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Relationship between serum bilirubin concentration and sarcopenia in patients with type 2 diabetes: a cross-sectional study

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Abstract

Objective: The prevalence of sarcopenia is high in patients with type 2 diabetes mellitus (T2DM). Oxidative stress and inflammation play important roles in the pathogenesis of sarcopenia in diabetes. Bilirubin has been shown to possess anti-oxidative activity. We aimed to explore the relationship between bilirubin and sarcopenia in patients with T2DM.

Methods: A total of 251 patients (124 men and 127 postmenopausal women) with T2DM, aged $\geq$50 years, participated in a cross-sectional study. The serum concentrations of bilirubin (TBIL), direct bilirubin (DBIL) and indirect bilirubin (IBIL) were measured. Muscle mass was measured using dual-energy X-ray absorptiometry.

Results: TBIL and IBIL were positively associated with appendicular skeletal muscle mass index (SMI) in men, but not in women. After adjustment for multiple factors in multiple linear regression analysis, TBIL and IBIL were also significantly associated with SMI in men. In multiple logistic regression analysis, participants in the highest quartile of IBIL demonstrated a lower odds ratio for sarcopenia in men.

Conclusions: Both TBIL and IBIL are positively associated with muscle mass in men with T2DM. Furthermore, IBIL may protect against sarcopenia in men with T2DM.

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Introduction

Sarcopenia is characterized by low muscle mass, poor muscle strength and poor physical performance.[1] The prevalence of sarcopenia ranges from 10% to 40% in healthy adults aged ≥60 years,[2] and patients with sarcopenia are at higher risk of frailty, falls, fracture and even mortality.[3,4] Therefore, screening for risk factors and the development of effective treatments for sarcopenia are extremely important. In 2017, it was estimated that 451 million people worldwide had diabetes and approximately 90% of these people had type 2 diabetes mellitus (T2DM).[5] T2DM increases the risk of sarcopenia,[6–9] but the mechanism whereby this sarcopenia develops remains unclear.

Oxidative stress and inflammation play important roles in the pathogenesis of diabetic sarcopenia.[10] Bilirubin, the end product of haem metabolism, has been recently confirmed to have protective effects in metabolic syndrome and diabetes by antagonizing oxidative stress and chronic inflammation.[11–14] To date, few studies have explored the relationship between bilirubin concentration and sarcopenia and no definitive conclusions have been reached.[15,16] Kawamoto *et al.*[15] found that high serum total bilirubin (TBIL) concentration is associated with higher handgrip strength in community-dwelling Japanese adults. In addition, Kim *et al.*[16] found no association between TBIL and sarcopenia in patients with chronic liver diseases after adjustment for multiple potential confounding factors. However, the relationship between bilirubin concentration and sarcopenia in patients with T2DM has not been characterized. Therefore, we conducted a cross-sectional study to determine whether bilirubin concentration is associated with sarcopenia in middle-aged and elderly patients with T2DM.

Materials and methods

Study participants

Sarcopenia is more common in middle-aged and elderly patients, and most of the inpatients with T2DM in our hospital were >50 years old. Therefore, we performed a cross-sectional study of consecutively recruited patients with T2DM who were aged ≥50 years at Qilu Hospital, Shandong University, between January 2017 and December 2019. The exclusion criteria were severe liver disease (liver cirrhosis or serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) activities >120 U/L), severe kidney disease (estimated glomerular filtration rate [eGFR] <60 mL/minute/1.73 m^2), malignant disease, haematological disease and serum bilirubin concentration greater than two times the upper limit of the normal range. Diabetes was diagnosed according to the 2006 World Health Organization criteria:[17] fasting blood glucose (FBG) ≥7.0 mmol/L and/or a 2-hour postprandial blood glucose concentration ≥11.1 mmol/L. The study was performed in strict accordance with the provisions of the Declaration of

Helsinki and its amendments, and was approved by the ethics committee of Qilu Hospital of Shandong University (approval no. KYLL-2019-270). All the participants provided their written informed consent.

Data collection

The basic clinical and demographic data for all the participants, including their age, sex, height, body mass, blood pressure (BP) and smoking history, were collected from the computerized patient medical record system of Qilu Hospital of Shandong University. Fasting venous blood samples were collected and immediately used for the measurement of FBG, HbA1c, total cholesterol (TC), triglyceride (TG), ALT, AST, creatinine, TBIL, direct bilirubin (DBIL) and indirect bilirubin (IBIL). Homeostasis model of assessment 2- insulin resistance (HOMA2-IR) was calculated using the FBG and fasting C-peptide concentrations and the formula at http: //www.ocdem.ox.ac.uk/.[18] Appendicular skeletal muscle mass (ASM) and body fat percentage were measured using dual-energy X-ray absorptiometry (Discovery Wi, Hologic, Boston, MA, USA). The appendicular skeletal muscle mass index (SMI) was calculated as: SMI $(kg/m^2) = ASM\ (kg)/height^2\ (m^2)$. Sarcopenia was defined as a SMI $<7.0\,kg/m^2$ in men and $<5.4\,kg/m^2$ in women, according to the Asian Working Group for Sarcopenia recommendations.[1] eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[19]

Statistical analysis

The Kolmogorov–Smirnov test was used to determine whether datasets were normally distributed. Continuous data that were normally distributed or non-normally distributed are expressed as the mean ± standard deviation (SD) and the median (interquartile range), respectively. Student's *t*-test (normally distributed data) or Mann–Whitney U-test (non-normally distributed data) were used to compare differences between the two groups. The relationships between serum bilirubin concentration and SMI were assessed using Pearson's correlation analysis and multiple linear regression analysis in both men and women. Multiple logistic regression analysis was also used to characterize the relationship between the highest quartile of serum bilirubin and sarcopenia. All the statistical analyses were performed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA) and $P < 0.05$ was considered to represent statistical significance.

Results

General characteristics of the participants

A total of 251 patients (124 men and 127 postmenopausal women) were enrolled. As shown in Table 1, the men with sarcopenia were older and had significantly lower BMI, DBP, fasting C-peptide, HOMA2-IR and TG than those without sarcopenia. Women with sarcopenia were also older and had lower BMI and TC than those without sarcopenia. However, the serum bilirubin (TBIL, DBIL and IBIL) concentrations did not significantly differ between sarcopenic and non-sarcopenic men or women.

Relationships between serum bilirubin and SMI in men and women

To explore the relationship between serum bilirubin concentration and SMI, Pearson's correlation analyses were performed. As shown in Figure 1, TBIL ($r = 0.211$, $p = 0.019$) and IBIL ($r = 0.249$, $p = 0.005$) positively correlated with SMI in men. However, there were no correlations between serum bilirubin concentrations and SMI in women. We next conducted

Table 1. General characteristics of the study participants, categorized according to sex and the presence of sarcopenia.

	Men			Women		
Characteristic	Sarcopenia	Non-sarcopenia	P value	Sarcopenia	Non-sarcopenia	P value
N	51	73	–	38	89	–
Age (years)	62.61±6.93	59.68±5.97	**0.013**	65.32±6.22	61.40±6.47	**0.002**
BMI (kg/m^2)	24.13±2.94	28.07±3.23	**<0.001**	22.29±2.83	25.96±3.65	**<0.001**
SBP (mmHg)	131.86±20.01	137.22±19.68	0.141	136.87±23.30	140.16±17.10	0.377
DBP (mmHg)	76.10±10.94	81.11±11.02	**0.014**	75.03±12.41	77.91±10.88	0.192
FBG (mmol/L)	7.69±2.91	8.20±2.62	0.317	8.53±2.84	8.11±2.89	0.463
HbA1c (%)	8.17±1.60	8.36±1.92	0.562	8.87±2.17	8.69±1.86	0.632
Fasting C-peptide (ng/mL)	0.93 (0.71–1.72)	1.55 (1.00–2.09)	**0.010**	1.23 (0.64–1.63)	1.13 (0.71–1.71)	0.836
HOMA2-IR (units)	0.80 (0.56–1.41)	1.29 (0.79–1.92)	**0.013**	1.02 (0.55–1.50)	0.99 (0.57–1.46)	0.945
TG (mmol/L)	1.32 (0.98–1.86)	1.58 (1.19–2.28)	**0.026**	1.46 (0.97–2.12)	1.40 (1.06–1.95)	0.941
TC (mmol/L)	4.13±0.95	4.49±1.01	0.050	5.09±1.06	4.68±1.01	**0.044**
Creatinine (μmol/L)	72.64±17.54	74.51±19.63	0.590	55.37±15.66	56.03±12.88	0.803
eGFR (mL/minute/1.73 m^2)	93.84±15.02	94.28±15.55	0.876	92.53±14.24	94.31±12.46	0.482
SMI (kg/m^2)	6.33±0.48	7.72±0.58	**<0.001**	4.88±0.30	6.14±0.65	**<0.001**
Body fat percentage (%)	27.97±4.61	29.14±4.42	0.158	35.32±5.11	36.04±4.60	0.433
Smoking (n, %)	20 (39.2%)	40 (54.8%)	0.102	0 (0.0%)	0 (0.0%)	1.000
AST (U/L)	19.06±8.97	20.48±8.39	0.369	20.84±13.53	19.73±7.17	0.634
ALT (U/L)	19.12±13.20	22.51±13.09	0.160	16.74±12.33	19.72±12.13	0.209
TBIL (μmol/L)	11.19±4.94	12.32±4.69	0.199	10.25±4.64	9.73±4.13	0.530
DBIL (μmol/L)	3.86±1.60	3.94±1.45	0.763	3.56±2.05	3.23±1.15	0.265
IBIL (μmol/L)	7.33±3.48	8.37±3.42	0.099	6.70±3.14	6.49±3.07	0.729

Continuous data that were normally distributed or non-normally distributed are expressed as the mean ± standard deviation (SD) or the median (interquartile range), respectively. Student's *t*-test (normally distributed data) or Mann–Whitney U-test (non-normally distributed data) were used to compare differences between the two groups. Significant *P* values (< 0.05) are indicated in bold.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HOMA2-IR, homeostasis model assessment of insulin resistance; TG, triglyceride; TC, total cholesterol; eGFR, estimated glomerular filtration rate; SMI, appendicular skeletal muscle mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin.

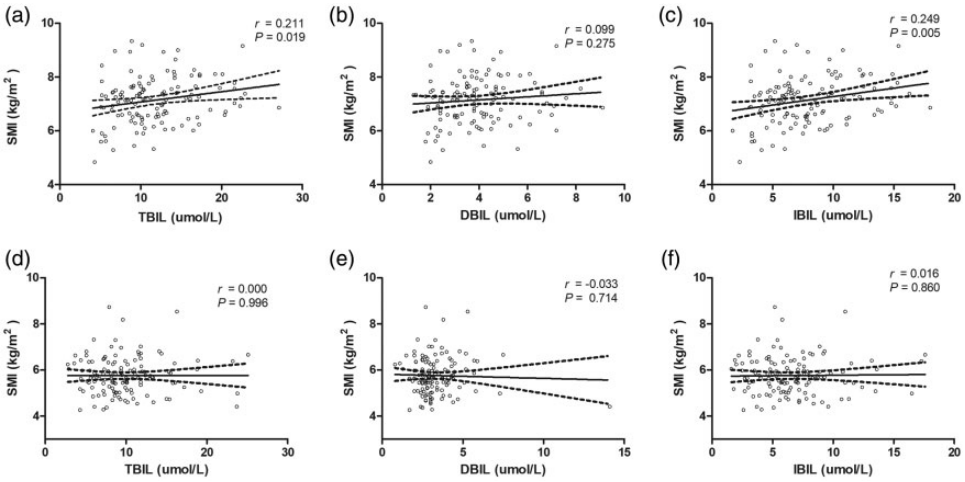

Figure 1. Pearson's correlations between serum bilirubin concentrations and appendicular skeletal muscle mass index (SMI) in men (a, b, c) and women (d, e, f).

Table 2. Results of multiple linear regression analysis of the relationships between serum bilirubin concentration and SMI in men and women.

	Model 1		Model 2	
Independent variable	β Coefficient (95% CI)	*P*	β Coefficient (95% CI)	*P*
Men				
TBIL	0.038 (0.006–0.070)	**0.019**	0.037 (0.005–0.070)	**0.023**
DBIL	0.057 (−0.046–0.160)	0.275	0.079 (−0.029–0.187)	0.152
IBIL	0.063 (0.019–0.107)	**0.005**	0.057 (0.013–0.101)	**0.012**
Women				
TBIL	7.846E-5 (−0.034–0.034)	0.996	0.006 (−0.027–0.039)	0.729
DBIL	−0.018 (−0.115–0.079)	0.714	2.936E-5 (−0.095–0.096)	1.000
IBIL	0.004 (−0.043–0.051)	0.860	0.011 (−0.035–0.058)	0.631

Model 1: unadjusted. Model 2: adjusted for age, SBP, HbA1c, HOMA2-IR, TG, TC, eGFR, smoking, and body fat percentage.

SMI, appendicular skeletal muscle mass index; CI, confidence interval; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; SBP, systolic blood pressure; HbA1c, glycated haemoglobin; HOMA2-IR, homeostasis model assessment 2-insulin resistance; TG, triglycerides; TC, total cholesterol; eGFR, estimated glomerular filtration rate.

multiple linear regression analysis to further explore the associations between serum bilirubin concentration and SMI. As shown in Table 2, close relationships of TBIL and IBIL with SMI were only present in men. Furthermore, after adjustment for age, SBP, HbA1c, HOMA2-IR, TG, TC, eGFR, smoking and body fat percentage, TBIL ($p = 0.023$) and IBIL ($p = 0.012$) remained significantly associated with SMI in men.

Multiple logistic regression analysis of the relationships between serum bilirubin and sarcopenia in men and women

Finally, we conducted multiple logistic regression analyses to explore the

Table 3. Results of multiple logistic regression analysis of the relationships between serum bilirubin concentration and sarcopenia in men and women.

	Model 1		Model 2	
Independent variable	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Men				
Highest quartile of TBIL	0.646 (0.273–1.530)	0.321	0.424 (0.153–1.175)	0.099
Highest quartile of IBIL	0.380 (0.155–0.933)	**0.035**	0.243 (0.084–0.703)	**0.009**
Women				
Highest quartile of TBIL	0.841 (0.348–2.031)	0.700	0.825 (0.297–2.290)	0.711
Highest quartile of IBIL	0.891 (0.367–2.159)	0.798	0.829 (0.305–2.254)	0.714

Model 1: unadjusted. Model 2: adjusted for age, SBP, HbA1c, HOMA2-IR, TG, TC, eGFR, smoking, and body fat percentage.

CI, confidence interval; TBIL, total bilirubin; IBIL, indirect bilirubin; SBP, systolic blood pressure; HbA1c, glycated haemoglobin; HOMA2-IR, homeostasis model assessment 2-insulin resistance; TG, triglycerides; TC, total cholesterol; eGFR, estimated glomerular filtration rate.

relationships of TBIL and IBIL with sarcopenia, because only TBIL and IBIL were significantly associated with SMI. The male and female participants were allocated to groups according to quartiles of TBIL and IBIL. The highest quartiles of TBIL and IBIL were included in the analysis as independent variables. As shown in Table 3, before adjustment, the highest quartile of IBIL was associated with a low odds ratio (OR) for sarcopenia ($OR = 0.380$, $p = 0.035$) in men. After adjustment for age, SBP, HbA1c, HOMA2-IR, TG, TC, eGFR, smoking and body fat percentage, this low OR remained ($OR = 0.243$, $p = 0.009$). However, TBIL was not significantly associated with sarcopenia after adjustment in men, and neither TBIL nor IBIL was associated with sarcopenia in women.

Discussion

The prevalences of metabolic and musculoskeletal diseases, including T2DM and sarcopenia, increase with age.[5,20] The bidirectional relationship between T2DM and sarcopenia has been discussed in a previous review.[10] T2DM is characterized by insulin resistance, an inflammatory phenotype, oxidative stress and higher concentrations of advanced glycation end-products (AGEs), which may lead to cell death and further reductions in skeletal muscle mass, strength and function, ultimately resulting in sarcopenia.[10] Bilirubin is one of the most active endogenous antioxidant molecules and has been shown to have beneficial effects in the prevention and treatment of T2DM.[11–14,21] However, the role of bilirubin in the pathophysiology of sarcopenia in patients with T2DM has not been explored. To clarify the relationship between serum bilirubin concentration and sarcopenia, we conducted the present cross-sectional study in participants of both sexes.

Unexpectedly, we found that the serum TBIL, DBIL and IBIL concentrations did not significantly differ between participants who did or did not have sarcopenia. The serum bilirubin concentration is lower in smokers and is negatively associated with BMI.[22–24] As shown in Table 1, male participants with sarcopenia had lower BMIs and were less likely to smoke than those without sarcopenia. In theory, the serum bilirubin concentrations of the sarcopenia group would be expected to be higher than those of the non-sarcopenia group. However, the opposite findings were

made, which suggested that the effects of smoke and BMI on serum bilirubin could be modified by the inverse relationship between serum bilirubin and sarcopenia. As shown in Figure 1, we found that TBIL and IBIL positively correlated with SMI in men, but not in women, and the multiple linear regression analysis yielded consistent results. However, in this analysis, only the highest quartile of IBIL was associated with a low OR for sarcopenia after adjustment for multiple factors in men. These data indicate that IBIL, but not DBIL, may play a role in the protection against sarcopenia in men with T2DM.

TBIL is the sum of DBIL and IBIL, and most serum bilirubin is IBIL.[25] Therefore, it is logical that IBIL would play the major role in the protection against sarcopenia, although both IBIL and DBIL have antioxidant properties.[26] Other differences in the clinical significance of DBIL and IBIL have also been identified with respect to other diseases. Patients with Gilbert syndrome who have slightly high IBIL concentrations have lower risks of coronary vascular diseases, whereas this association is not present in patients with Dubin–Johnson syndrome and high DBIL.[27]

A close relationship between serum IBIL and sarcopenia was only identified in men. Several previous studies have shown that men have higher serum bilirubin concentrations than women,[2]and serum bilirubin may also be affected by oestrogen.[29] In addition, sex-specific patterns of aging involve differing changes in muscle mass and quality, as well as in sex hormones.[30] Sex hormones have been shown to be involved in the maintenance of skeletal muscle homoeostasis: both testosterone and oestrogen promote muscle protein synthesis, whereas low levels of testosterone and oestrogen are associated with sarcopenia.[31,32] Moreover, in women, age-associated changes in muscle are mostly reflected in changes in muscle quality, whereas in men they are mostly reflected in a decline in muscle mass.[32] All these differences might influence the sex-specific relationship between bilirubin and sarcopenia.

The present study had some limitations. First, because of the cross-sectional nature of the study, conclusions regarding causal relationships between serum bilirubin concentration and sarcopenia cannot be drawn. Second, the participants in the study were Chinese and aged $\geq$50 years, and the sample size was relatively small. Therefore, the present findings require confirmation in prospective studies with larger sample sizes and in different age groups. Third, sarcopenia was defined using SMI alone; muscle strength and physical performance were not assessed. Fourth, we did not collect data regarding, or adjust the analyses for the use of medication, despite some anti-diabetic drugs possibly affecting bilirubin concentration and skeletal muscle mass. For example, sodium-glucose co-transporter 2 inhibitors have been shown to increase bilirubin concentration and reduce skeletal muscle mass, and insulin may increase skeletal muscle mass.[33,34] Finally, some other factors that might influence sarcopenia, such as sex hormones, pro-inflammatory mediators, exercise and smoking history were not fully adjusted for, which might have affected the accuracy of the results.

Conclusions

We have shown that TBIL and IBIL are positively associated with SMI in men with T2DM and that IBIL might be more effective at protecting against the development and progression of sarcopenia. More detailed studies are necessary to further define the interactions between bilirubin and sarcopenia.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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Author contributions

JBL and CW designed the study and drafted the manuscript. JDL and CJ collected the data. CW and XFY conducted the data analysis. All the authors approved the final version of the manuscript.

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