal muscle and adipose tissue to systemic BCAA metabolism.[18] In general, plasma BCAAs are positively correlated with total fat mass, visceral fat mass, and skeletal muscle mass in the general population, obese individuals, and older people.[19] However, the relative contribution of muscle and adipose tissue to plasma BCAA levels remains to be determined because the association with BCAAs was examined separately for each body composition in most previous studies. The issue would be even more complicated in diabetic patients by the coexistence of body compositional changes that affect plasma BCAA levels differently: visceral fat accumulation and predisposition of skeletal muscle loss.[20] Furthermore, there have been few studies on the relationship between BCAAs and body composition in HF patients. A deeper understanding of metabolic alterations in HF patients with diabetes could lead to novel therapeutic strategies to improve their morbidity and mortality.

In the present study, we first examined whether co-morbid diabetes affects the prognostic value of plasma valine in HF patients. We then examined the relative contributions of skeletal muscle and fat mass to plasma BCAA levels by incorporating both indexes simultaneously in a single logistic regression model, in which analyses were conducted separately for patients with and those without diabetes. In the latter analyses, we separated fat mass into visceral and appendicular compartments because of the possibility that regional fat tissues have different effects on plasma BCAA levels.

Methods

Study subjects

This study was a single-center, retrospective, and observational study. We enrolled 436 consecutive patients admitted to our institute for diagnosis and management of HF from 15 February 2018 to 31 July 2020 and started to follow-up from the admission day. The inclusion criterion was HF that was diagnosed according to the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic HF.[21] Exclusion criteria were pulmonary artery hypertension, acute myocarditis, and chronic kidney disease at stage 5 that was defined as estimated glomerular filtration rate (eGFR) $<$ 15 mL/min/1.73 m^2, missing data for amino acid profiling and a follow-up period of $<$30 days, resulting in 301 patients for final analyses as we previously described.[16] This study was conducted strictly in accordance with the principles of the Declaration of Helsinki and was approved by the Clinical Investigation Ethics Committee of Sapporo Medical University Hospital (approval number 302-243).

Biochemical analyses, echocardiography, and body composition analyses

Data for blood tests and left ventricular ejection fraction (LVEF), measured by the modified Simpson method, were retrieved from the patients' medical records. eGFR was calculated using equations developed for Japanese subjects as follows: eGFRcre (mL/min/1.73 m^2) = 194 × $Scr^{-1.094}$ × $age^{-0.287}$(×0.739 if female).[22] Chronic kidney disease (CKD) was defined as eGFRcre of $<$60 mL/min/1.73 m^2.

Whole and regional fat/lean masses of patients were analysed during hospitalization using the Horizon A DXA System (HOLOGIC, Waltham, MA, USA) as previously reported,[16,23,24] and lean mass was defined as an index of skeletal muscle mass. Appendicular skeletal muscle mass (ASM) was calculated as the sum of bone-free lean masses in the arms and legs. Systemic fat mass (SFM) was separated into appendicular fat mass (AFM) and trunk fat mass (TFM). Visceral adipose tissue (VAT) area at the level of the L4 lumbar vertebra was also estimated, as previously reported.[25] According to previous large-scale dual-energy X-ray absorptiometry (DXA) studies examining body composition reference range,[26,27] systemic skeletal muscle mass (SSM) index (SSMI), ASM index (ASMI), SFM index (SFMI), AFM index (AFMI), and TFM index (TFMI) were defined as SSM/$height^2$, ASM/$height^2$, SFM/$height^2$, AFM/$height^2$, and TFM/$height^2$, respectively. Muscle wasting was defined as ASMI $<$ 7.0 kg/m^2 for males and ASMI $<$ 5.4 kg/m^2 for females according to the criteria of the Asian Working Group for Sarcopenia.[28]

Measurements of plasma branched-chain amino acids and 3-methylhistidine concentration

Plasma concentrations of BCAAs and 3-methylhistidine, a marker of myofibrillar degradation, were measured in a fasting state after stabilization of HF during hospitalization using ultraperformance liquid chromatography (UPLC, LSI Medience Corporation, Tokyo, Japan), and UPLC analysis was conducted using the Acquity™ UPLC system with a TUV detector and MassTrak™ AAA Solutions Kit (Waters Corporation, Milford, MA) and validated as we previously described.[16]

Clinical endpoint

The clinical endpoint in this study was an adverse event that was defined as a composite of all-cause death and unscheduled readmission due to worsening HF or lethal arrhythmia after discharge as we previously described.[16]

Assessment of lipid infiltration into psoas muscle by computed tomography imaging

In a post-hoc analysis, lipid infiltration into the psoas muscle was evaluated by single slice computed tomography (CT) imaging at L3/4 level in randomly selected 100 patients from the study subjects who underwent abdominal CT imaging within 2 months from plasma amino acid profiling. The psoas muscle area was separated into areas of lean muscle, fatty muscle, and intermuscular adipose tissue (IMAT) using CT value thresholds of 30 to 100 Hounsfield units (HU), −30 to 29 HU, and −190 to −31 HU, respectively, as previously reported.[29] Mean muscle attenuation was defined as the mean CT value of the muscle area with −30 to 100 HU. Data were analysed using SYNAPSE VINCENT (Fujifilm, Tokyo, Japan).

Statistical analysis

Data are presented as means ± standard deviation or medians (interquartile range: 25th–75th percentiles) and expressed as frequency and percentage. In comparing baseline characteristics between patients with and those without diabetes, Student's *t*-test or Mann–Whitney test were used for continuous variables, and the χ^2 test was used for categorical variables. Survival curves were calculated using the Kaplan–Meier method, and the statistical significance of differences between the curves was assessed using log-rank statistics. Multivariate Cox proportional regression analyses were used to evaluate the predictive ability of valine level adjusted for age, sex, and log NT-proBNP, in which the interaction with the presence of diabetes was evaluated. The impact of diabetes on plasma metabolites and body compositions was assessed by analysis of covariance (ANCOVA) adjusted for potential confounders, including age, sex, New York Heart Association (NYHA) functional classification, ischaemic aetiology, hypertension, dyslipidaemia, eGFR (only in the comparison of 3-methylhistidine), and statin use.

The associations between BCAA levels and body compositions were assessed by multivariate linear regression analyses adjusted for potential confounders including age, sex, and serum albumin. In these models, the absence of significant multicollinearity among each body composition was confirmed by variance inflation factors ranging from 1.10 to 4.38. The relative contribution of each body composition to plasma BCAA levels was assessed by calculating the standardized β coefficients. Univariate and multivariate logistic regression analyses were performed to determine factors associated with muscle wasting. The relationship between ASMI and AFMI was evaluated by linear or binomial regression analysis to obtain the highest R^2 value separately in patients with and those without diabetes. A P value < 0.05 was considered statistically significant. Statistical analyses were conducted using JMP Pro version 17.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline clinical characteristics of heart failure patients with diabetes

In our study cohort, 109 patients (36%) were diagnosed with diabetes. As shown in *Table 1*, patients with diabetes were significantly older than patients without diabetes and had higher prevalences of NYHA III/IV symptoms, ischaemic aetiology, hypertension, and dyslipidaemia than patients without diabetes. Other characteristics were similar in the two groups except for higher fasting glucose and HbA1c levels in diabetic patients. Heart failure medications and device therapies were similarly used in the two groups, but statins were more frequently prescribed in patients with diabetes. Among diabetic medications, dipeptidyl peptidase 4 (DPP-4) inhibitors were most frequently used, followed by sodium-glucose cotransporter 2 (SGLT2) inhibitors and insulin. Because patients were enrolled in this study before the era of SGLT2 inhibitors being recommended as standard HF therapy, no patients without diabetes used SGLT2 inhibitors.

Plasma levels of BCAAs are elevated in HF patients with diabetes

In ANCOVA analyses with adjustment for potential confounders, patients with diabetes had significantly higher levels of plasma total BCAAs, valine, leucine, and isoleucine

Table 1 Baseline characteristics

	All patients	Non-DM	DM	
	N = 301	*N* = 192	*N* = 109	*P* value
Age (years)	70.2 (14.5)	68.0 (15.3)	74.1 (12.1)	0.0004
Male sex	177 (59)	110 (57)	67 (61)	0.4792
BMI (kg/m^2)	23.2 (4.1)	22.9 (4.0)	23.6 (4.3)	0.1718
NYHA III or IV	84 (28)	46 (24)	38 (35)	0.0427
LVEF (%)	46.6 (16.0)	46.2 (15.7)	47.4 (16.5)	0.5191
LVEF <40%	117 (39)	75 (39)	42 (39)	0.9277
Ischaemic aetiology	43 (14)	11 (6)	32 (29)	<0.0001
Co-morbidity				
Hypertension	185 (61)	103 (54)	82 (75)	0.0002
Dyslipidaemia	163 (54)	91 (47)	72 (66)	0.0018
Chronic kidney disease	174 (58)	113 (59)	61 (56)	0.6255
Atrial fibrillation	114 (38)	74 (39)	40 (37)	0.7512
Laboratory data				
Haemoglobin (g/dL)	12.5 (2.1)	12.6 (2.1)	12.4 (2.0)	0.3750
NT-proBNP (pg/mL)	995 (467–2,150)	978 (477–2,176)	996 (443–2,110)	0.9434
eGFR (mL/min/1.73 m^2)	55.5 (20.5)	56.9 (20.0)	53.1 (21.3)	0.1174
Albumin (g/dL)	3.7 (0.5)	3.7 (0.5)	3.6 (0.5)	0.2064
Fasting glucose (mg/dL)	96 (27)	87 (15)	113 (34)	<0.0001
HbA1c (%)	6.1 (0.7)	5.8 (0.4)	6.7 (0.8)	<0.0001
Medication				
ACE-I or ARB	168 (56)	101 (53)	67 (61)	0.1356
Beta-blocker	199 (66)	127 (66)	72 (66)	0.9872
MRA	123 (41)	78 (41)	45 (41)	0.9109
Loop diuretics	165 (55)	99 (52)	66 (61)	0.1321
Statin	141 (47)	72 (38)	69 (63)	<0.0001
DPP-4 inhibitor		0 (0)	46 (42)	
SGLT2 inhibitor		0 (0)	25 (23)	
Insulin		0 (0)	14 (13)	
α-glucosidase inhibitors		0 (0)	11 (10)	
Metformin		0 (0)	7 (6)	
Sulphonylureas		0 (0)	6 (6)	
GLP-1 receptor agonist		0 (0)	3 (3)	
Pioglitazone		0 (0)	1 (1)	
Glinide		0 (0)	1 (1)	
Device				0.9050
Pacemaker	24 (8)	17 (9)	7 (6)	
ICD	41 (14)	26 (14)	15 (14)	
CRT-P or CRT-D	30 (10)	19 (10)	11 (10)	

Values are presented as mean (± standard deviation), median (interquartile range), or *n* (%) wherever appropriate. *N* refers to the number of patients for whom the parameter was available.
ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CRT-D, cardiac resynchronization therapy defibrillator; CRT-P, cardiac resynchronization therapy pacemaker; DM, diabetes, DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SGLT2, sodium glucose cotransporter 2.

than those in patients without diabetes (*Figure 1*). Although it was reported that plasma BCAAs were increased by administration of empagliflozin in patients with type 2 diabetes (T2DM),[30] similar trends were confirmed even after excluding patients with diabetes receiving SGLT2 inhibitors (*Figure S1*).

Lower plasma valine similarly predicts adverse events in heart failure patients with and those without diabetes

During the mean follow-up period of 379 ± 212 days, 24 (13%) of the patients without diabetes and 16 (15%) of the patients with diabetes had adverse events. When the patients were divided into two groups according to the cut-off value of plasma valine concentration of 222.1 nmol/mL, which we derived and validated in our previous study,[16] patients with low valine had significantly lower event-free survival rates both in patients with and those without diabetes (*Figure 2A,B*). In multivariate Cox proportional regression analysis adjusted for age, gender, and NT-proBNP, the hazard ratios (HRs) of low plasma valine for adverse events were comparable in patients with and those without diabetes (Model 1, *Figure 2C*). The trend was consistent in another model in which NT-proBNP was replaced by CKD [adjusted HR of 2.7, 95% confidence interval (CI) of 1.1–6.3 for

Figure 1 Comparison of plasma branched-chain amino acid (BCAA) levels by analysis of covariance (ANCOVA) in patients with and those without diabetes (DM). Data are presented with the least square mean and 95% confidence interval. Age, gender, NYHA III or IV, ischaemic aetiology, hypertension, dyslipidaemia, and statin use were incorporated into the ANCOVA as potential cofounders. The *P* values obtained for comparisons of the groups at both ends of the line are shown.

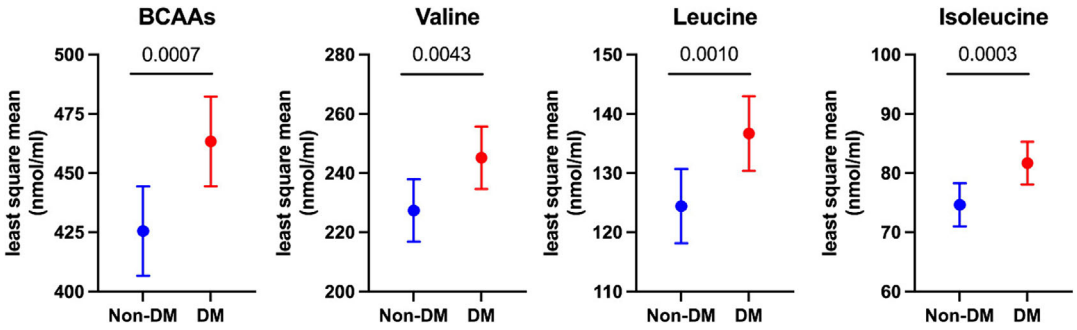
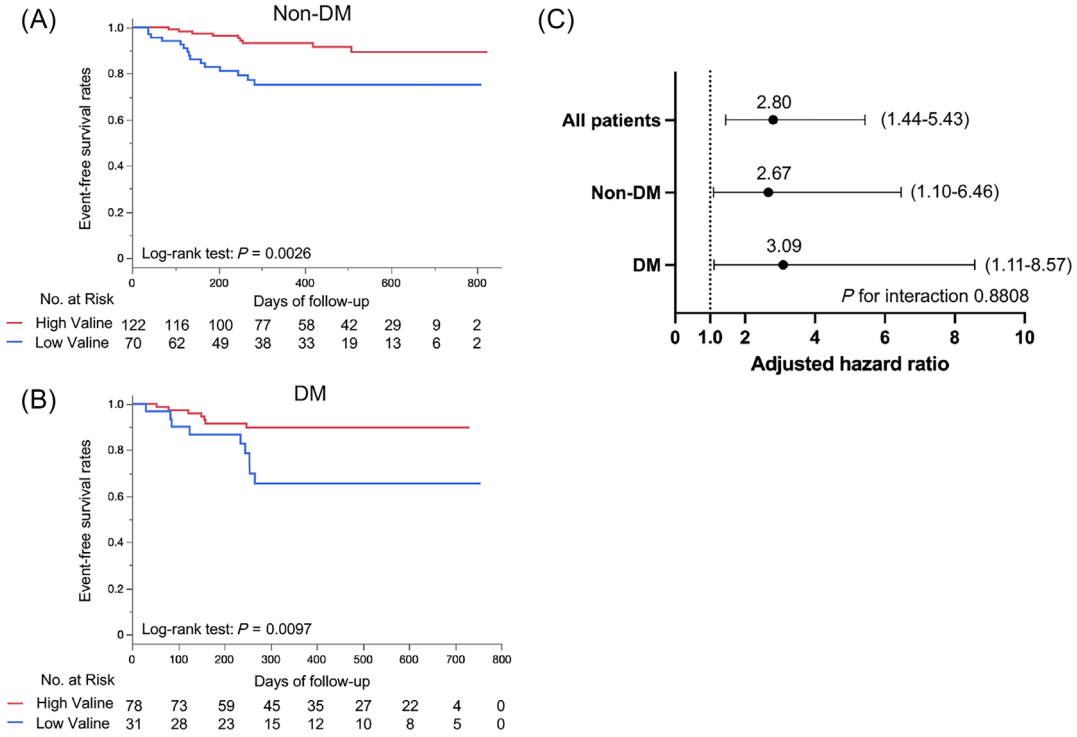


Figure 2 Comparison of the prognostic value of plasma valine in patients with and those without diabetes (DM). Kaplan–Meier event-free survival curves in patients without DM (A) and those with DM (B). HF patients were divided into a high valine group (red) and a low valine group (blue) according to the cut-off value of 222.1 nmol/mL, which we previously derived.[16] Multivariate Cox proportional regression analyses assessing the predictive value of low valine (☆222.1 nmol/mL) for adverse events with adjustment for age, gender, and log NT-proBNP (C). Data are presented with an adjusted hazard ratio and 95% confidence interval in all patients and patients stratified by the status of DM.



non-diabetic patients and adjusted HR of 2.5, 95% CI of 0.9–6.8 for diabetic patients, *P* value for interaction = 0.93]. Even after the addition of use of SGLT2 inhibitors in Model 1, there was no significant interaction between patients with and those without diabetes (*P* = 0.86), and lower valine was still associated with adverse events (adjusted HR: 3.3, 95% CI 1.2–9.2) in patients with diabetes. No significant interaction between patients with and those without diabetes was also consistently observed when patients receiving SLGT2 inhibitors were excluded in Model 1 (*P* = 0.72). Lower valine remained predictive after adjustment for age, gender, NT-proBNP, and HbA1c in the whole study population (adjusted HR: 2.7, 95% CI 1.3–5.3), suggesting that diabetes management does not affect the prognostic value of plasma valine.

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These results indicate that, although diabetes is associated with elevated plasma levels of BCAAs even after HF development, it does not devalue the prognostic role of lower valine in HF patients.

Associations between plasma branched-chain amino acids and regional body compositions are different in heart failure patients with and those without diabetes

ANCOVA analyses revealed that SSMI, ASMI, SFMI, and AFMI were comparable in patients with and those without diabetes. On the other hand, TFMI and VAT area were significantly larger in diabetic patients than in non-diabetic patients (*Figure 3*). The least square mean of VAT area in diabetic patients (101.4 cm^2, 95% CI 91.0–111.8 cm^2) exceeded the cut-off value of visceral obesity (VAT area $\geq$ 100 cm^2) for Japanese subjects,[31] indicating that a significant proportion of HF patients with diabetes had visceral obesity.

We next investigated how regional body compositions influence plasma BCAA levels in non-diabetic and diabetic patients using multivariate logistic regression with adjustment for age and sex (*Table 2*). In non-diabetic patients, BCAA levels were correlated positively with SFMI but not with SSMI. Conversely, in diabetic patients, BCAA levels were correlated positively with SSMI and negatively with SFMI (Model 1). Further analysis with separation of adipose tissue into AFMI and TFMI and inclusion of ASMI (Model 2) revealed that BCAA levels were correlated positively with TFMI and negatively with AFMI in non-diabetic patients, with a more potent impact of TFMI indicated by its higher absolute β coefficients. In diabetic patients, BCAA levels showed no association with TFMI but were correlated negatively with AFMI and positively with ASMI. Similar trends persisted when TFMI was replaced with VAT area (Model 3) and after adjustment for serum albumin, a measure of nutritional status (*Table S1*). However, the associations between BCAA levels and ASMI in diabetic patients were attenuated by the adjustment for serum albumin. An adjustment for SGLT2 inhibitors, which reportedly reduce VAT in T2DM[32] and lean mass in patients with HF with reduced LVEF (HFrEF),[33] did not alter the distinct association between BCAAs and body compositions in diabetic patients (*Table S2*). An additional adjustment for

Figure 3 Comparison of body compositions by analysis of covariance (ANCOVA) in patients with and those without diabetes (DM). Data are presented with the least square mean and 95% confidence interval. Age, gender, NYHA III or IV, ischaemic aetiology, hypertension, dyslipidaemia, and statin use were incorporated into the ANCOVA as potential cofounders. The *P* values obtained for comparisons of the groups at both ends of the line are shown. AFMI, appendicular fat mass index; ASMI, appendicular skeletal muscle mass index; SFMI, systemic fat mass index; SSMI, systemic skeletal muscle mass index; TFMI, trunk fat mass index; VAT, visceral adipose tissue.

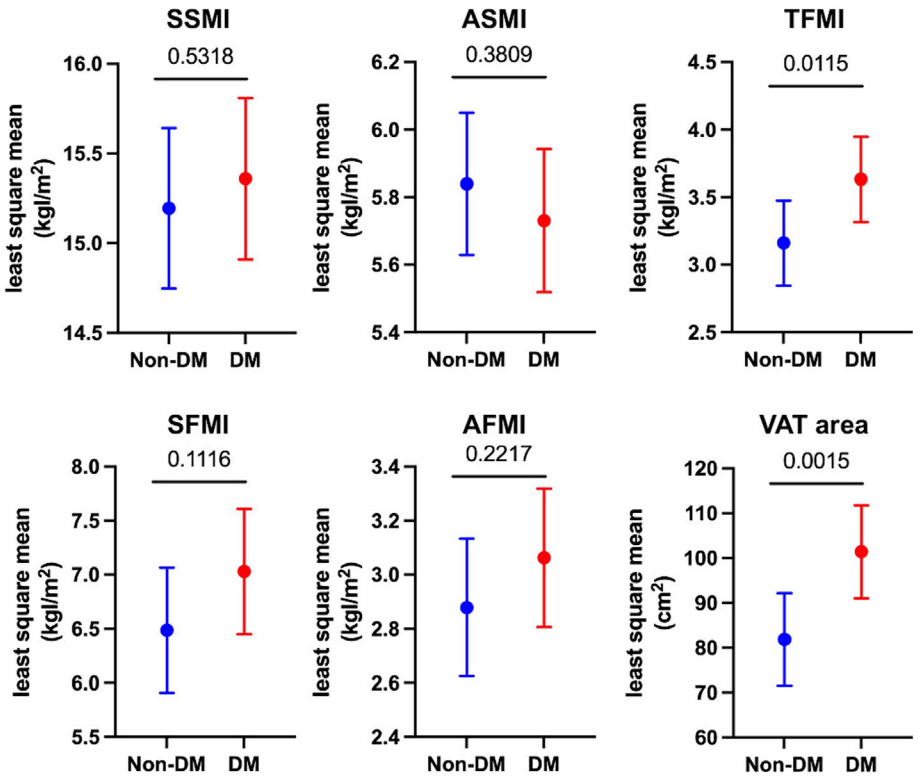


Table 2 Standardized β coefficients between BCAA and body compositions assessed by DXA

Model 1: age, sex, SSMI, SFMI			R^2	SSMI	*P* value	SFMI	*P* value		
	Non-DM	BCAAs	0.123	−0.082	0.3941	0.323	0.0003		
		Val	0.085	−0.102	0.3019	0.299	0.0011		
		Leu	0.140	−0.032	0.7359	0.294	0.0009		
		Ile	0.104	−0.066	0.7748	0.272	0.0030		
	DM	BCAAs	0.115	0.463	0.0010	−0.308	0.0095		
		Val	0.077	0.433	0.0026	−0.307	0.0116		
		Leu	0.125	0.434	0.0019	−0.293	0.0131		
		Ile	0.145	0.457	0.0010	−0.252	0.0305		
Model 2: age, sex, ASMI, AFMI, TFMI			R^2	ASMI	*P* value	AFMI	*P* value	TFMI	*P* value
	Non-DM	BCAA	0.213	0.076	0.4195	−0.520	0.0001	0.722	<0.0001
		Val	0.153	0.040	0.6826	−0.464	0.0011	0.651	<0.0001
		Leu	0.237	0.121	0.1913	−0.536	<0.0001	0.714	<0.0001
		Ile	0.135	0.065	0.5093	−0.380	0.0076	0.560	<0.0001
	DM	BCAA	0.126	0.508	0.0008	−0.423	0.0145	0.188	0.2423
		Val	0.085	0.504	0.0011	−0.360	0.0408	0.106	0.5172
		Leu	0.149	0.491	0.0010	−0.460	0.0071	0.232	0.1426
		Ile	0.136	0.399	0.0075	−0.398	0.0204	0.266	0.0962
Model 3: age, sex, ASMI, AFMI, VAT area			R^2	ASMI	*P* value	AFMI	*P* value	VAT area	*P* value
	Non-DM	BCAAs	0.158	0.107	0.2723	−0.236	0.0402	0.439	<0.0001
		Val	0.110	0.067	0.4990	−0.211	0.0731	0.401	0.0001
		Leu	0.172	0.152	0.1146	−0.234	0.0402	0.406	<0.0001
		Ile	0.113	0.088	0.3767	−0.185	0.1155	0.374	0.0003
	DM	BCAAs	0.139	0.494	0.0010	−0.419	0.0038	0.225	0.0895
		Val	0.091	0.494	0.0014	−0.369	0.0127	0.145	0.2867
		Leu	0.162	0.478	0.0013	−0.439	0.0022	0.254	0.0531
		Ile	0.162	0.380	0.0098	−0.392	0.0060	0.319	0.0155

AFMI, appendicular fat mass index; ASMI, appendicular skeletal muscle mass index; BCAA, branched-chain amino acid; DM, diabetes; DXA, dual-energy X-ray absorptiometry; Ile, isoleucine; Leu, leucine; R^2, coefficient of determination; SFMI, systemic fat mass index; SSMI, systemic skeletal muscle mass index; TFMI, trunk fat mass index; Val, valine; VAT, visceral adipose tissue.

HbA1c in Model 3 confirmed that diabetes management does not significantly affect the distinct association between BCAA and body compositions in diabetic patients (*Table S3*).

Appendicular skeletal muscle wasting with fat deposition is more prevalent in heart failure patients with diabetes

In the whole study population, 166 patients (55%) were diagnosed as having appendicular skeletal muscle wasting according to the criteria of the Asian Working Group for Sarcopenia. In univariate analysis, older age, diabetes, ischaemic aetiology, NYHA III or IV, higher NT-proBNP, lower haemoglobin, lower serum albumin, and MRA use were associated with muscle wasting. In multivariate analysis, diabetes, NYHA III or IV, and lower albumin were selected as independent predictors for muscle wasting (*Table 3*). Patients with diabetes showed a significantly higher plasma 3-methylhistidine concentration, a marker of myofibrillar degradation (*Figure 4A*). ASMI and AFMI showed a modest linear relationship in non-diabetic patients, but a J-shaped relationship fitted more robustly in diabetic patients (*Figure 4B*). Furthermore, in patients with muscle wasting, AFMI was significantly higher in diabetic patients than in non-diabetic patients despite comparable ASMI levels in the two groups (*Figure 4C*). One hundred patients (33% had diabetes) were randomly selected to assess lipid infiltration into the psoas muscle using CT imaging (*Figure S2*). In non-diabetic patients, muscle wasting reduced all areas of IMAT, fatty muscle, and lean muscle, with no change in mean muscle attenuation, suggesting that lipid content per muscle area is not altered by muscle wasting. Conversely, in diabetic patients, muscle wasting reduced only lean muscle area with lower mean muscle attenuation, suggesting that lipid content per muscle area is increased with muscle wasting. The results suggest that HF patients with diabetes are more predisposed to muscle wasting with fatty degeneration and that lower plasma levels of BCAAs in diabetic patients specifically mirror such body compositional changes.

Discussion

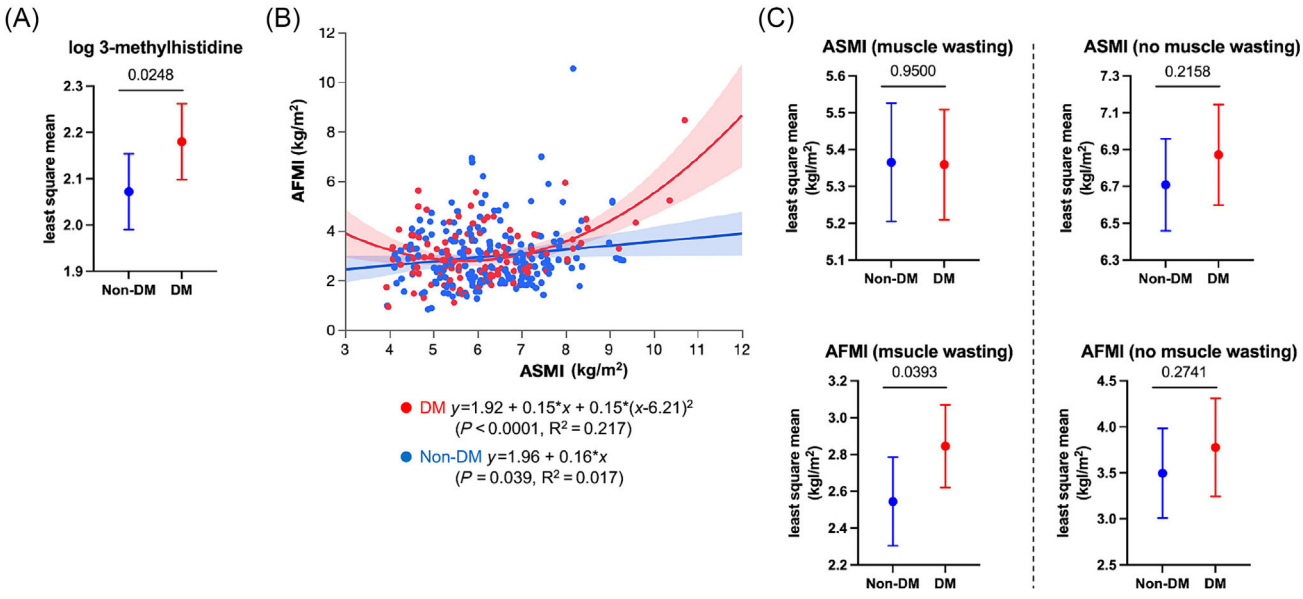
Beyond the well-established association of elevated plasma BCAA levels with insulin resistance and diabetes,[1–6] diabetic patients with high plasma levels of BCAAs but with no history of CVD have been reported to be at high risk for developing myocardial infarction, stroke, coronary revascularization, and heart failure.[8,9] On the other hand, we found the opposite association between plasma valine level and clinical outcomes in HF patients with diabetes. Furthermore, despite significantly higher plasma BCAA levels in diabetic patients (*Figure 1*), the prognostic value of lower valine level (☆222.1 nmol/mL) was comparable to that in patients with-

Table 3 Logistic regression analysis for muscle wasting

	Univariate model		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.02 (1.00–1.04)	0.0185	1.01 (0.98–1.03)	0.5314
Male	1.14 (0.72–1.81)	0.5744	1.42 (0.75–2.71)	0.2813
Diabetes mellitus	2.30 (1.41–3.76)	0.0009	2.14 (1.13–4.04)	0.0191
Smoking history	1.02 (0.65–1.60)	0.9422	0.82 (0.45–1.50)	0.5157
Ischaemic aetiology	2.69 (1.30–5.57)	0.0076	1.99 (0.79–4.99)	0.1429
NYHA III or IV	3.93 (2.21–6.99)	<0.0001	3.07 (1.61–5.83)	0.0006
LVEF <40%	1.53 (0.96–2.45)	0.0762	1.47 (0.79–2.75)	0.2225
Log NT-proBNP	1.33 (1.11–1.60)	0.0024	1.09 (0.85–1.40)	0.4786
eGFR	0.99 (0.98–1.00)	0.1499	1.01 (0.99–1.02)	0.4972
Haemoglobin	0.89 (0.79–0.99)	0.0346	0.99 (0.85–1.16)	0.9377
Albumin	0.30 (0.18–0.52)	<0.0001	0.36 (0.19–0.69)	0.0020
ACE-I or ARB	0.82 (0.52–1.30)	0.3944	0.58 (0.34–1.00)	0.0504
Beta-blocker	0.96 (0.59–1.55)	0.8548	0.98 (0.54–1.80)	0.9493
MRA	1.77 (1.11–2.84)	0.0170	1.72 (0.97–3.04)	0.0616
SGLT2 inhibitor	1.49 (0.64–3.49)	0.3553	0.81 (0.27–2.39)	0.7020
Statin	1.07 (0.68–1.69)	0.7735	0.96 (0.55–1.69)	0.8978

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; OR, odds ratio; SGLT2, sodium glucose cotransporter 2.

Figure 4 Comparison of levels of 3-methylhistidine, a marker of myofibrillar degradation (A), correlation of ASMI with AFMI (B), and body compositions stratified by the presence of muscle wasting (C) in patients with and those without diabetes (DM). Data are presented with the least square mean (A, C), regression line or curve (B), and their 95% confidence interval. Age, gender, NYHA III or IV, ischaemic aetiology, hypertension, dyslipidaemia, and statin use were incorporated into the analysis of covariance (ANCOVA) as potential cofounders in (A) and (C). In (A), eGFR was also incorporated. The *P* values obtained for comparisons of the groups at both ends of the line are shown. Abbreviations are the same as those shown in the legend of *Figure 3*.



out diabetes (*Figure 2C*). Thus, the negative impact of elevated plasma levels of BCAAs in diabetes appears to be overridden once HF develops. In a secondary analysis of the ADVANCE trial in which high-risk T2DM patients were enrolled (35% of the patients having macrovascular diseases and 5% of the patients having HF), elevated plasma levels of BCAAs had no association with macrovascular events; instead, they were associated with lower risk of all-cause mortality.[34] Similarly, plasma metabolomics in the FIGHT trial in which the effect of liraglutide was examined in patients with advanced HFrEF, 58% of whom had T2DM, revealed that a principal component factor level comprising BCAAs correlated negatively with NT-proBNP and positively with 6-min walk distance regardless of co-morbid diabetes,[14] being consistent

with our findings. These results indicate that higher circulating levels of BCAAs are protective rather than detrimental in patients with advanced diabetic complications. The reversed impact of plasma BCAA levels on prognosis across the trajectory of diabetes is analogous to the 'obesity paradox' in HF[35] and in T2DM with established CVD,[36] suggesting that lower plasma BCAA levels might mirror adverse tissue wasting through enhanced catabolism in advanced chronic diseases.

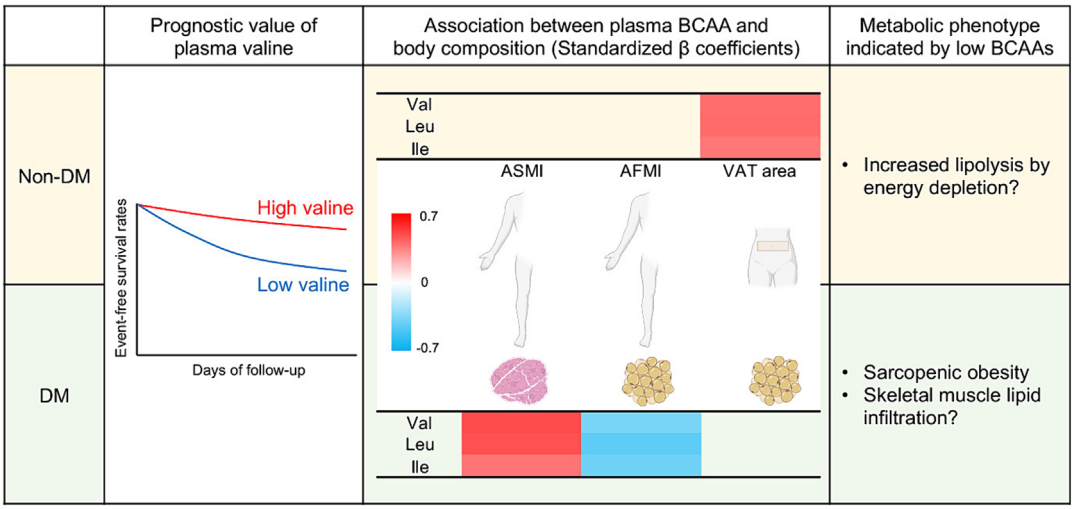
In the present study, plasma levels of BCAAs were positively correlated with VAT area in HF patients without diabetes (*Table 2*), in line with results of previous studies conducted in community-dwelling subjects in which significant proportions of overweight and obese subjects were included.[31,37,38] Adipose tissue is the major organ metabolizing circulating BCAAs.[39] However, obesity is associated with decreased expression of genes related to BCAA metabolism in adipose tissue (especially in VAT),[40–42] partly explaining the well-established association between elevated plasma BCAA levels and obesity. In contrast, a plausible explanation for the association between BCAAs and VAT in relatively lean subjects observed in our study is that lower BCAA levels may indicate malnutrition, which is accompanied by increased lipolysis through enhanced catabolism. This hypothesis is supported by the results of a previous study showing that plasma BCAA levels positively correlate with VAT area in mostly non-obese patients with hepatocellular carcinoma undergoing hepatectomy and that a larger VAT area is associated with a better prognosis.[43] Nevertheless, in the present study, the correlation between VAT area and BCAAs remained almost unchanged even with adjustment for serum albumin as a nutritional index (*Table S1*), suggesting that factors other than malnutrition may also be involved in the association between VAT and BCAAs.

On the other hand, in HF patients with diabetes, plasma BCAA levels showed no significant association with VAT area but were correlated positively with ASMI and negatively with AFMI. Several studies have shown correlations between BCAA and muscle mass in older adults and HF patients,[11,44–48] but the relative contribution of fat mass has not been explored. In one recent study, plasma BCAAs showed a positive correlation with both lean and fat mass index independently in older adults.[49] The discrepancy with our findings in diabetic patients might be explained by the condition termed 'sarcopenic obesity'. Given the higher plasma BCAA levels and more prevalent visceral obesity (*Figure 3*), the effect of VAT on plasma BCAAs is possibly 'saturated', attenuating its impact in diabetic patients. Instead, fat deposition in the extremities, possibly through lipid infiltration into atrophic muscle, appears to become a more potent determinant of plasma BCAA levels, as discussed later. The association between ASMI and fasting plasma BCAAs in diabetic patients may stem from disturbed protein dynamics as skeletal muscle is the largest reservoir of protein (i.e., BCAAs) and has a mutual regulatory relationship with circulating BCAAs for protein synthesis.[18] The mechanistic target of rapamycin complex 1 (mTORC1), which is crucial for switching from protein breakdown to synthesis, is activated by insulin signalling and amino acids, especially leucine. Systemic insulin resistance induced by heart failure is exacerbated by co-morbid diabetes,[50] likely intensifying the impact of latent BCAA deficiency on skeletal muscle turnover in HF patients with diabetes. This hypothesis is supported by our finding that muscle wasting was more prevalent in patients with diabetes, with higher circulating 3-methylhistidine (*Table 3*, *Figure 4A*).

Previous reports indicate that the biological effects of increased fat deposition to the extremities are context-dependent. A study in which body composition was assessed by DXA in the general population revealed that adipose tissue expansion in the gynoid region and the extremities, especially in the legs, is metabolically protective in contrast to the detrimental effect of visceral fat accumulation.[51] In this scenario, subcutaneous adipose tissue (SAT) in the lower extremities serves as a reservoir for excess lipids, preventing ectopic fat deposition. On the other hand, lipid infiltration into skeletal muscle leads to metabolic disturbances and impaired muscle function. For example, fat infiltration into thigh skeletal muscles assessed by CT imaging has been shown to be associated with mobility limitation in the elderly,[52] insulin resistance in obese and diabetic patients,[53] and decreased peak oxygen uptake and poor prognosis in patients with HFrEF.[54] A recent large-scale DXA study revealed that AFM increases with aging, and it has been speculated to involve this harmful fat deposition.[26] Indeed, in a small validation study with CT imaging, DXA-estimated leg fat mass correlated more strongly with IMAT than with SAT in older men with an increased IMAT/SAT ratio.[55] Furthermore, using CT imaging, we observed a higher tendency of lipid infiltration into the psoas muscle in diabetic patients with muscle wasting (*Figure S2*). Taken together with our findings, co-morbid diabetes in HF patients may accelerate muscle aging with fatty degeneration, with lower plasma levels of BCAAs somehow mirroring such adverse muscle remodelling.

The modest positive linear relationship between ASMI and AFMI in non-diabetic patients may be explained by the increased catabolism of muscle and adipose tissue with HF progression. Indeed, we previously showed that a high per cent body fat was associated with a better prognosis in HF patients as an explanation for the obesity paradox,[56] and per cent body fat was strongly correlated with AFMI. In contrast, AFMI tended to increase in diabetic patients once a decline in ASMI reaches muscle wasting levels, resulting in the unique J-shape relationship between the two parameters. This negative association between ASMI and AFMI is consistent with the findings observed in aging in community-dwelling adults,[26] supporting the hypothesis that diabetes promotes appendicular muscle aging, as discussed above.

Figure 5 A graphical summary of the present study. Despite higher levels of plasma branched-chain amino acids (BCAAs) in heart failure (HF) patients with diabetes (DM), low plasma valine level consistently predicts adverse events regardless of co-morbid diabetes. However, plasma BCAAs correlate with body fat and muscle mass differently in diabetic and non-diabetic HF patients. Low plasma BCAAs in diabetic HF patients are uniquely associated with fatty muscle degradation in the extremities. Ile, isoleucine; Leu, leucine; Val, valine. Other abbreviations are the same as those shown in the legend of *Figure 3*. The figure was created partly with BioRender.com.



Our results suggest that prevention of fatty muscle degeneration is a potential therapeutic strategy in HF patients with diabetes. DXA is unsuitable for assessing body compositions in daily clinical settings. Instead, plasma BCAA levels could become simple biomarkers to screen adverse tissue remodelling. We propose that diabetic patients with low BCAA levels should be evaluated for body compositional changes quantitatively and qualitatively using imaging modalities, including DXA and CT. Identifying patients with adverse tissue remodelling, such as fatty muscle degeneration, may allow personalized cardiac rehabilitation. Since the correlation between BCAAs and ASMI in diabetic patients was partially attenuated by the adjustment for serum albumin in the present study (*Table S1*), restoring malnutrition could be one potential intervention. Resistance training has also been reported to reduce thigh intermuscular adipose tissue in older adults.[57,58] The hypothesis that diabetic HF patients, especially those with lower levels of BCAAs, benefit more from the combination of nutritional support and resistance training seems plausible but requires further investigations.

There are several limitations in the present study. First, selection bias might have existed because subjects were retrospectively enrolled in a single centre. Second, SGLT2 inhibitors, which reportedly affect the plasma amino acid profile and body compositions,[30,32,33] were prescribed only in diabetic patients. However, the analyses excluding patients receiving SGLT2 inhibitors or adjusting for the use of SGLT2 inhibitors did not alter the general trend of our primary results. Third, because DXA cannot separate SAT from IMAT and intramuscular fat, the compartments of fat reflected by AFMI remain to be determined. Validation studies with CT or magnetic resonance imaging in HF patients are needed. Fourth, although the logistic regression models consisting of previously reported plasma BCAA determinants (i.e., age, sex, and body compositions) were statistically valid, these variables explained <20% of the variance of plasma BCAA levels based on the R^2 values. Other factors such as protein intake and absorption, BCAA oxidation in the whole body, and gut microbiota compositions might also be involved.[18] Fifth, plasma valine had the highest prognostic value for adverse events among BCAAs in our previous study[16] but was less associated with body composition than leucine (*Table 2*). We hypothesize that plasma leucine is more reflective of skeletal muscle degeneration and dysfunction, while valine predicts broader adverse events independently of sarcopenia. Further studies are needed to elucidate whether such distinct roles exist among BCAAs. Sixth, insulin resistance could also affect aromatic amino acid metabolism.[1,2] The prognostic value of combined assessments of BCAAs and aromatic amino acids in HF patients has been reported,[59–61] though we did not explore the utility in the current study. Finally, the generalizability of our findings to other races, particularly obese Western people, remains to be examined. However, a robust correlation between BCAA dysmetabolism and insulin resistance has been well-established in the non-obese Asian population as well.[62]

In conclusion, although co-morbid diabetes was associated with higher plasma levels of BCAAs even in HF patients, low plasma valine level predicted adverse events similarly in patients with and those without diabetes. However, in HF patients with diabetes, low levels of BCAAs were distinctly associated with appendicular skeletal muscle wasting and

fat deposition (*Figure 5*). Further studies are needed to elucidate whether such adverse tissue remodelling with plasma BCAA reductions is reversible with interventions such as comprehensive cardiac rehabilitation.

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Conflict of interest

None declared.

Supporting information

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

Figure S1. Comparison of plasma branched-chain amino acid (BCAA) levels by analysis of covariance (ANCOVA) in patients with and those without diabetes (DM), excluding those receiving SGLT2 inhibitors (N = 25). Data are presented with the least square mean and 95% confidence interval. Age, gender, NYHA III or IV, ischemic aetiology, hypertension, dyslipidemia, and statin use were incorporated into the ANCOVA as potential cofounders. The *p* values obtained for comparisons of the groups at both ends of the line are shown.

Figure S2. Comparison of psoas muscle area stratified by the presence of diabetes (DM) in patients with and those without muscle wasting (MW) by analysis if covariance (ANCOVA). According to the computed tomography (CT) value, the psoas muscle area was separated into areas of lean muscle (30 to 100 HU), fatty muscle (−30 to 29 HU), and intermuscular adipose tissue (−190 to −31 HU). Mean muscle attenuation was defined as the mean CT value of the muscle area with −30 to 100 HU. Data are presented with the least square mean and 95% confidence interval. Age and gender were incorporated into the ANCOVA as potential cofounders. The *p* values obtained for comparisons of the groups at both ends of the line are shown. HU, Houndsfield units.

Table S1. Standardized β coefficients between BCAA and body compositions assessed by DXA under the adjustment for serum albumin.

Table S2. Standardized β coefficients between BCAA and body compositions assessed by DXA under the adjustment for SGLT2 inhibitors in diabetic patients.

Table S3. Standardized β coefficients between BCAA and body compositions assessed by DXA under the adjustment for HbA1c.

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