Correlation between lower extremity arterial disease and skeletal muscle mass in patients with type 2 diabetes mellitus

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Abstract

Objectives: To evaluate skeletal muscle mass in patients with both type 2 diabetes mellitus (T2DM) and concomitant lower extremity arterial disease (LEAD) and determine the contribution of skeletal muscle mass to macrovascular diseases.

Methods: In total, 112 patients with T2DM were divided into the T2DM and T2DM+LEAD groups. Hepatic function, renal function, uric acid, blood glucose, and glycated hemoglobin (HbA1C) were measured. Dual-energy X-ray absorptiometry was used to measure visceral fat area and skeletal muscle mass index (SMI).

Results: Waist-to-hip ratio, uric acid, and body fat percentage were significantly higher in the T2DM+LEAD group than in the T2DM group; SMI was significantly lower in the T2DM+LEAD group than in the T2DM group. There were no significant differences in albumin, creatinine, fasting blood glucose, HbA1C, or blood lipids. Uric acid, SMI, and body fat percentage were significantly positively correlated with T2DM and concomitant LEAD. Logistic regression analyses suggested that SMI is an independent risk factor for LEAD in T2DM (odds ratio = 1.517; 95% confidence interval: 1.082–2.126).

Conclusions: Skeletal muscle mass is lower in patients with T2DM and concomitant LEAD than in patients with T2DM who do not exhibit LEAD. SMI is an important risk factor for LEAD.

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Introduction

Increasing age is associated with a loss of skeletal muscle mass and progressive deterioration in muscle strength, power, and endurance. These changes reduce the capacity and quality of skeletal muscle coordination, which can hinder elderly individuals from completing simple daily tasks. In patients with severe loss of skeletal muscle mass, gait and body balance can be impacted, and affected individuals are likely to experience falls.1

Skeletal muscle loss is also associated with arterial stiffness, which may result in an increased risk of macrovascular disease. Sarcopenia has been identified as an important factor in cardiovascular diseases.2 Lower extremity arterial disease (LEAD) is also an age-related disease that can lead to reduced blood flow to lower extremities. It can indirectly affect blood supply to the muscle, leading to mobility dysfunction in lower extremities and reduced skeletal muscle mass.3 LEAD is a common complication in patients with diabetes mellitus (DM) and is the major cause of disability and mortality in type 2 diabetes mellitus (T2DM).4 In this study, we aimed to evaluate skeletal muscle mass in patients with T2DM and concomitant LEAD and determine the contribution of skeletal muscle mass to macrovascular diseases.

Patients and methods

Patients

Between August 2017 and May 2018, patients with T2DM admitted to the Department of Endocrinology in The Second Hospital of Shandong University were selected for this study. All patients were using oral hypoglycemic medications or insulin. The inclusion criteria were as follows: i) patients met the diagnostic criteria for DM and LEAD in the “2010 China Guidelines for the Prevention and Treatment of Diabetes Mellitus,” formulated by the Chinese Diabetes Society; ii) patients had a) lower extremity weakness, rest pain, chills, and intermittent claudication, b) sensations of pain, coldness, or numbness in the tips of the toes or back of the feet, and c) weakened or no pulse in the dorsalis pedis, posterior tibial, or popliteal arteries; iii) patients manifested LEAD on Doppler ultrasonography, characterized by thickening, roughness, and thrombosis in the vascular endothelium, as well as atherosclerotic plaque formation, blood flow reduction, and narrowing of the inner vessel diameter; and iv) ankle-brachial index of <0.9. Based on the inclusion criteria, the patients were divided into the T2DM group and T2DM + LEAD group. Exclusion criteria were as follows: i) other types of DM; ii) acute diabetic complications and severe neuropathy; iii) severe diabetic foot ulcer; iv) hepatic function impairment, renal function impairment, or severe chronic obstructive pulmonary disease; v) severe arrhythmia or acute cardiac insufficiency; vi) autoimmune diseases; vii) history of cancer or recently diagnosed tumors; viii) pregnancy; and ix) history of severe mental illness. This study was approved by the Ethics Committee of The Second Hospital of Shandong University,
and written informed consent to participate was obtained from all patients.

**Assessment of patient characteristics**

Height, weight, waist circumference, hip circumference, blood pressure, and other parameters were measured by resident physicians. Body mass index (BMI) was calculated using the following formula: $\text{BMI} = \frac{\text{weight}}{\text{height} \ (\text{kg/m}^2)}$. Venous blood was drawn after 8 to 10 hours of fasting and the serum was extracted as follows: whole blood without anticoagulant was incubated at room temperature for 30 minutes, then centrifuged at 1500 × g for 5 minutes at 4°C; the supernatant was stored at −80°C. Biochemical parameters (e.g., total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol [LDL-C], fasting blood glucose, postprandial blood glucose, triglycerides, uric acid, and creatinine) were measured using an automatic biochemical analyzer (Beckman Coulter, Brea, CA, USA). Fasting blood glucose was measured using the hexokinase method; glycated hemoglobin was measured by high performance liquid chromatography. The Hologic Discovery Wi (S/N 88803) dual-energy X-ray absorptiometry system was used to measure various parameters, including the visceral fat area and skeletal muscle mass index (SMI; muscle content of limbs after height correction [kg/m²]). Vascular Doppler (Huntleigh Healthcare, Cardiff, UK) was used to measure the blood pressure of the bilateral forearms in patients in the supine position; the highest brachial artery pressure recorded was used in this study. The ankle artery pressure was recorded as the highest systolic blood pressure value of the bilateral dorsalis pedis and posterior tibial arteries. The ankle-brachial index was regarded as ankle artery pressure divided by brachial artery pressure. Color Doppler ultrasound (GE-LOGIQ-E9, GE Healthcare, Wauwatosa, WI, USA) was used to perform vascular ultrasound of lower extremities. The inner vessel diameter, peak blood flow velocity, intima-media thickness, and blood flow spectrum were measured for the bilateral femoral, popliteal, tibial, and dorsalis pedis arteries.

**Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). Quantitative data are expressed as mean ± standard deviation. The Kolmogorov–Smirnov test was used to assess the normality of all quantitative data. The $t$-test was used for pairwise comparisons. Quantitative data that did not follow a normal distribution are expressed as median (range), and were analyzed using the rank-sum test. Differences with $P < 0.05$ were considered statistically significant.

**Results**

**Comparison of baseline characteristics between groups**

In total, 112 patients with T2DM were included in the study: 52 in the T2DM-alone group and 60 in the T2DM+LEAD group. There were no significant differences between the two groups in age, sex, systolic blood pressure, diastolic blood pressure, duration of DM, or BMI (Table 1). There were significant differences between the groups regarding uric acid level ($t = 2.168$, $P = 0.034$), SMI ($t = 2.330$, $P = 0.026$), waist-to-hip ratio (WHR) ($t = −2.604$, $P = 0.009$), and body fat percentage ($t = −2.381$, $P = 0.017$). There were no statistically significant differences between the groups in albumin, creatinine, fasting blood glucose, glycated hemoglobin, triglycerides, total cholesterol, high-density lipoprotein cholesterol, or LDL-C.
Spearman analysis of factors correlated with T2DM and LEAD

Spearman correlation analysis showed that uric acid ($r = 0.244$, $P = 0.056$), SMI ($r = 0.367$, $P = 0.002$), and body fat percentage ($r = 0.285$, $P = 0.016$) were significantly positively correlated with T2DM and concomitant LEAD (Table 2), while abdominal obesity was not.

Logistic regression analysis of risk factors for T2DM and LEAD

Logistic regression analysis was performed with group as the dependent variable and parameters significantly associated with LEAD as independent variables. The analysis showed that SMI was an independent risk factor for LEAD in patients with T2DM (odds ratio $= 1.517$; 95% confidence interval: $1.082–2.126$; $P = 0.016$) (Table 3).

Discussion

LEAD is the primary cause of lower extremity amputations in patients with
DM, and its morbidity rate is 20-fold higher in patients with DM than in non-diabetic individuals. LEAD is present in 8.0% of patients at the time of DM diagnosis and is an important factor that leads to disability and mortality in patients with DM. The fundamental pathological change in LEAD that occurs in patients with DM is atherosclerosis of peripheral blood vessels; this process may be related to genetic factors, chronic inflammation, and lipid metabolism. In the present study, patients with T2DM and concomitant LEAD had significantly elevated LDL-C and serum uric acid levels, as well as body fat percentage, compared with those parameters in patients with T2DM alone. Uric acid has received increasing attention because of its role as a risk factor for macrovascular diseases. A prospective cohort study revealed that an elevated uric acid level was independently associated with the onset of arteriosclerosis. Elevated uric acid may lead to increased platelet adhesion and oxygen free radical generation. Long-term uric acid elevation can damage vascular endothelial cells, ultimately leading to the formation of atherosclerotic plaques. Lipid metabolism disorders are also an important pathogenic factor for LEAD. In a state of hyperglycemia, glycosylation of collagen also lead to increased LDL-C deposition in the collagen matrix within the vasculature. In an Italian study, the LDL-C level was significantly higher in patients with T2DM and concomitant LEAD than in patients with T2DM alone, suggesting that LDL-C plays a key role in the pathogenesis of LEAD. Therefore, appropriate control of serum uric acid and LDL-C levels in patients with DM will be of great significance in delaying the development of LEAD in these patients.

WHR and BMI are common clinical parameters that reflect the degree of obesity. From the perspective of fat distribution, BMI reflects the overall fat percentage in the whole body, while WHR reflects the local fat percentage in the abdomen. WHR and BMI are reportedly correlated with vascular complications in patients with T2DM. There are significant differences in waist circumferences and hip circumferences among populations of different ages, ethnicities, and sexes. Nonetheless, WHR is relatively stable and is an effective parameter for determining the degree of obesity. WHR is closely related to the level of visceral fat. A high level of visceral fat can cause adipocytes to release a variety of cytokines, such as interleukin-1 and tumor necrosis factor-α; therefore, it is an important factor in the development

|                 | B   | Odds ratio | P   | Lower limit | Upper limit |
|-----------------|-----|------------|-----|-------------|-------------|
| Weight-adjusted SMI | 0.417 | 1.517     | 0.016 | 1.082       | 2.126       |
| Uric acid       | 0.007 | 1.007     | 0.049 | 1.000       | 1.014       |
| Body fat percentage | 0.088 | 1.092     | 0.259 | 0.937       | 1.271       |
| Abdominal obesity | 0.426 | 1.531     | 0.592 | 0.323       | 7.263       |
| Constant        | −15.226 | 0.000    | 0.023 |             |             |

*P < 0.05 denotes statistical significance.

Abbreviations: SMI, skeletal muscle mass index; T2DM, type 2 diabetes mellitus; LEAD, lower extremity arterial disease.
of atherosclerosis.\textsuperscript{15} WHR is presumed to be a strong predictor for cardiovascular and cerebrovascular diseases in patients with DM.\textsuperscript{16} Notably, DM is regarded as a state of chronic inflammation. The amount of visceral fat in patients with abdominal obesity is significantly elevated, which significantly increases insulin resistance and the levels of chronic inflammatory factors, thereby causing increased morbidity of vascular diseases.\textsuperscript{17} In the present study, there was a more substantial increase in WHR than in BMI in the T2DM + LEAD group, demonstrating that abdominal obesity, represented by WHR, plays a greater role in DM-associated LEAD.

Furthermore, SMI was closely correlated with LEAD. Skeletal muscle mass constitutes approximately 40% of human body weight and is regarded as the largest secretory organ in the human body. Skeletal muscle can express, synthesize, and secrete various biological signaling molecules,\textsuperscript{18} including interleukin-1, interleukin-6, irisin, FGF-21, leptin, and adiponectin; these molecules can regulate skeletal muscle function and its associated microenvironment in a paracrine and/or autocrine manner. Moreover, these molecules can regulate the function of distant body organs by entering the blood circulation. Reduced skeletal muscle mass is a relatively newly identified type of complication in patients with DM and is associated with increased rates of hospitalization, cardiovascular events, and mortality.\textsuperscript{19} To the best of our knowledge, there have relatively few studies regarding skeletal muscle mass in patients with DM and concomitant LEAD. The incidence of sarcopenia in men with LEAD is reportedly significantly higher than that of normal men of the same age,\textsuperscript{20} which may be explained by multiple factors associated with LEAD, including lower limb ischemia, reduced skeletal muscle capillary density, and poor mobility.

There are bidirectional relationships between sarcopenia and diabetes-related macrovascular diseases. First, insulin resistance, advanced glycation end-product accumulation, and increased inflammation and oxidative stress are fundamental pathogenic characteristics in patients with T2DM. These factors can negatively affect muscle health, including muscle mass, strength, quality, and function. Diabetic lower extremity vascular disease is an important complication of diabetes. Thickening of the vascular basement membrane, nonenzymatic glycosylation, and reduction of nascent blood vessels all further aggravate the development of skeletal muscle diseases. In a state of long-term hyperglycemia, nonenzymatic protein glycosylation causes thickening of skeletal muscle capillary basement membrane, increases exchange distance between blood and tissues, hinders oxygen diffusion and metabolite exchange, and aggravates ischemia and hypoxia in skeletal muscles; these changes ultimately lead to nutritional and metabolic disorders of muscle tissue.\textsuperscript{21,22}

Second, many pathophysiological changes occur in skeletal muscle during long-term hyperglycemia, including abnormal autophagy, enhanced apoptosis, reduced secretion of growth factors and adiponectin, and mitochondrial dysfunction. Furthermore, the exocrine function of skeletal muscle is severely impacted.\textsuperscript{23} Changes in the skeletal muscle microenvironment may lead to peripheral vascular endothelial dysfunction, thereby promoting atherosclerosis.\textsuperscript{24} However, there have been few clinical or basic science studies regarding sarcopenia and diabetes-related macrovascular diseases; thus, further investigations are needed. The present study demonstrated that SMI was significantly lower in patients with T2DM and concomitant LEAD than in patients with T2DM alone, consistent with the findings of previous studies. Logistic regression analysis
showed that SMI was an important risk factor for LEAD in our patients. These changes further demonstrate the significance of skeletal muscle function in LEAD. The present study was limited in that it focused on clinical parameters and did not assess inflammatory factors, oxidative stress, or other parameters. The underlying pathogenic mechanism should be further investigated in subsequent studies.

In conclusion, in patients with T2DM and concomitant LEAD, uric acid and WHR were significantly elevated, while SMI was significantly reduced. Moreover, SMI was independently associated with LEAD in logistic regression analyses. Future studies should focus on clinical analyses of skeletal muscle mass and peripheral vascular disease, and should include investigations of the underlying mechanisms by which skeletal muscle mass affects peripheral vascular disease.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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