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Serum Myostatin Mediates Muscle-Kidney Axis and is an Independent Risk Factor for Diabetic Kidney Disease: A Cross-Sectional Study and Mediation Analysis

Haotian Gu
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Chenyu Han
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Haiying Jin
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Yarong Wang
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Aowen Luo
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Siyuan Shen
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Yaxuan Zhang
Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University
Min Zhang
18116016187@163.com

Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University

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Abstract

Aims

The relationship of serum myostatin, implicated in sarcopenia and diabetic kidney disease (DKD), with DKD is not understood. This study explores the independent association between myostatin levels and DKD, its relationship with renal injury severity in type 2 diabetes mellitus (T2DM) patients, and its role as a muscle-kidney axis biomarker.

Materials and methods

In total, 216 T2DM patients were enrolled. Hierarchical multivariable logistic and linear regression analyses were conducted for independent associations of serum myostatin with DKD and renal injury markers. Causal mediation analysis with bootstrap resampling was performed for mediating effects of myostatin on the bidirectional relationship between renal impairment and skeletal muscle dysfunction. ROC curve analysis was used for the discriminative performance of serum myostatin in identifying DKD.

Results

Myostatin levels were higher in DKD patients compared to DKD (3.47 vs. 1.66 ng/mL, $p < 0.001$). Log-transformed serum myostatin levels were associated with DKD prevalence (OR = 1.93, 95% CI: 1.29–2.95, $p = 0.002$) and urinary albumin-to-creatinine ratio (UACR) (β = 0.308, $p = 0.007$). Indirect effects of myostatin in the renal injury-to-muscle dysfunction and the reciprocal muscle dysfunction-to-renal injury pathways were identified. Incorporating serum myostatin into traditional risk prediction models improved diagnostic discrimination for DKD, increasing the AUC from 0.665 to 0.803 ($p < 0.001$).

Conclusion

Higher serum myostatin levels are associated with increased DKD risk and severe renal injury in T2DM patients. Myostatin may be a molecular component of the muscle-kidney axis and a biomarker for early DKD risk stratification, disease monitoring, and targeted intervention.

Trial registration:

Chinese Clinical Trial Registry (ChiCTR2500106905, 2025-07-31)

1. Introduction

Diabetic kidney disease (DKD) is one of the most prevalent and clinically relevant microvascular complications of type 2 diabetes mellitus (T2DM) and has emerged as a main contributor to end-stage renal disease globally.[1] The pathophysiological progression of DKD involves intricate metabolic dysregulation, persistent inflammatory activation, and progressive fibrotic remodeling, ultimately leading to irreversible deterioration of renal function.[2] Despite effective glycemic and blood pressure management, many patients still face rapid disease progression, suggesting that the main pathogenic mechanisms beyond conventional risk factors are not yet fully understood. Sarcopenia, a muscle disorder common in older adults characterized by a gradual decline in skeletal muscle mass, strength, and physical performance, shows a significantly higher prevalence among T2DM patients.[3]

Many studies suggest that DKD and sarcopenia frequently coexist within the same patients, and their interaction negatively impacts quality of life, increases the risk of physical disability, and raises overall mortality rates.[4–6] This clinical coexistence indicates shared biological mechanisms connecting renal and skeletal muscle tissues within the diabetic environment, known as the "muscle-kidney axis." However, the precise molecular mediator responsible for this inter-organ communication remains poorly understood.

Myostatin, also known as growth differentiation factor-8 (GDF-8), is a member of the transforming growth factor-beta (TGF-β) superfamily and has attracted considerable scientific interest due to its potent inhibition of skeletal muscle growth and regeneration.[7] Previous studies have shown that myostatin plays a pivotal role in regulating muscle mass and functional integrity by suppressing myoblast proliferation and differentiation and by promoting ubiquitin-proteasome system (UPS)-mediated protein catabolism.[8,9] The biological effects of myostatin extend beyond skeletal muscle. Studies have suggested that myostatin is closely involved in systemic inflammatory activity, insulin resistance, and fibrotic remodeling across multiple organs, including the myocardium and kidneys. For example, in a diabetic rat model, myostatin showed significant positive associations with IL-6, TNF-α, and IL-1β.[10] Genetic or pharmacological inhibition of myostatin has been shown to increase AMP-activated protein kinase (AMPK) expression and activity, increasing insulin sensitivity in skeletal muscle.[11] In a mouse sepsis model, myostatin deficiency not only prevented skeletal muscle wasting but also suppressed acute kidney injury and hepatic dysfunction, thus improving survival.[12] These observations support the concept that myostatin may function as a key molecular integrator linking chronic inflammation, metabolic disturbances, tissue fibrosis, and inter-organ communication.

Although preclinical studies suggest that myostatin may play a role in various multisystem disease processes, there remains a lack of clear evidence regarding its clinical importance in patients with T2DM, particularly in the initiation and progression of DKD. The majority of existing clinical literature primarily emphasizes associations between myostatin levels and skeletal muscle mass or strength, or examines variation in non-diabetic chronic kidney disease groups. However, strong clinical evidence demonstrating an independent association between circulating myostatin levels and both the risk and severity of DKD-related kidney damage remains limited. Moreover, the potential role of myostatin as a biological intermediary in the "muscle-kidney axis," which links DKD and sarcopenia, has not been studied or confirmed in human clinical populations. Its proposed function as a signaling mediator, through which renal impairment may lead to skeletal muscle loss, or reduced muscle function may exacerbate renal dysfunction, remains poorly explored. Addressing this knowledge gap is important to understand the shared pathogenic mechanisms behind diabetic complications, discover new prognostic biomarkers, and develop targeted therapies to disrupt pathological communication between organs.

Therefore, this study used an observational cross-sectional design with several specific objectives. First, it aimed to observe whether higher circulating myostatin levels in patients with T2DM are independently and positively associated with increased DKD risk and a higher urine albumin-to-creatinine ratio (UACR), a well-known marker of renal injury severity, beyond traditional risk factors. Second, the study conducted an exploratory investigation into whether myostatin mediates the bidirectional relationship between markers of renal injury and measures of muscle mass and function, thus providing a novel understanding of the potential mechanisms underlying the coexistence of DKD and sarcopenia. Third, to examine the incremental diagnostic value of serum myostatin as a biomarker for DKD and to explore its potential to improve current risk stratification models. Through these objectives, the study aims to generate clinically relevant epidemiological evidence clarifying the role of myostatin within the network of diabetic complications and to establish a basis for future interventions targeting the harmful muscle-kidney cycle.

2. Materials and methods

2.1. Study design and participants

A total of 416 T2DM patients admitted to the Department of Endocrinology at the Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University, between June 2024 and February 2026 were included in the analysis. The primary analysis was conducted in 216 participants with complete muscle-related measurements. For sensitivity analysis, an independent cohort of 200 participants who lacked these muscle-related indicators was used. Eligibility criteria included: (1) age between 18 and 90 years; (2) a confirmed diagnosis of T2DM according to World Health Organization (WHO) diagnostic criteria. Exclusion criteria comprised: (1) type 1 diabetes mellitus, gestational diabetes, or other specific diabetes subtypes; (2) chronic kidney disease due to etiologies other than DKD; (3) severe cardiac, hepatic, or pulmonary insufficiency; (4) active malignant disease; (5) chronic thyroid dysfunction; (6) major surgical intervention within the preceding 3 months; (7) current administration of medications known to affect body composition or metabolisms; and (8) the presence of metallic implants. DKD diagnosis was established in accordance with the 2007 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines.[13]

Sample size was estimated using PASS software with α = 0.05 and statistical power (1-β) = 0.90. Based on preliminary data, mean serum myostatin levels were 1.66 ± 1.82 ng/mL in the non-DKD group and 3.47 ± 3.00 ng/mL in the DKD group. The minimum required sample size was 43 participants per group. Accounting for a 20% attrition rate, at least 54 participants per group were required, yielding a minimum total of 108 participants. The final primary cohort included 147 non-DKD and 69 DKD participants, exceeding the required sample size.

All participants provided written informed consent. The study protocol was approved by the Ethics Committee of the Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University.

2.2. Clinical and biochemical data acquisition

Demographic, clinical, and biochemical variables were extracted from electronic medical records, including sex, age, body mass index (BMI), waist circumference, diabetes duration, fasting insulin, fasting C-peptide, homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-β), glycated hemoglobin (HbA1c), lipid profile [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), small dense LDL (sdLDL)], hepatic enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT)], serum albumin, serum creatinine, estimated glomerular filtration rate (eGFR), serum uric acid, 25-hydroxyvitamin D [25(OH)D], high-sensitivity C-reactive protein (hs-CRP), IL-6, procalcitonin (PCT), urinary microalbumin, UACR, comorbid diabetic complications, and pharmacologic treatment history.

2.3. Body composition-related indicators

2.3.1. Skeletal muscle and adipose tissue quantification

Body composition was analyzed using dual-energy X-ray absorptiometry (DXA). Whole-body scans were conducted by trained operators using a GE Lunar Prodigy system, following standardized acquisition protocols. Participants wore light, standardized clothing, removed all metallic objects, and remained supine with minimal movement and normal respiration during scanning. Quantified parameters included total and regional lean and fat mass. All scans were analyzed by a single technician who was blinded to clinical data.

2.3.2. Handgrip strength

Muscle strength was evaluated using a Jamar Plus+ digital hand dynamometer. Participants were seated with hips and knees at 90°, feet flat on the floor, elbows flexed at 90°, and forearms in a neutral position. Three maximal voluntary contractions were performed for each dominant hand, with a 60-second rest between trials. The highest recorded value, in kilograms (kg), was used for analysis. Standardized verbal encouragement was provided to minimize performance variability.

2.3.3. Appendicular skeletal muscle mass index (ASMI) and muscle quality index (MQI)

ASMI was calculated as appendicular lean mass divided by height squared (kg/m²). MQI was defined as total skeletal muscle mass normalized to handgrip strength.[14]

2.3.4. Serum myostatin quantification

Fasting venous blood samples (5 mL) were collected, allowed to clot at ambient temperature for 30 min, and then centrifuged at 3000 rpm for 15 min to obtain serum. Aliquots were stored at -80°C until analysis, with strict avoidance of repeated freeze-thaw cycles. Serum myostatin concentrations were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-H1437) following the manufacturer's protocol. Optical density was measured at 450 nm with wavelength correction at 570 nm using a Thermo Scientific Varioskan Lux microplate reader. Concentrations were determined from a standard calibration curve generated from known standards. All samples were assayed in duplicate, and mean values were used for statistical analysis. The assay detection limit was 0.47 ng/mL, with a measurable range of 0.78-50 ng/mL and an intra-assay coefficient of variation < 10%.

2.4. Statistical analysis

Normality of continuous variables was evaluated using the Shapiro-Wilk test. Normally distributed variables were reported as mean ± standard deviation (SD) and compared using an independent-samples t-test or a one-way analysis of variance (ANOVA). Non-normally distributed variables were reported as median (interquartile range [IQR]) and analyzed with the Mann-Whitney U test or Kruskal-Wallis H test. Categorical variables were reported as counts and percentages [n (%)] and compared with Pearson's chi-square test or Fisher's exact test. Trend analyses were conducted with the Jonckheere–Terpstra test for continuous variables and the Cochran-Armitage test for categorical variables.

Associations between continuous variables were evaluated using Spearman's rank correlation. Hierarchical multivariable logistic regression models were constructed to identify independent determinants of DKD (binary outcome), and hierarchical multivariable linear regression models were used to assess determinants of UACR (continuous outcome). Stepwise adjustment models (Models 1–5) were implemented to sequentially control for potential confounders, including demographic characteristics, lifestyle factors, metabolic & inflammatory markers, and medication exposure. Multicollinearity was observed using the variance inflation factor (VIF) analysis. Variables with non-normal distributions (i.e., serum myostatin, UACR, TG, and IL-6) were natural log-transformed before inclusion in regression models.

Bidirectional mediation analyses were conducted by alternately specifying log-transformed UACR and muscle-related indices as the exposure and outcome variables to evaluate the mediating role of serum myostatin. Mediation effects, including the average causal mediation effect (ACME) and the average direct effect (ADE), were estimated using a nonparametric bootstrap with 1000 resampling iterations. The diagnostic performance of serum myostatin for DKD was evaluated using ROC curve analysis. Area under the curve (AUC) with 95% confidence intervals (CI) was calculated, and optimal cut-off thresholds were determined using the maximum Youden index, with corresponding sensitivity and specificity reported.

All analyses were conducted in R (v4.4.1). Key analytical packages included MASS, mediation, and pROC. All statistical tests were two-tailed, and significance was set at $p < 0.05$.

3. Results

3.1. Clinical characteristics of the primary analysis population and comparison stratified by myostatin

In the primary analysis cohort (N = 216), serum myostatin levels were significantly higher in participants with DKD (N = 69) than in those without DKD (N = 147) [3.47 (1.71, 5.76) ng/mL vs. 1.66 (0.96, 3.42) ng/mL, $p < 0.001$]. Individuals in the DKD group also had a longer duration of diabetes and higher levels of TG, serum creatinine, IL-6, urinary microalbumin, and UACR, whereas HbA1c levels were lower (all $p < 0.05$) (Table 1).

Table 1
Clinical characteristics of the study population stratified by the presence of diabetic kidney disease (DKD).

Variable	Overall (N = 216)	Non-DKD (N = 147)	DKD (N = 69)	*P* value
Sex [n (%)]				> 0.999
Male	143(66%)	97(66%)	46(67%)	
Female	73(34%)	50(34%)	23(33%)	
Age [years, M (Q1, Q3)]	58(44,67)	59(44,67)	57(48,67)	0.834
BMI [kg/m², Mean ± SD]	26.5 ± 3.7	26.6 ± 3.8	26.2 ± 3.4	0.349
Waist circumference [cm, Mean ± SD]	95 ± 11	95 ± 11	95 ± 10	0.929
Smoking [n (%)]	78(36%)	56 (38%)	22 (32%)	0.463
Alcohol consumption [n (%)]	52 (24%)	38 (26%)	14 (20%)	0.471
Hypertension [n (%)]	118 (55%)	81 (55%)	37 (54%)	0.955
Systolic BP [mmHg, Mean ± SD]	137 ± 17	136 ± 17	141 ± 19	0.051
Diastolic BP [mmHg, Mean ± SD]	82 ± 12	81 ± 12	84 ± 13	0.085
Diabetes duration [months, M (Q1, Q3)]	84(9,144)	60(4,120)	120(60,240)	< 0.001
Fasting insulin [mU/L, M (Q1, Q3)]	7(3,11)	6(3,11)	8(4,13)	0.218
Fasting C-peptide [nmol/L, M (Q1, Q3)]	0.59(0.39,0.89)	0.56(0.38,0.84)	0.65(0.39,0.93)	0.283
HOMA-IR [M (Q1, Q3)]	2.4(1.2,4.3)	2.3(1.2,3.9)	2.7(1.4,5.2)	0.231
HOMA-β [M (Q1, Q3)]	28(14,62)	25(14,62)	28(13,63)	0.437
HbA1c [%, M (Q1, Q3)]	10.0(8.5,11.5)	10.1(8.7,11.7)	9.7(8.1,10.7)	0.038
TC [mmol/L, M (Q1, Q3)]	4.42(3.75,5.09)	4.42(3.78,5.00)	4.43(3.61,5.15)	0.917
TG [mmol/L, M (Q1, Q3)]	1.56(1.15,2.27)	1.46(1.10,2.01)	2.03(1.32,2.57)	0.006
HDL-C [mmol/L, M (Q1, Q3)]	0.97(0.80,1.20)	0.97(0.81,1.20)	0.94(0.78,1.17)	0.272
LDL-C [mmol/L, M (Q1, Q3)]	2.69(2.06,3.28)	2.70(2.14,3.28)	2.61(1.95,3.23)	0.415
sdLDL [mmol/L, M (Q1, Q3)]	0.84(0.58,1.12)	0.79(0.57,1.12)	0.94(0.63,1.17)	0.130
ALT [U/L, M (Q1, Q3)]	23(16,36)	23(15,36)	22(16,32)	0.571
AST [U/L, M (Q1, Q3)]	21(17,28)	22(18,29)	21(17,26)	0.566
γ-GT [U/L, M (Q1, Q3)]	26(18,47)	25(17,45)	26(19,48)	0.859

Data are presented as mean ± SD for normally distributed continuous variables, median (Q1, Q3) for skewed continuous variables, and n (%) for categorical variables.

P values: Kruskal-Wallis test or t-test for continuous variables; χ^2 test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

Variable	Overall (N = 216)	Non-DKD (N = 147)	DKD (N = 69)	*P* value
Albumin [g/L, M (Q1, Q3)]	42.1(39.0,44.5)	42.2(39.0,44.4)	41.4(38.9,44.6)	0.624
Creatinine [μmol/L, M (Q1, Q3)]	62(51,73)	60(51,70)	65(51,80)	0.046
eGFR [mL/min/1.73m², M (Q1, Q3)]	103(90,117)	104(92,117)	99(75,117)	0.108
Uric acid [μmol/L, M (Q1, Q3)]	310(258,366)	310(253,355)	318(269,395)	0.097
25(OH)D [ng/mL, M (Q1, Q3)]	17(13,22)	18(13,23)	16(13,21)	0.260
hs-CRP [mg/L, M (Q1, Q3)]	1.55(0.60,3.33)	1.55(0.65,3.37)	1.55(0.45,2.84)	0.401
IL-6 [pg/mL, M (Q1, Q3)]	4.6(2.8,6.7)	4.3(2.7,6.1)	5.2(3.5,7.1)	0.034
PCT [μg/L, M (Q1, Q3)]	0.045(0.033,0.060)	0.045(0.033,0.061)	0.044(0.033,0.056)	0.564
Urine microalbumin [mg/L, M (Q1, Q3)]	15(7,42)	10(5,18)	83(38,231)	< 0.001
UACR [mg/g, M (Q1, Q3)]	14(7,46)	9(6,15)	87(48,304)	< 0.001
Body composition				
Total body muscle mass [kg, M (Q1, Q3)]	52(45,58)	52(44,59)	52(47,57)	0.976
Total body fat mass [kg, M (Q1, Q3)]	18(15,23)	19(15,23)	17(14,24)	0.481
Trunk muscle mass [kg, M (Q1, Q3)]	26.4(23.0,29.5)	26.2(22.2,29.5)	26.7(24.0,29.7)	0.458
Trunk fat mass [kg, M (Q1, Q3)]	12.3(9.1,15.3)	12.5(9.1,15.0)	11.5(9.2,15.4)	0.700
ASM [kg, M (Q1, Q3)]	26(21,29)	26(21,30)	25(22,28)	0.504
Grip strength [kg, M (Q1, Q3)]	34(25,43)	35(25,44)	33(25,40)	0.200
ASMI [kg/m², M (Q1, Q3)]	9.0(8.3,10.1)	9.1(8.2,10.2)	8.8(8.4,9.5)	0.184
MQI [kg/m², Mean ± SD]	0.65 ± 0.15	0.66 ± 0.15	0.62 ± 0.14	0.091
Myostatin [ng/mL, M (Q1, Q3)]	2.14(1.05,4.18)	1.66(0.96,3.42)	3.47(1.71,5.76)	< 0.001
Complications and comorbidities [n (%)]				
Diabetic retinopathy	51(24%)	25(17%)	26(38%)	0.002
Diabetic peripheral neuropathy	72(33%)	41(28%)	31(45%)	0.020
Diabetic peripheral vascular disease	158(73%)	104(71%)	54(78%)	0.319
Fatty liver	156(72%)	107(73%)	49(71%)	0.914

Data are presented as mean ± SD for normally distributed continuous variables, median (Q1, Q3) for skewed continuous variables, and n (%) for categorical variables.

P values: Kruskal-Wallis test or t-test for continuous variables; χ^2 test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

Variable	Overall (N = 216)	Non-DKD (N = 147)	DKD (N = 69)	*P* value
Coronary heart disease	26(12%)	13(8.8%)	13(19%)	0.060
Medication history [n (%)]				
Metformin	120(56%)	78(53%)	42(61%)	0.352
Sulfonylureas	70(32%)	45(31%)	25(36%)	0.505
Thiazolidinediones	22(10%)	13(8.8%)	9(13%)	0.478
α-Glucosidase inhibitors	63(29%)	47(32%)	16(23%)	0.244
Glinides	12(5.6%)	7(4.8%)	5(7.2%)	0.671
DPP-4 inhibitors	62(29%)	42(29%)	20(29%)	> 0.999
GLP-1 receptor agonists	7(3.2%)	3(2.0%)	4(5.8%)	0.298
SGLT2 inhibitors	65(30%)	33(22%)	32(46%)	< 0.001
Insulin	75(35%)	53(36%)	22(32%)	0.655
ACEI/ARB/ARNI	92(43%)	65(44%)	27(39%)	0.577
Statins	66(31%)	41(28%)	25(36%)	0.279
Data are presented as mean ± SD for normally distributed continuous variables, median (Q1, Q3) for skewed continuous variables, and n (%) for categorical variables.				
P values: Kruskal-Wallis test or t-test for continuous variables; χ^2 test for categorical variables.				
DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.				

When participants were categorized into tertiles by myostatin levels, the prevalence of DKD increased progressively across tertiles with higher myostatin levels (17% vs. 31% vs. 49%, p for trend < 0.001). Participants in the highest tertile (T3) had a longer duration of diabetes and lower grip strength. Higher myostatin levels were associated with progressive increases in serum creatinine, urinary microalbumin, and UACR, whereas eGFR showed a significant decline (all p for trend < 0.05). In the case of body composition and comorbidity profiles, individuals in the high-myostatin group had lower ASMI and MQI values and a higher prevalence of coronary heart disease (Table 2).

Table 2
Clinical characteristics of the study population stratified by serum myostatin tertiles.

Variable	Overall (N = 216)	Tertiles of serum myostatin				
		T1 (N = 72)	T2 (N = 72)	T3 (N = 72)	*P* value	*P* for trend
Sex [n (%)]					0.764	0.481
Male	143(66%)	50(69%)	47(65%)	46(64%)		
Female	73(34%)	22(31%)	25(35%)	26(36%)		
DKD [n (%)]					< 0.001	< 0.001
No	147(68%)	60(83%)	50(69%)	37(51%)		
Yes	69(32%)	12(17%)	22(31%)	35(49%)		
Age [years, M (Q1, Q3)]	58(44,67)	49(37,61)	61(51,69)	61(50,69)	< 0.001	< 0.001
BMI [kg/m², Mean ± SD]	26.5 ± 3.7	27.1 ± 3.9	26.2 ± 3.8	26.3 ± 3.3	0.373	0.269
Waist circumference [cm, Mean ± SD]	95 ± 11	95 ± 11	95 ± 11	96 ± 10	0.454	0.212
Smoking [n (%)]	78(36%)	28 (39%)	24 (33%)	26 (36%)	0.786	0.729
Alcohol consumption [n (%)]	52 (24%)	16 (22%)	17 (24%)	19 (26%)	0.838	0.559
Hypertension [n (%)]	118 (55%)	32 (44%)	40 (56%)	46 (64%)	0.063	0.019
Systolic BP [mmHg, Mean ± SD]	137 ± 17	136 ± 17	137 ± 18	139 ± 18	0.705	0.531
Diastolic BP [mmHg, Mean ± SD]	82 ± 12	83 ± 11	80 ± 13	82 ± 12	0.396	0.536
Diabetes duration [months, M (Q1, Q3)]	84(9,144)	60(6,120)	84(6,150)	120(36,240)	0.001	< 0.001
Fasting insulin [mU/L, M (Q1, Q3)]	7(3,11)	7(3,12)	6(3,12)	7(4,11)	0.956	0.900
Fasting C-peptide [nmol/L, M (Q1, Q3)]	0.59(0.39,0.89)	0.61(0.38,0.89)	0.60(0.32,0.81)	0.55(0.41,0.91)	0.889	0.937

T1, Myostatin ≤ P33; T2, P33 < Myostatin ≤ P67; T3, Myostatin > P67.

Data are presented as mean ± SD for normally distributed continuous variables and median (Q1, Q3) for skewed continuous variables; categorical variables are shown as n (%).

P values: Kruskal-Wallis test or one-way ANOVA for continuous variables; χ^2 test for categorical variables. *P* for trend: Jonckheere-Terpstra test for continuous variables; Cochran-Armitage test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

Variable	Overall (N = 216)	Tertiles of serum myostatin				
		T1 (N = 72)	T2 (N = 72)	T3 (N = 72)	*P* value	*P* for trend
HOMA-IR [M (Q1, Q3)]	2.4(1.2,4.3)	2.7(1.2,4.8)	2.3(1.1,3.7)	2.3(1.3,4.4)	0.804	0.963
HOMA-β [M (Q1, Q3)]	28(14,62)	28(13,57)	28(14,64)	26(14,64)	0.929	0.917
HbA1c [%, M (Q1, Q3)]	10.0(8.5,11.5)	10.0(8.2,11.4)	10.0(8.8,11.5)	10.0(8.3,11.4)	0.823	0.938
TC [mmol/L, M (Q1, Q3)]	4.42(3.75,5.09)	4.54(3.98,4.96)	4.36(3.68,5.02)	4.28(3.70,5.18)	0.594	0.521
TG [mmol/L, M (Q1, Q3)]	1.56(1.15,2.27)	1.60(1.26,2.15)	1.40(1.03,2.35)	1.59(1.16,2.35)	0.630	0.917
HDL-C [mmol/L, M (Q1, Q3)]	0.97(0.80,1.20)	0.97(0.81,1.14)	0.97(0.80,1.20)	0.97(0.81,1.28)	0.874	0.676
LDL-C [mmol/L, M (Q1, Q3)]	2.69(2.06,3.28)	2.77(2.41,3.28)	2.61(1.97,3.23)	2.56(1.92,3.27)	0.137	0.106
sdLDL [mmol/L, M (Q1, Q3)]	0.84(0.58,1.12)	0.91(0.67,1.14)	0.82(0.58,1.03)	0.77(0.50,1.17)	0.334	0.201
ALT [U/L, M (Q1, Q3)]	23(16,36)	25(18,38)	21(15,41)	23(15,32)	0.192	0.087
AST [U/L, M (Q1, Q3)]	21(17,28)	22(19,27)	20(17,30)	22(17,27)	0.482	0.427
γ-GT [U/L, M (Q1, Q3)]	26(18,47)	28(19,63)	22(17,38)	25(18,55)	0.124	0.181
Albumin [g/L, M (Q1, Q3)]	42.1(39.0,44.5)	42.7(40.2,44.9)	41.6(38.9,44.0)	41.6(38.5,44.3)	0.147	0.079
Creatinine [μmol/L, M (Q1, Q3)]	62(51,73)	60(50,69)	60(51,70)	66(54,86)	0.021	0.010
eGFR [mL/min/1.73m², M (Q1, Q3)]	103(90,117)	114(100,120)	101(89,113)	96(73,109)	< 0.001	< 0.001
Uric acid [μmol/L, M (Q1, Q3)]	310(258,366)	316(255,364)	306(251,357)	312(269,381)	0.640	0.714
25(OH)D [ng/mL, M (Q1, Q3)]	17(13,22)	17(13,22)	17(13,22)	16(12,23)	0.884	0.811
hs-CRP [mg/L, M (Q1, Q3)]	1.55(0.60,3.33)	1.73(0.75,3.94)	1.18(0.54,2.80)	1.60(0.60,3.19)	0.161	0.316

T1, Myostatin ≤ P33; T2, P33 < Myostatin ≤ P67; T3, Myostatin > P67.

Data are presented as mean ± SD for normally distributed continuous variables and median (Q1, Q3) for skewed continuous variables; categorical variables are shown as n (%).

P values: Kruskal-Wallis test or one-way ANOVA for continuous variables; χ^2 test for categorical variables. *P* for trend: Jonckheere-Terpstra test for continuous variables; Cochran-Armitage test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

Variable	Overall (N = 216)	Tertiles of serum myostatin				
		T1 (N = 72)	T2 (N = 72)	T3 (N = 72)	*P* value	*P* for trend
IL-6 [pg/mL, M (Q1, Q3)]	4.6(2.8,6.7)	4.1(2.8,5.8)	4.6(2.6,7.1)	5.0(3.3,7.3)	0.088	0.027
PCT [μg/L, M (Q1, Q3)]	0.045(0.033,0.060)	0.045(0.032,0.060)	0.043(0.032,0.056)	0.047(0.035,0.067)	0.450	0.411
Urine microalbumin [mg/L, M (Q1, Q3)]	15(7,42)	11(6,21)	14(7,38)	33(9,85)	< 0.001	< 0.001
UACR [mg/g, M (Q1, Q3)]	14(7,46)	10(6,19)	12(7,42)	29(10,85)	< 0.001	< 0.001
Body composition						
Total body muscle mass [kg, M (Q1, Q3)]	52(45,58)	53(46,62)	52(44,56)	53(44,58)	0.278	0.302
Total body fat mass [kg, M (Q1, Q3)]	18(15,23)	18(15,24)	19(13,23)	19(16,24)	0.718	0.879
Trunk muscle mass [kg, M (Q1, Q3)]	26.4(23.0,29.5)	26.1(23.5,30.4)	26.4(22.3,28.2)	26.9(23.1,29.8)	0.687	0.782
Trunk fat mass [kg, M (Q1, Q3)]	12.3(9.1,15.3)	12.5(8.8,15.1)	12.1(8.7,14.7)	12.3(9.7,15.4)	0.843	0.880
ASM [kg, M (Q1, Q3)]	26(21,29)	27(23,31)	24(21,28)	26(20,29)	0.013	0.040
Grip strength [kg, M (Q1, Q3)]	34(25,43)	40(26,47)	31(24,41)	32(23,40)	0.020	< 0.001
ASMI [kg/m², M (Q1, Q3)]	9.0(8.3,10.1)	9.5(8.8,10.6)	8.7(8.1,9.6)	8.9(8.1,9.5)	0.001	0.003
MQI [kg/m², Mean ± SD]	0.65 ± 0.15	0.70 ± 0.14	0.63 ± 0.15	0.61 ± 0.13	< 0.001	< 0.001
Complications and comorbidities [n (%)]						
Diabetic retinopathy	51(24%)	13(18%)	20(28%)	18(25%)	0.367	0.327
Diabetic peripheral neuropathy	72(33%)	19(26%)	27(38%)	26(36%)	0.305	0.216

T1, Myostatin ≤ P33; T2, P33 < Myostatin ≤ P67; T3, Myostatin > P67.

Data are presented as mean ± SD for normally distributed continuous variables and median (Q1, Q3) for skewed continuous variables; categorical variables are shown as n (%).

P values: Kruskal-Wallis test or one-way ANOVA for continuous variables; χ^2 test for categorical variables. *P* for trend: Jonckheere-Terpstra test for continuous variables; Cochran-Armitage test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

Variable	Overall (N = 216)	Tertiles of serum myostatin				
		T1 (N = 72)	T2 (N = 72)	T3 (N = 72)	*P* value	*P* for trend
Diabetic peripheral vascular disease	158(73%)	46(64%)	57(79%)	55(76%)	0.088	0.091
Fatty liver	156(72%)	50(69%)	54(75%)	52(72%)	0.758	0.710
Coronary heart disease	26(12%)	1(1.4%)	11(15%)	14(19%)	0.020	< 0.001
Medication history [n (%)]						
Metformin	120(56%)	38(53%)	42(58%)	40(56%)	0.799	0.737
Sulfonylureas	70(32%)	22(31%)	27(38%)	21(29%)	0.519	0.859
Thiazolidinediones	22(10%)	8(11%)	9(13%)	5(6.9%)	0.518	0.408
α-Glucosidase inhibitors	63(29%)	21(29%)	22(31%)	20(28%)	0.935	0.855
Glinides	12(5.6%)	3(4.2%)	4(5.6%)	5(6.9%)	0.767	0.467
DPP-4 inhibitors	62(29%)	17(24%)	25(35%)	20(28%)	0.330	0.581
GLP-1 receptor agonists	7(3.2%)	2(2.8%)	3(4.2%)	2(2.8%)	0.863	> 0.999
SGLT2 inhibitors	65(30%)	21(29%)	18(25%)	26(36%)	0.340	0.364
Insulin	75(35%)	24(33%)	25(35%)	26(36%)	0.941	0.726
ACEI/ARB/ARNI	92(43%)	23(32%)	30(42%)	39(54%)	0.026	0.007
Statins	66(31%)	14(19%)	28(39%)	24(33%)	0.033	0.070

T1, Myostatin ≤ P33; T2, P33 < Myostatin ≤ P67; T3, Myostatin > P67.

Data are presented as mean ± SD for normally distributed continuous variables and median (Q1, Q3) for skewed continuous variables; categorical variables are shown as n (%).

P values: Kruskal-Wallis test or one-way ANOVA for continuous variables; χ^2 test for categorical variables. *P* for trend: Jonckheere-Terpstra test for continuous variables; Cochran-Armitage test for categorical variables.

DKD, diabetic kidney disease; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; 25(OH)D, 25-hydroxyvitamin D; ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

3.2. Correlation analysis between myostatin and various clinical indicators

Spearman rank correlation analyses revealed that serum myostatin levels were significantly positively associated with age (r = 0.237), duration of diabetes (r = 0.248), serum creatinine (r = 0.181), urinary microalbumin (r = 0.263), UACR (r = 0.270), and IL-6 (r = 0.155) (all $p < 0.05$). Serum myostatin levels were significantly inversely associated with eGFR (r = -0.283), grip strength (r = -0.199), ASMI (r = -0.165), and MQI (r = -0.242) (all $p < 0.05$) (Table 3).

Table 3
Spearman correlation analysis of serum myostatin levels with clinical parameters.

Variable	Spearman r	*P* value
Age	0.237	< 0.001
Diabetes duration	0.248	< 0.001
Creatinine	0.181	0.0076
eGFR	-0.283	< 0.001
Urine microalbumin	0.263	< 0.001
UACR	0.27	< 0.001
IL-6	0.155	0.0224
ASMI	-0.165	0.0153
Grip strength	-0.199	0.0034
MQI	-0.242	< 0.001

eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; IL-6, interleukin-6; ASMI, appendicular skeletal muscle mass index; MQI, muscle quality index.

3.3. Hierarchical regression analysis of the independent association between myostatin and kidney injury

(1) Association between myostatin and DKD: Multivariable logistic regression analyses indicated that in the unadjusted model (Model 1), Log(myostatin) was positively associated with the risk of DKD (OR = 1.86, 95% CI: 1.34–2.63, $p < 0.001$). This association remained significant after sequential adjustment for demographic variables, diabetes duration, lifestyle factors, metabolic and inflammatory markers, and medication history (Models 2–4). In the fully adjusted model (Model 5), which additionally incorporated muscle-related variables, including ASMI and grip strength, myostatin remained independently associated with DKD risk (OR = 1.93, 95% CI: 1.29–2.95, $p = 0.002$). Multicollinearity assessments showed that all covariates had VIF values below 5, indicating the absence of multicollinearity within the model (Table 4).

Table 4
Association between serum myostatin and the risk of diabetic kidney disease (DKD).

Model	OR (95% CI)	Wald	*P* value
Model 1	1.86 (1.34, 2.63)	3.64	< 0.001
Model 2	1.90 (1.36, 2.71)	3.64	< 0.001
Model 3	1.85 (1.26, 2.79)	3.07	0.002
Model 4	1.95 (1.31, 2.97)	3.23	0.001
Model 5	1.93 (1.29, 2.95)	3.12	0.002
OR, odds ratio; CI, confidence interval. Myostatin was analyzed as a continuous variable (per 1 ng/mL increase).			
Model 1: unadjusted.			
Model 2: adjusted for age and sex.			
Model 3: adjusted for Model 2 + diabetes duration, BMI, smoking, alcohol consumption, hypertension, HbA1c, eGFR, 25(OH)D, and natural logarithm of TG and IL-6.			
Model 4: adjusted for Model 3 + ACEI/ARB/ARNI, SGLT2 inhibitors, GLP-1 receptor agonists, and statins.			
Model 5: adjusted for Model 4 + ASMI and grip strength.			
P < 0.05 indicates statistical significance.			

(2) Association between myostatin and UACR: To further evaluate the relationship between myostatin and renal injury severity, hierarchical multivariable linear regression analyses were conducted with Log(UACR) as the dependent variable. In the unadjusted model, Log(myostatin) showed a highly significant positive association with UACR (β = 0.443, 95% CI: 0.224–0.662, $p < 0.001$). After sequential adjustment for age, sex, lifestyle-related variables, metabolic parameters, and inflammatory markers, this association remained stable (Model 3: β = 0.327, 95% CI: 0.107–0.546, $p = 0.004$). After further adjustment for medication use and muscle-related indicators (ASMI and grip strength), myostatin continued to show a statistically significant, independent positive association with UACR (Model 5: β = 0.308, 95% CI: 0.086–0.532, $p = 0.007$) (Table 5).

Table 5
Association between serum myostatin and urine albumin-to-creatinine ratio (UACR).

Model	β (95% CI)	t	*P* value
Model 1	0.443 (0.224, 0.662)	3.99	< 0.001
Model 2	0.466 (0.239, 0.694)	4.05	< 0.001
Model 3	0.327 (0.107, 0.546)	2.94	0.004
Model 4	0.325 (0.108, 0.542)	2.96	0.003
Model 5	0.308 (0.086, 0.532)	2.73	0.007
β, regression coefficient; CI, confidence interval. UACR was natural log-transformed before analysis. Myostatin was analyzed as a continuous variable (per 1 ng/mL increase).			
Model 1: unadjusted.			
Model 2: adjusted for age and sex.			
Model 3: adjusted for Model 2 + diabetes duration, smoking, alcohol consumption, hypertension, BMI, HbA1c, eGFR, 25(OH)D, and natural logarithm of TG and IL-6.			
Model 4: adjusted for Model 3 + ACEI/ARB/ARNI, SGLT2 inhibitors, GLP-1 receptor agonists, and statins.			
Model 5: adjusted for Model 4 + ASMI and grip strength.			
P < 0.05 indicates statistical significance.			

3.4. Mediation effect analysis

To investigate the potential mechanistic role of myostatin in linking diabetic kidney injury and muscle deterioration, a bidirectional mediation model was developed. After adjustment for age, sex, lifestyle factors, metabolic, and inflammatory markers, the analyses showed the following findings. In the pathway examining the effect of kidney injury on muscle outcomes (UACR → myostatin → muscle indicators), although no significant total effect was detected at the overall model level (all $p > 0.05$), the indirect effect mediated through myostatin (ACME) was statistically significant for both grip strength (β = -0.2128, $p < 0.001$) and MQI (β = -0.0045, $p < 0.001$). In the reverse pathway (muscle indicators → myostatin → UACR), the indirect effects mediated by myostatin were also statistically significant for grip strength (ACME: β = -0.0092, $p < 0.001$), ASMI (ACME: β = -0.0126, $p = 0.040$), and MQI (ACME: β = -0.7178, $p < 0.001$) (Table 6) (Fig. 1).

Table 6
Bidirectional mediation analysis results.

Path direction	Effect type	Estimate	(95% CI)	*P* value	Mediation proportion (%)
Direction 1: UACR → Myostatin → Muscle					
UACR → Myostatin → Grip strength	Total effect	-0.3889	(-0.9281, 0.2689)	0.2	100
	Direct effect (ADE)	-0.1761	(-0.7461, 0.4819)	0.58	45.3
	Indirect effect (ACME)	-0.2128	(-0.4137, -0.0556)	< 0.001	54.7
UACR → Myostatin → ASMI	Total effect	-0.0764	(-0.252, 0.0705)	0.34	100
	Direct effect (ADE)	-0.0498	(-0.225, 0.0967)	0.46	65.2
	Indirect effect (ACME)	-0.0266	(-0.0686, 0.0062)	0.16	34.8
UACR → Myostatin → MQI	Total effect	-0.007	(-0.0154, 0.0025)	0.16	100
	Direct effect (ADE)	-0.0024	(-0.0114, 0.0071)	0.66	35.1
	Indirect effect (ACME)	-0.0045	(-0.0087, -0.002)	< 0.001	64.9
Direction 2: Muscle → Myostatin → UACR					
Grip strength → Myostatin → UACR	Total effect	-0.0169	(-0.0424, 0.0082)	0.28	100
	Direct effect (ADE)	-0.0077	(-0.0305, 0.0177)	0.7	45.6
	Indirect effect (ACME)	-0.0092	(-0.0205, -0.0026)	< 0.001	54.4
ASMI → Myostatin → UACR	Total effect	-0.0341	(-0.1066, 0.0367)	0.34	100
	Direct effect (ADE)	-0.0214	(-0.0918, 0.0491)	0.54	62.9
	Indirect effect (ACME)	-0.0126	(-0.0336, -4e-04)	0.04	37.1
MQI → Myostatin → UACR	Total effect	-1.131	(-2.8223, 0.2853)	0.22	100
	Direct effect (ADE)	-0.4132	(-2.0935, 1.0558)	0.6	36.5
	Indirect effect (ACME)	-0.7178	(-1.4301, -0.24)	< 0.001	63.5

All path effect estimates for continuous variables in the causal mediation model, along with their 95% confidence intervals (CI), represent differences in regression coefficients (β) on a linear scale. All statistics were derived from 1,000 non-parametric bootstrap resamples after adjustment for covariates. UACR was natural log-transformed prior to inclusion in the analysis. Two-sided *P* values were used, with $P < 0.05$ considered statistically significant.

UACR, urine albumin-to-creatinine ratio; MQI, muscle quality index; ASMI, appendicular skeletal muscle mass index.

3.5. ROC curve-based predictive analysis

To further assess the models' discriminatory performance, ROC curves were generated for individual predictors and the combined model. The results showed that the AUC for detecting DKD using Log(myostatin) alone was 0.665 (95% CI: 0.588–0.741) (sensitivity = 82.6% and specificity = 47.6%). After inclusion of covariates in the fully adjusted logistic regression model (Model 5), the AUC of the combined model increased to 0.803 (95% CI: 0.742–0.864). The DeLong test indicated a significant difference ($Z = -3.72$, $p < 0.001$), demonstrating that the combined model had better discriminatory power than the myostatin-only model. The optimal probability threshold, determined by the Youden index, was 0.321 (sensitivity = 73.9% and specificity = 72.1%) (Fig. 2).

These results indicate that integrating myostatin alongside conventional risk factors can enhance the identification of DKD risk to some extent.

3.6. Sensitivity analysis

(1) Homogeneity test

To evaluate sample representativeness and consistency, baseline characteristics were compared between the sensitivity-analysis cohort (N = 200) and the primary-analysis cohort (N = 216). The results showed no significant differences between the two cohorts in key variables (sex, age, diabetes duration, HbA1c, uric acid, 25(OH)D, urinary microalbumin, and DKD prevalence) (all $p > 0.05$). However, differences were identified in several metabolic and body composition parameters. Compared with the sensitivity analysis cohort, the primary analysis cohort had higher BMI, fasting insulin, fasting C-peptide, HOMA-IR, and IL-6 levels ($p < 0.05$), and lower HDL-C and LDL-C levels ($p < 0.05$). Statistically significant differences were observed between the two cohorts in the use of certain glucose- and lipid-lowering medications (Supplementary Table 1).

(2) Comparison of sensitivity analyses: The fully adjusted model (Model 4) derived from the sensitivity analysis also demonstrated a positive association of Log(myostatin) with both DKD (OR = 5.89, 95% CI: 3.11–12.05, $p < 0.001$) and Log(UACR) (β = 0.605, 95% CI: 0.371–0.840, $p < 0.001$), consistent with the findings from the primary analysis (Supplementary Tables 2, 3). The high consistency between the sensitivity and the primary analyses supports the stability of this positive association.

4. Discussion

This study is the first to comprehensively demonstrate that circulating myostatin levels are positively associated with the presence of DKD and the extent of renal damage (as reflected by UACR) in T2DM patients, and that they are also associated with reduced skeletal muscle mass and impaired muscle function. These observations closely align with previous findings in patients with chronic kidney disease (CKD), in whom increasing serum myostatin levels were related to worsening renal function and independently predicted the onset of sarcopenia.[15] In pathophysiological mechanisms, myostatin can directly induce myotube atrophy and activate the Smad2/Smad3 signaling cascade, which has been reported to be abnormally upregulated in the lower-extremity muscles of patients with COPD-associated sarcopenia. Pathway activity shows an inverse relationship with the fat-free mass index and muscle strength.[16] Mechanistic evidence in DKD further indicates that high glucose levels and glycated albumin stimulate both myostatin mRNA and protein production in human renal tubular epithelial cells (HK-2), particularly within the tubulointerstitial compartment, where expression levels are positively correlated with the severity of interstitial fibrosis ($r = 0.54$, $p < 0.01$). Exogenous myostatin treatment promotes NF-κB activation, increases CCL-2 and SMAD2/3 expression, and induces ERK pathway-dependent generation of reactive oxygen species (ROS) in these cells.[17] These molecular processes provide a mechanistic basis for how myostatin contributes to glomerulosclerosis and tubulointerstitial fibrosis, directly supporting the positive associations between myostatin, UACR, and serum creatinine identified in this study. Moreover, upregulation of IL-6 observed in the high-myostatin group suggests that myostatin may further compromise renal function indirectly by boosting chronic systemic inflammation. The comparatively lower HbA1c levels observed in the DKD group may be due to several factors: shortened erythrocyte lifespan and low erythropoietin production resulting from renal dysfunction may affect the reliability of HbA1c as an indicator of glycemic control; additionally, myostatin exerts complex effects on insulin sensitivity and glucose homeostasis,[18] and we hypothesize that under certain pathological conditions it may promotes glucose consumption and cellular uptake, enhance glycolytic activity, and suppress glycogen synthesis, thus affecting glucose metabolism and contributing to lower HbA1c levels. These results collectively provide provisional evidence that myostatin is a key pathophysiological mediator linking renal injury and muscle loss in individuals with T2DM.

In the hierarchical regression analyses, Log(myostatin) remained independently and positively associated with DKD risk even after sequential adjustment for demographic variables, diabetes duration, metabolic and inflammatory parameters, and medication history (OR = 1.93, $p = 0.002$). After further incorporating muscle-related measures, including ASMI and grip strength, the independent association between myostatin and Log (UACR) also remained statistically significant (β = 0.308, $p = 0.007$). Positioning muscle indicators in the final adjustment step strongly suggests that the adverse renal effect of myostatin is partially independent of its role in promoting muscle atrophy, consistent with experimental evidence indicating that myostatin can directly

exert pro-inflammatory and pro-fibrotic actions within the kidney.[17] The strong alignment between the logistic and linear regression findings, which converge on the same conclusion from the perspectives of "disease risk" and "injury severity," further strengthens the robustness of the evidence. In all models, VIF values were below 5, indicating the absence of serious multicollinearity, supporting the precision of the regression coefficient estimates and allowing reliable differentiation of the relatively independent contribution of myostatin from established traditional risk factors. The hierarchical regression findings rigorously minimized the influence of major confounding factors and provided strong statistical support for myostatin as an independent risk biomarker for DKD.

The mediation analysis further demonstrated the bidirectional mediating role of myostatin between renal injury and muscle deterioration, providing preliminary statistical evidence supporting causal inference for the "muscle-kidney axis" hypothesis. In the "kidney injury → myostatin → muscle indicators" pathway, myostatin significantly mediated the negative association between UACR and grip strength and MQI, whereas the mediating effect of myostatin was not significant in the ASMI pathway. This finding revealed that advanced renal injury, as reflected by elevated UACR and reduced eGFR, may contribute to impaired insulin signaling, chronic inflammation, metabolic acidosis, and increased angiotensin II levels, all of which have been shown to upregulate myostatin expression.[9] Increased myostatin may suppress protein synthesis and enhance proteolysis in skeletal muscle by activating the Smad2/Smad3 signaling pathway and the UPS, ultimately resulting in muscle atrophy and reduced muscle strength.[9,16] During the progression of DKD-related myopathy, activation of myostatin signaling may initially cause intrinsic impairment of muscle function; along with inflammatory mediators, myostatin may directly disrupt mitochondrial energy metabolism and excitation-contraction coupling, leading to a decline in muscle strength before overt muscle atrophy becomes evident.[19] The reduction in MQI, which reflects strength output relative to muscle mass, more directly captures impairment in muscle function. Clinical investigations by Hirano et al. similarly reported that diabetic kidney disease was associated with increased muscle fatigue, whereas the skeletal muscle index (SMI) did not differ significantly.[20] Similarly, Chatrenet et al. observed that muscle strength progressively declined with CKD progression, whereas muscle mass remained relatively unchanged.[21] It should also be recognized that ASMI, as assessed by DXA, cannot distinguish functional muscle fibers from infiltrating adipose tissue, potentially obscuring true muscle loss.[22] This observation provides clinicians with important guidance for early detection: monitoring functional measures, such as grip strength, together with biomarkers such as myostatin, may enable earlier identification and intervention for sarcopenia in patients with DKD than relying on changes in muscle mass alone. In the reverse "muscle indicators → myostatin → kidney injury" pathway, myostatin also significantly mediated the effects of grip strength, ASMI, and MQI on UACR. Skeletal muscle is a major metabolic organ and has been shown in previous research to exert protective effects on the kidney. For example, reduced rectus femoris cross-sectional area has been associated with an increased risk of DKD, and diabetic patients with sarcopenia have a higher likelihood of developing severe DKD than those without sarcopenia.[5,6] Based on these mediation results, this study further suggests that deterioration in muscle mass and function may, in turn, worsen renal injury and proteinuria through dysregulation of myokines, including increased myostatin secretion. In summary, through bidirectional mediation effects, this study provides further evidence for a myostatin-mediated vicious cycle linking DKD and sarcopenia.

In the case of biomarker diagnostic performance, this study found that Log(myostatin) alone predicted DKD with an AUC of 0.665, a sensitivity of 82.6%, and a specificity of 47.6%, indicating moderate discriminative capacity. However, when myostatin was combined with traditional risk factors, the AUC of the combined model increased significantly to 0.803, with a sensitivity of 73.9% and a specificity of 72.1%, and the DeLong test yielded $p < 0.001$. This improvement has clear clinical relevance, indicating that incorporating serum myostatin levels into DKD risk stratification based on conventional risk factors, such as BMI, disease duration, blood pressure, lipid parameters, and inflammatory markers, can substantially improve the accuracy of identifying high-risk individuals. The high sensitivity of myostatin alone, 82.6%, was achieved at the expense of low specificity, 47.6%, which may still hold clinical utility in DKD screening strategies: as an initial screening marker, high sensitivity can help minimize missed DKD diagnoses, whereas the combined model provides a more balanced sensitivity and specificity profile, making it more appropriate for confirmatory assessment or risk stratification. However, it has also been reported that an AUC of 0.665 remains insufficient for a single biomarker, likely due to the multifactorial complexity of DKD. The incremental diagnostic contribution of myostatin supports its potential role as a supplementary biomarker for improving early DKD risk stratification. The sensitivity analysis further confirmed the robustness of the main results. Although there were statistical differences between the sensitivity cohort (N = 200) and the primary cohort (N = 216) in some metabolic variables, such as BMI, lipid levels, and IL-6, and in medication history, no

differences were found in key indicators, including demographic details, diabetes duration, or DKD prevalence. These metabolic differences might be due to seasonal or dietary changes associated with different enrollment times, or to minor systematic differences between lab assay batches. However, these variations did not affect the study's primary conclusions. After adjusting for demographic factors, disease duration, metabolic and inflammatory markers, and medication history, the sensitivity analysis cohort confirmed a significant positive link between Log(myostatin) and DKD risk (OR = 5.89, $p < 0.001$), as well as between Log(myostatin) and Log (UACR) (β = 0.605, $p < 0.001$). Both the direction and strength of these associations stayed consistent with the primary results. These aligned results across subgroups with different metabolic traits suggest that the link between myostatin and renal injury is not influenced or confounded by specific metabolic factors such as lipid levels or inflammatory burden, but rather reflects a relatively generalizable biological phenomenon. Therefore, from an internal validity perspective, the sensitivity analysis supports the reliability of the study's conclusions. However, external validity, generalizability to populations with different races, ages, or disease severities, still needs validation through larger, more diverse cohorts.

Although this study confirmed the independent association between serum myostatin and DKD through multilevel statistical adjustment and sensitivity analyses, several limitations have also been reported. First, the cross-sectional design restricts causal interpretation. Although the mediation analyses suggest possible bidirectional relationships within the "kidney injury → myostatin → muscle decline" pathway and its reverse, the exposure, mediator, and outcome variables were assessed at a single time point; therefore, reverse causality or unmeasured time-dependent confounding factors, such as glycemic variability or the timing of inflammatory changes, cannot be excluded as potential sources of distortion in the path coefficients. Therefore, this statistically inference-based causal indication should be considered only as a hypothesis-generating tool and cannot replace longitudinal exposure-outcome assessments in prospective cohorts. Second, although the primary analytic sample (N = 216) was sufficient to identify key associations, some pathways in the bidirectional mediation analysis, like ASMI → myostatin → UACR, showed significant ACME with relatively small effect sizes, and the total effect was not statistically significant. This pattern suggests limited statistical power, which may limit accurate effect-size estimation and robust subgroup testing, such as analyses stratified by sex or renal function stage. Third, single-center enrollment and the inclusion and exclusion criteria, including participants' willingness to complete muscle strength and body composition assessments, may have introduced selection bias, reducing the generalizability of the findings to broader, multi-ethnic T2DM populations. Finally, although inflammatory markers such as IL-6 were included in the adjustment models, other molecules that may participate in the "muscle-kidney axis," including TNF-α, irisin, fibroblast growth factor 21, and Klotho protein, were not examined, leaving the possibility of residual confounding.[23–28]

5. Conclusion

This study demonstrates an independent positive association between serum myostatin levels and both DKD risk and elevated UACR in patients with T2DM. It preliminarily reveals that myostatin may function as a bidirectional mediator between renal injury and muscle deterioration, providing clinical-level statistical evidence supporting the "muscle-kidney axis" hypothesis. Incorporation of myostatin increased the AUC of the DKD risk model from 0.665 to 0.803, indicating incremental diagnostic value. Myostatin may serve as a novel biomarker for assessing the comorbid relationship between DKD risk and decline in muscle function, while also providing a target-based rationale for developing combined interventions targeting the "muscle-kidney axis," such as exercise rehabilitation or myostatin inhibitors. Future studies should use multicenter prospective cohorts to validate whether dynamic changes in myostatin can predict DKD progression. *In vitro* cellular models should be integrated with animal models to precisely identify the molecular mechanisms by which myostatin promotes renal fibrosis through TGF-β/Smad or NF-κB signaling pathways, ultimately advancing a closed-loop translational pathway from clinical observation to mechanistic validation.

Abbreviations

DKD
Diabetic kidney disease
T2DM
Type 2 diabetes mellitus
GDF-8
Growth differentiation factor-8
TGF-β

Transforming growth factor-beta
UPS
Ubiquitin-proteasome system
AMPK
AMP-activated protein kinase
UACR
Urine albumin-to-creatinine ratio
WHO
World Health Organization
NKF-KDOQI
National Kidney Foundation Kidney Disease Outcomes Quality Initiative
BMI
Body mass index
HOMA-IR
Homeostasis model assessment of insulin resistance
HOMA-β
Homeostasis model assessment of β-cell function
HbA1c
Glycated hemoglobin
TC
Total cholesterol
TG
Triglycerides
HDL-C
High-density lipoprotein cholesterol
LDL-C
Low-density lipoprotein cholesterol
SdLDL
Small dense LDL
ALT
Alanine aminotransferase
AST
Aspartate aminotransferase
γ-GT
γ-glutamyl transferase
eGFR
Estimated glomerular filtration rate
25(OH)D
25-hydroxyvitamin D
hs-CRP
High-sensitivity C-reactive protein
PCT
Procalcitonin
DXA
Dual-energy X-ray absorptiometry
ASMI
Appendicular skeletal muscle mass index
MQI
Muscle quality index
ELISA

Enzyme-linked immunosorbent assay
SD
Standard deviation
ANOVA
A one-way analysis of variance
IQR
Interquartile range
VIF
Variance inflation factor
ACME
Average causal mediation effect
ADE
Average direct effect
AUC
Area under the curve
CI
Confidence intervals
CKD
Chronic kidney disease
ROS
Reactive oxygen species
SMI
Skeletal muscle index

Declarations

Author information

Authors and Affiliations

Haotian Gu[a], Chenyu Han[a], Haiying Jin[a], Yarong Wang[a], Aowen Luo[a], Siyuan Shen[a], Yaxuan Zhang[a], Min Zhang[a]

[a] Department of Endocrinology, Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University

Corresponding author

Correspondence to Min Zhang.

Ethics approval and consent to participate

The studies involving humans were approved by Ethical Committee of Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

CRediT authorship contribution statement

H-TG: Conceptualization, Writing-review and editing, Investigation, Writing original draft. C-YH: Writing-review and editing, Data curation, Methodology. H-YJ: Writing-review and editing, Validation. Y-RW: Writing-review and editing, Formal analysis. A-WL: Writing-review and editing, Formal analysis. S-YS: Writing-review and editing, Resources. Y-XZ: Resources, Writing-review and editing. MZ: Writing-review and editing, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

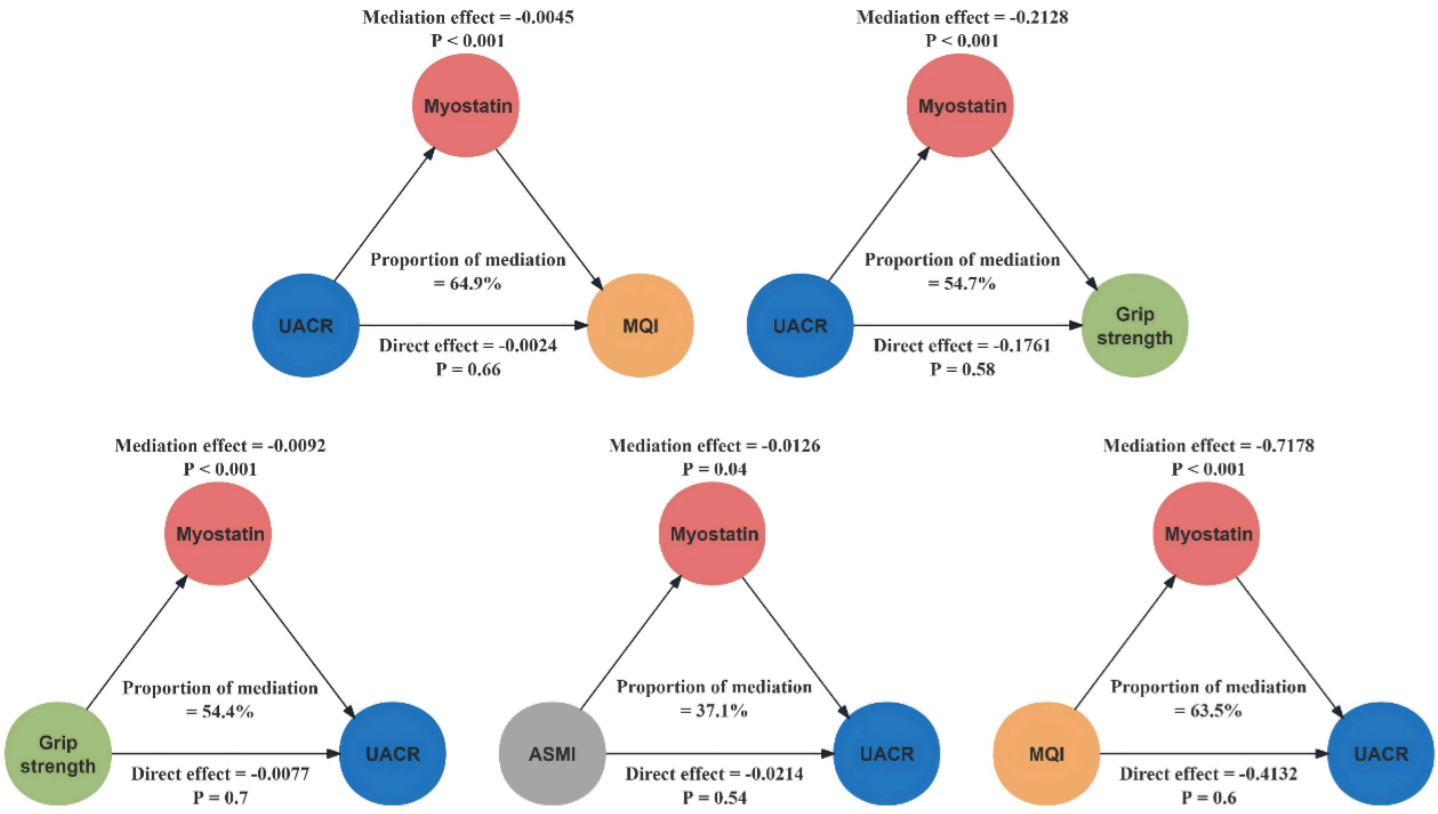


Figure 1

Schematic diagram of the bidirectional mediation analysis.

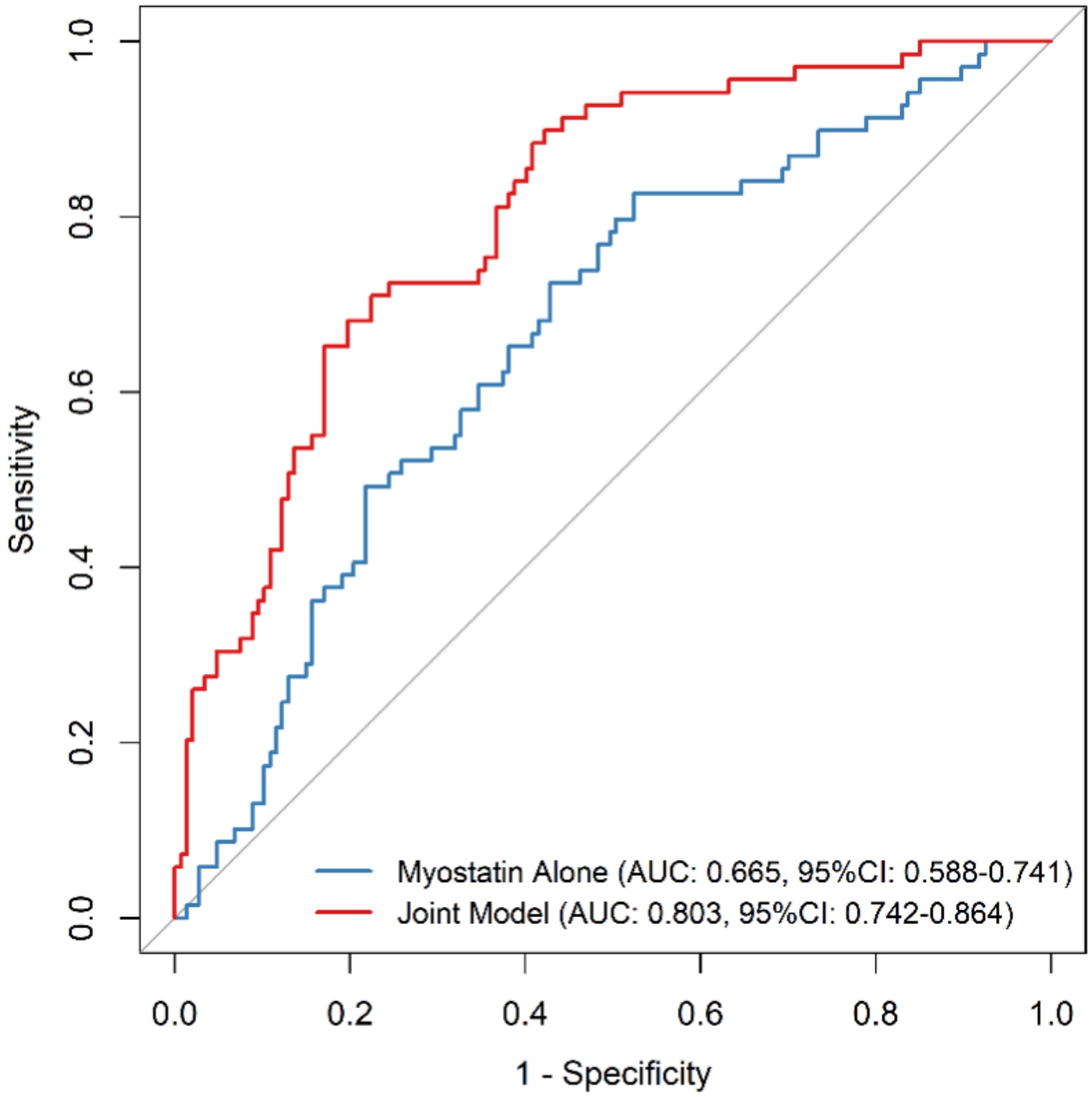


Figure 2

Receiver operating characteristic (ROC) curve of myostatin for identifying diabetic kidney disease (DKD).

Supplementary Files

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