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ORIGINAL RESEARCH

Elevated Serum Tsukushi Levels and Their Association with Glycolipid Metabolism in Gestational Diabetes Mellitus Patients

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Objective: Tsukushi (TSK) is a recently identified hepatic factor that has gained prominence for its distinctive function in glycolipid metabolism and energy homeostasis. However, the role of TSK in gestational diabetes mellitus (GDM) remains largely unexplored. In this study, we conducted a pioneering investigation by determining serum TSK levels in patients with GDM and examining the correlation between these levels and various metabolic parameters in gestational diabetes patients.

Methods: A total of 176 pregnant women (mean age 29.5 ± 3.2 years) were recruited from September 2023 to December 2024. Serum TSK levels and clinical markers related to glycolipid metabolism were measured at 24–28 weeks of gestation. All subjects underwent an oral glucose tolerance test (OGTT).

Results: Serum TSK was significantly higher in patients with GDM than in the normal glucose tolerance group [0.60(0.47,0.73 vs 0.52(0.42,0.67) ng/mL; P <0.05]. Correlation analysis showed that TSK was significantly and positively correlated with diastolic blood pressure (DBP), alanine aminotransferase (ALT), direct bilirubin (Dbil), free triiodothyronine (FT3), 1-h post-OGTT glucose (1hPG), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC). Logistic regression analysis showed that Logistic regression analysis showed that higher TSK levels were independently associated with increased odds of GDM.

Conclusion: TSK levels were higher in GDM than in NGT (P=0.013), and higher TSK tertiles were independently associated with increased odds of GDM (Model 3 OR=2.883, 95% CI 1.173–7.084; P=0.021). After FDR correction, only the association with total cholesterol remained significant (FDR-adjusted P=0.023).

Keywords: Tsukushi, gestational diabetes mellitus, pregnancy, insulin resistance

Introduction

Gestational Diabetes Mellitus (GDM) is one of the common chronic metabolic diseases during pregnancy. In recent years, the incidence of GDM has shown an increasing trend year by year, with the delay in the age of childbearing and the rise in obesity rates worldwide.[1] The 8th Global Diabetes Map released by the International Diabetes Federation reveals that 16.2% of pregnant women experience varying degrees of elevated blood glucose levels. GDM not only increases the odds of adverse pregnancy outcomes such as gestational hypertension, preeclampsia, placental abruption, preterm birth, macrosomia, hyperbilirubinemia, neonatal hypoglycemia, in addition to adverse perinatal outcomes, women who have experienced gestational diabetes mellitus (GDM) face a significantly increased long-term risk of type 2 diabetes, with a cumulative incidence typically reaching approximately 10–20% within ten years of delivery. GDM also imposes a significant healthcare and economic burden on the population.[2] Furthermore, the substantial economic burden of GDM and its long-term complications underscores the urgent need for developing early biomarkers and interventions. Consequently, GDM has become a core public health concern, with prevention and treatment now requiring immediate action.[2] In recent years, the liver is no longer simply regarded as a digestive organ but as an endocrine organ that secretes a variety of cytokines, also known as hepatic factors. Several studies have found that the

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liver can regulate the systemic metabolic state by secreting hepatic factors through influence feeding, energy metabolism, etc.,[3,4] and is involved in the onset and development of GDM.[5,6] TSK, a newly discovered hepatic factor, belongs to the non-classical subgroup of the leucine-rich proteoglycan family of small proteoglycans,[7,8] and has recently been found to play a role in the regulation of glycolipid metabolism and energy homeostasis, glycolipid metabolism and energy homeostasis.[7,9] Experimental studies have shown that TSK regulates high-density lipoprotein (HDL) metabolism and hepatic conversion of cholesterol to bile acids. Its elevation as a circulating marker of hepatic stress links NAFLD to atherogenic dyslipidemia. Clinically, elevated serum TSK levels are associated with metabolic syndrome, type 2 diabetes, proteinuria, and the severity of liver fibrosis, suggesting its association with metabolic abnormalities. However, limited evidence exists during pregnancy, and the inflammatory and dyslipidemic milieu of gestational diabetes mellitus (GDM) provides a biologically plausible context in which TSK may play a role. Therefore, we propose a scientific hypothesis: GDM patients exhibit elevated TSK levels, which are associated with dyslipidemia and glucose metabolism disorders. This study aims to investigate the characteristic changes in serum TSK levels in GDM patients and their impact on lipid metabolism, providing new scientific evidence for risk assessment and personalized treatment strategies. Additionally, this research offers novel therapeutic approaches for GDM prevention and management, enabling early and effective interventions to alleviate the substantial burden of GDM on society and families.

Methods

Population

A total of 176 pregnant women were enrolled between 24–28 weeks of gestation for regular prenatal check-ups at the Department of Obstetrics and Gynecology, Affiliated Hospital of Jiangsu University, from September 2023 to December 2024. Among them, 83 pregnant women diagnosed with GDM served as the study group (GDM group), and 93 healthy pregnant women of the same period served as the control group (normal control group). The diagnostic criteria for GDM were based on the American Diabetes Association's 2025 diagnostic criteria.[10] Participants with pre-existing conditions, including diabetes, hypertension, cardiovascular disease, metabolic syndrome, and those taking medications that affect glucose metabolism, were excluded from the study. This study complied with the Declaration of Helsinki and was approved by the Biomedical Research Ethics Committee of the Affiliated Hospital of Jiangsu University. All participants provided written informed consent.

Anthropometric and Biochemical Measurements

Data collection of all subjects was performed by trained medical professionals, including age, past medical history, and other factors. Height (without shoes in a standing position), body weight (in light clothing), waist circumferences (WC, at the midpoint between the inferior costal margin and the iliac crest) and blood pressure were measured using standard protocols, and body mass index (BMI) was calculated as weight in kilograms(kg)/height in meters squared(m^2), the waist-to-height ratio (WHtR) was expressed as the ratio of waist and height. Blood samples were withdrawn from a forearm vein after an 8–10 hour overnight fast followed by an oral glucose tolerance test(OGTT) in resting conditions. Fasting plasma glucose (FPG), 1-h post-OGTT glucose (1hPG) and 2-h post-OGTT glucose (2hPG) were detected using glucose oxidase method; The levels of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), glutamic pyruvic transaminase (ALT), glutamic oxaloacetic transaminase (AST),Creatinine (CR) and blood urea nitrogen (BUN) were detected by enzymatic method. Apolipoprotein A (APOA) and Apolipoprotein B (APOB) were measured by immunoturbidimetric assay. Free Triiodothyronine (FT3), Free Thyroxine (FT4) and Thyroid-Stimulating Hormone (TSH) were measured by chemiluminescence immunoassay. Direct Bilirubin (Dbil) and Total Bilirubin (Tbil) were measured by the diazocouple method.Additional serum samples were stored in microtubes in the −80°C degree refrigerator until the analysis of TSK. Serum TSK levels were measured via the commercially available human ELISA kit (catalog number E15159h; Eiaab Science, Wuhan, China) with intra- and inter-assay coefficients of variation of < 6.3% and < 9.8%, respectively. Conversions between different units were carried out to fit the formula calculation requirements.

Statistical Analysis

Normality of continuous variables was assessed using the Shapiro–Wilk test together with inspection of Q–Q plots and histograms. Levene's test was used to assess homogeneity of variances; where violated, Welch's *t*-test or non-parametric tests were applied. Between-group comparisons used an independent Student's *t*-test or Mann–Whitney *U*-test as appropriate; comparisons among tertiles used one-way ANOVA or Kruskal–Wallis tests. Correlations between TSK and clinical indicators were evaluated using Spearman's rank correlation. P-values for correlation analyses were adjusted using the Benjamini–Hochberg FDR method. Binary logistic regression was used to examine the associations between TSK tertiles and GDM (reported as odds ratios with 95% CIs). Although no a priori sample-size calculation was performed, a post hoc power analysis (two-sided α=0.05) indicated >80% power to detect medium effects (Cohen's d≈0.5) for group comparisons (n1=83, n2=93) and ~80% power to detect OR≈2.0 in logistic regression given the observed event rate.

Result

The Clinical and Biochemical Parameters in Two Groups

Weight, BMI, SBP, WC, WhtR, ALT, AST, FT3, TSH, FPG, 1hPG and 2hPG were significantly higher in the GDM group than in the NGT group (P<0.05 or P<0.001), while height and CR levels were significantly lower than those in the NGT group (P<0.05).Serum TSK levels were significantly higher in the GDM group than in the NGT group [0.52 (0.42,0.67) vs 0.60 (0.47,0.73)] ($P < 0.05$) (Table 1).

Table 1 Comparisons of the Basic Data and Clinical Indicators Between the GDM Group and the Normal Control Group

Variables	NGT (n=93)	GDM (n=83)	T/Z/x^2	P value	Effect Size (95% CI)
Age (years)	29.00(27.00,31.00)	30.00(27.00,33.00)	−1.736	0.083	0.15 (−0.03, 0.31)
Height (cm)	163.00(160.00,167.00)	160.00(158.00,165.00)	−2.036	0.042*	−0.18 (−0.35, −0.02)
Weight (kg)	63.51±8.85	67.96±10.23	−3.203	0.001**	0.28 (0.11, 0.43)
BMI (kg/m^2)	23.87±2.86	26.03±3.18	−4.726	<0.001***	0.71 (0.40, 1.01)
SBP (mmHg)	111.00(103.50,118.50)	119.00 (115.00,130.00)	−5.006	<0.001***	0.44 (0.28, 0.57)
DBP (mmHg)	72.52±8.12	74.38±7.45	−1.541	0.123	0.24 (−0.07, 0.54)
WC (cm)	93.50(89.00,99.00)	94.00(92.00,102.00)	−1.982	0.047*	0.17 (0.01, 0.33)
WHtR	0.58(0.55,0.61)	0.59(0.57,0.62)	−2.587	0.010*	0.23 (0.06, 0.38)
ALT (U/L)	18.00(12.95,24.70)	23.10(15.40,32.50)	−2.793	0.005**	0.24 (0.07, 0.41)
AST (U/L)	17.10(14.25,20.75)	19.70(15.40,25.50)	−2.015	0.044*	0.18 (0.01, 0.35)
Tbil (μmol/L)	10.46(8.10,12.11)	10.37(8.31,11.64)	−0.387	0.699	−0.03 (−0.20, 0.13)
Dbil (μmol/L)	2.80(2.20,3.40)	2.65(2.15,3.30)	−0.365	0.715	−0.03 (−0.19, 0.13)
CR (μmol/L)	47.00(42.55,50.35)	44.50(41.30,49.00)	−1.977	0.048*	−0.17 (−0.33, −0.00)
BUN (mmol/L)	2.89(2.35,3.53)	3.01(2.44,3.57)	−0.984	0.325	0.09 (−0.08, 0.26)
FT3 (pmol/L)	4.74(4.19,5.12)	5.11(4.69,5.39)	−3.777	<0.001***	0.33 (0.18, 0.48)
FT4 (pmol/L)	15.76(13.98,17.37)	16.18(14.55,18.05)	−1.283	0.200	0.11 (−0.06, 0.28)
TSH (mIU/L)	1.50(0.93,1.91)	1.77(1.23,2.28)	−2.681	0.007**	0.23 (0.08, 0.40)
FPG (mmol/L)	4.24(4.11,4.56)	4.69(4.48,4.94)	−6.722	<0.001***	0.59 (0.46, 0.71)
1hPG (mmol/L)	7.31(6.48,8.50)	10.25(9.31,11.04)	−9.758	<0.001***	0.85 (0.77, 0.92)
2hPG (mmol/L)	6.59(5.87,7.54)	8.81(8.35,9.56)	−9.186	<0.001***	0.80 (0.70, 0.90)
LDL-C (mmol/L)	3.62(3.07,4.23)	3.59(2.93,4.08)	−0.285	0.776	−0.02 (−0.19, 0.15)
HDL-C (mmol/L)	2.03(1.77,2.31)	2.00(1.72,2.21)	−1.147	0.251	−0.10 (−0.28, 0.08)
TC (mmol/L)	7.11±1.04	7.15±1.08	−0.304	0.761	0.03 (−0.27, 0.33)
TG (mmol/L)	3.80(2.85,4.30)	4.02(3.39,4.97)	−1.722	0.085	0.15 (−0.02, 0.32)

(*Continued*)

Table 1 (Continued).

Variables	NGT (n=93)	GDM (n=83)	T/Z/x^2	P value	Effect Size (95% CI)
APOA (g/L)	2.16±0.32	2.15±0.34	−0.055	0.956	−0.02 (−0.32, 0.28)
APOB (g/L)	1.22(1.05,1.41)	1.16(1.03,1.44)	−0.508	0.611	−0.04 (−0.21, 0.12)
TSK (ng/mL)	0.52(0.42,0.67)	0.60(0.47,0.73)	−2.477	0.013*	0.22 (0.05, 0.38)

Notes:Data are means±SD, n (%) and M (25^{th}, 75^{th}),*<0.05,**<0.01,***<0.001.

Abbreviations: NGT, normal glucose tolerance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; WHtR, the waist-to-height; ALT,alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; Dbil, direct bilirubin; CR, creatinine; BUN, blood urea nitrogen; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; 1hPG, 1-h post-OGTT glucose; 2hPG, 2-h post-OGTT glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; APOA, apolipoprotein a; APOB, apolipoprotein b; TSK, Tsukushi.

The Clinical and Biochemical Parameters of the Study Subjects According to the Tertiles of TSK

Tertile grouping according to serum TSK levels: T1 (TSK ≤ 0.46ng/mL, n=59), T2 (0.46ng/mL < TSK < 0.67ng/mL, n=59), T3 (TSK ≥ 0.67ng/mL, n=58).The results showed that with increasing serum TSK level, ALT, 1hPG, LDL-C, TC and prevalence of gestational diabetes mellitus gradually increased (P < 0.05). Compared with the T1 group, DBP was significantly higher in the T2 and T3 groups ($P < 0.001$), and BUN was significantly lower in the T2 group ($P < 0.05$) (Table 2).

Correlation of TSK with Clinical and Biochemical Parameters

Correlation analysis was performed to examine the associations between serum TSK levels and clinical/biochemical parameters. Before multiple testing correction, TSK levels showed positive correlations with DBP, ALT, Dbil, FT3, 1hPG, LDL-C, and TC. However, after adjustment for multiple comparisons using the False Discovery Rate (FDR) method, only the correlation with TC remained statistically significant (FDR-adjusted P = 0.023). These results suggest that TSK may be particularly associated with cholesterol metabolism in pregnant women (Table 3).

Table 2 Comparisons of Basic Data and Clinical Indicators After the Third Position of Serum TSK Level

Variables	T1	T2	T3	*F*/*Z*/x^2	*P* value
Age (years)	30.00(27.00,32.50)	29.00(27.00,31.00)	29.00(28.00,33.00)	1.099	0.577
Height (cm)	160.00(160.00,164.50)	163.00(158.75,167.25)	162.00(159.00,165.00)	0.976	0.614
Weight (kg)	64.24±9.55	66.19±9.20	66.36±10.54	0.836	0.435
BMI (kg/m^2)	24.34(22.02,26.39)	25.22(22.81,26.57)	25.39(22.26,26.83)	1.356	0.508
SBP (mmHg)	114.00(102.00,120.00)	116.50(109.00,122.25)	117.00(110.00,120.37)	3.188	0.203
DBP (mmHg)	71.02±7.43	75.29±8.54	73.71±6.91	4.664	<0.001
WC (cm)	95.17±8.43	94.63±7.47	95.19±7.28	0.102	0.903
WHtR	0.59±0.05	0.58±0.05	0.59±0.04	0.494	0.781
ALT (U/L)	17.00(11.35,24.50)	18.80(13.38,28.80)	24.00(16.00,32.75)	8.779	0.012
AST (U/L)	16.90(14.15,24.00)	18.00(15.00,22.60)	19.50(15.10,25.50)	3.188	0.203
Tail(μmol/L)	10.30(8.26,11.77)	10.09(8.05,11.90)	10.80(8.76,11.85)	1.255	0.534
Dbil (μmol/L)	2.70(2.20,3.39)	2.52(1.98,2.99)	3.04(2.40,3.89)	8.690	0.013
CR (μmol/L)	45.44(41.95,50.25)	45.95(41.83,50.55)	45.41(41.75,47.92)	0.875	0.646
BUN (mmol/L)	3.21(2.40,3.77)	2.70(2.26,3.37)	3.01(2.51,3.57)	6.022	0.049
FT3 (pmol/L)	4.70(4.23,5.08)	5.02(4.67,5.44)	4.89(4.50,5.22)	8.644	0.013
FT4 (pmol/L)	15.97(14.39,17.40)	15.76(13.94,18.67)	16.08(14.48,17.76)	0.070	0.966

(*Continued*)

Table 2 (Continued).

Variables	T1	T2	T3	$F/Z/x^2$	*P* value
TSH (mIU/L)	1.68(1.13,1.91)	1.64(1.12,2.24)	1.50(0.73,2.03)	1.208	0.547
FPG (mmol/L)	4.48(4.17,4.72)	4.51(4.18,4.74)	4.53(4.20,4.78)	1.219	0.544
1hPG (mmol/L)	8.35(6.76,9.55)	9.16(6.91,10.64)	9.25(7.64,10.26)	6.090	0.048
2hPG (mmol/L)	7.33±1.45	7.96±2.00	7.87±1.87	2.102	0.125
LDL-C (mmol/L)	3.54(2.82,3.95)	3.59(2.98,4.21)	3.76(3.18,4.43)	6.090	0.031
HDL-C (mmol/L)	1.95(1.70,2.20)	2(1.75,2.29)	2.08(1.82,2.41)	2.890	0.236
TC (mmol/L)	6.97(6.19,7.53)	7.15(6.36,7.53)	7.48(6.56,8.13)	12.092	0.002
TG (mmol/L)	3.80(3.10,5.02)	3.89(2.88,4.70)	3.94(3.27,4.83)	0.555	0.758
APOA (g/L)	2.10(1.93,2.38)	2.15(1.95,2.34)	2.16(2.00,2.41)	2.345	0.310
APOB (g/L)	1.21(1.03,1.41)	1.15(1.02,1.45)	1.22(1.05,1.44)	1.195	0.550
GDM (%)*	33.33	51.61	56.14	6.711	0.035

Notes: *Number of persons with gestational diabetes as a proportion of the group.
Abbreviations: NGT, normal glucose tolerance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; WHtR, the waist-to-height; ALT, glutamic pyruvic transaminase; AST, glutamic oxaloacetic transaminase; Tbil, total bilirubin; Dbil, direct bilirubin; CR, creatinine; BUN, blood urea nitrogen; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; 1hPG, 1-h post-OGTT glucose; 2hPG, 2-h post-OGTT glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; APOA, apolipoprotein a; APOB, apolipoprotein b; TSK, Tsukushi.

Table 3 Correlation Analysis of Serum TSK Level and Each Basic Data and Clinical Indicators

Variables	TSK		
	r	P value	FDR-adjusted P
Age (years)	−0.040	0.597	0.776
Height (cm)	0.030	0.693	0.783
Weight (kg)	0.046	0.548	0.750
BMI(kg/m^2)	0.027	0.726	0.786
SBP (mmHg)	0.130	0.086	0.279
DBP (mmHg)	0.154	0.042	0.182
WC (cm)	0.005	0.943	0.943
WHtR	−0.033	0.662	0.782
ALT (U/L)	0.209	0.005	0.065
AST (U/L)	0.119	0.115	0.299
Tbil (μmol/L)	0.058	0.447	0.684
Dbil (μmol/L)	0.169	0.025	0.162
CR (μmol/L)	−0.105	0.167	0.395
BUN (mmol/L)	−0.034	0.651	0.782
FT3 (pmol/L)	0.146	0.050	0.186
FT4 (pmol/L)	−0.015	0.846	0.880
TSH (mIU/L)	−0.072	0.346	0.600
FPG (mmol/L)	0.091	0.231	0.500
1hPG (mmol/L)	0.157	0.037	0.182
2hPG (mmol/L)	0.080	0.292	0.546
LDL-C (mmol/L)	0.197	0.009	0.078
HDL-C (mmol/L)	0.117	0.112	0.299
TC (mmol/L)	0.277	<0.001	0.023

(*Continued*)

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Table 3 (Continued).

Variables	TSK		
	r	P value	FDR-adjusted P
TG (mmol/L)	0.049	0.517	0.747
APOA (g/L)	0.067	0.376	0.611
APOB (g/L)	0.069	0.294	0.546

Abbreviations: NGT, normal glucose tolerance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; WHtR, the waist-to-height; ALT, glutamic pyruvic transaminase; AST, glutamic oxaloacetic transaminase; Tbil, total bilirubin; Dbil, direct bilirubin; CR, creatinine; BUN, blood urea nitrogen; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; 1hPG, 1-h post-OGTT glucose; 2hPG, 2-h post-OGTT glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; APOA, apolipoprotein a; APOB, apolipoprotein b; TSK, Tsukushi.

Association of the TSK with GDM

Binary logistic regression analyses were performed with the occurrence of GDM as the dependent variable, serum TSK level as the independent variable (model 1), and T1 group as the reference, which showed that the risk of GDM was higher in the T2 and T3 groups than in the T1 group (model 1), and after correction for age, BMI, DBP and other confounders (model 2), the risk of developing GDM was still significantly higher in the T3 group than in the T1 group. After further correction for ALT, AST, TG, TC and other factors based on model 2 (model 3), the results showed that compared with the T1 group, the OR values of subjects in the T2 and T3 groups for the occurrence of GDM were 2.481 (*95% CI*: 1.027–5.997,*P*=0.044) and 2.883 (*95% CI*: 1.173–7.084,*P*=0.021), showing a significantly higher trend ($P<0.05$) (Table 4).

Discussion

This study is the first to explore the role of TSK in GDM, and found that the serum TSK level of pregnant women with GDM was significantly higher than that of the normal pregnancy control group. Binary logistic regression analysis showed that higher serum TSK levels were associated with increased odds of GDM. In conclusion, TSK may serve as a biomarker associated with metabolic disturbances in GDM. However, since this study was a cross-sectional design and causal relationships cannot be inferred.

Table 4 Binary Logistic Regression Analysis with GDM as the Dependent Variable

	TSK	β	SE	Wald	OR	95% CI	*P* value
Model 1	T1				1		
	T2	0.758	0.379	4.000	2.133	1.015–4.483	0.046
	T3	0.940	0.388	5.883	2.560	1.198–5.472	0.015
Model 2	T1				1		
	T2	0.848	0.437	3.758	2.334	0.991–5.499	0.053
	T3	1.033	0.439	5.549	2.810	1.190–6.639	0.018
Model 3	T1				1		
	T2	0.909	0.450	4.074	2.481	1.027–5.997	0.044
	T3	1.059	0.459	5.330	2.883	1.173–7.084	0.021

Notes: Model 1: crude; Model 2: adjusted for age, BMI, DBP; Model 3: adjusted for all the factors in Model 2 and ALT, AST, TC and TG.
Abbreviation: TSK, Tsukushi.

At present, the specific pathogenesis of GDM has not been fully clarified. However, there is a consensus that the mechanism is similar to that of Type 2 Diabetes Mellitus (T2DM), which mainly involves the two core pathological aspects of insulin resistance (IR) and insufficient insulin secretion. During pregnancy, the maternal demand for glucose gradually increases in early pregnancy to accommodate the metabolic demands of foetal growth, while insulin sensitivity naturally decreases. As pregnancy progresses, hormones secreted by the placenta specifically antagonise the effects of insulin, leading to a gradual increase in insulin resistance.[11,12] Under normal conditions, the mother compensates for insulin resistance by increasing insulin secretion, but when pregnant women with GDM are unable to compensate effectively due to insufficient insulin secretion, it triggers an increase in blood glucose levels, which ultimately leads to the development of GDM.[13] In recent years, the study of the pathogenesis of GDM with liver factors has received more and more attention. The liver is not only an important organ for metabolic regulation, but also an endocrine organ capable of secreting a variety of hepatic factors, which may play an important role in the development of GDM by regulating insulin sensitivity, glucose metabolism, and lipid metabolism, among other mechanisms. For example, Fibroblast Growth Factor 21 (FGF21), a hormone secreted by the liver, is involved in the regulation of glucose and lipid metabolism, insulin sensitivity and energy balance. Clinical studies have found that FGF21 levels are significantly elevated in patients with GDM,[5,6] while animal experiments have shown that FGF21 can alleviate hyperglycaemia by enhancing the insulin signalling pathway and improving insulin sensitivity and glucose tolerance.[14] Existing studies indicate that TSK, as a hepatic-derived hormone, is closely associated with metabolic disorders such as metabolic syndrome, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD). Our findings align with Li et al's 2024 study, revealing a correlation between TSK and the urine albumin-to-creatinine ratio (UACR), suggesting higher TSK has been linked to urinary albumin–creatinine ratio, consistent with renal microvascular stress rather than a proven causal effect. Moreover, Lam et al reported that serum TSK levels were independently associated with the severity of liver fibrosis, further supporting the relevance of TSK to hepatic metabolic processes.While direct studies on TSK's relationship with GDM remain preliminary, our findings indicate that TSK is associated with multiple metabolic parameters, including insulin resistance, glucose-lipid metabolism, and inflammation. Based on these observations, we hypothesize that TSK is linked to GDM-related metabolic alterations. However, our study did not explore how TSK specifically affects lipid metabolism and liver function, which requires further investigation in future research.

Relevant studies have shown that TSK plays an important role in glucose metabolism, and Wang et al showed that TSK-deficient mice (KO mice) exhibited significantly lower blood glucose levels under high-fat dietary conditions, suggesting that TSK deficiency may ameliorate obesity-associated hyperglycaemia. In addition, TSK-deficient mice had significantly lower plasma insulin levels and exhibited significantly improved insulin sensitivity in the insulin tolerance test (ITT) compared to wild-type (WT) mice, suggesting that TSK may promote insulin resistance by modulating insulin secretion or sensitivity. Further studies showed that TSK-deficient mice exhibited significantly improved insulin tolerance in a high-fat diet-induced obesity model, suggesting that overexpression of TSK may promote metabolic disorders by exacerbating insulin resistance, which is mitigated by its deletion. TSK-deficient mice exhibited lower blood glucose and insulin levels, as well as better glucose tolerance (GTT) and insulin sensitivity, suggesting that TSK may be involved in the development and progression of diabetes, given its associations with insulin resistance and blood glucose levels.[9] In a recent clinical study, Li et al found that serum TSK levels were significantly elevated in patients with newly diagnosed type 2 diabetes mellitus and were closely associated with insulin resistance, decreased β-cell function, and disorders of glucose and lipid metabolism. The study showed that serum TSK levels were significantly higher in patients with type 2 diabetes than in normal glucose tolerance (NGT) controls, and that TSK levels were positively correlated with BMI, FPG, 2hPG, glycated haemoglobin (HbA1c), and index of insulin resistance (HOMA-IR), and positively correlated with index of insulin sensitivity ($ISI_{stumvoll}$) and glucose clearance ($MCR_{stumvoll}$) were negatively correlated. Further analysis showed that the prevalence of diabetes in the group with high TSK levels was significantly higher than that in the group with low TSK levels and exhibited more severe insulin resistance and lower pancreatic β-cell function.[15] TSK may be involved in the development and progression of type 2 diabetes through mechanisms such as interfering with the insulin signalling pathway, exacerbating chronic inflammation, and triggering dysregulation of hepatic lipid metabolism. In this study, we compared the serum TSK levels of pregnant women with GDM with those of pregnant women with normal pregnancies, and also analysed the relationship between serum TSK and other indicators of glucose

metabolism, and the results showed that the serum TSK levels of pregnant women in the GDM group were significantly higher than those of the normal control group, and after trichotomising the serum TSK, it was found that the prevalence of 1hPG and gestational diabetes mellitus gradually increased with the increase in serum TSK levels, and the results of the correlation analyses also showed a significant positive correlation between serum TSK levels and 1hPG in all study subjects.With the occurrence of GDM as the dependent variable and serum TSK levels as the independent variable, higher TSK levels were associated with increased odds of GDM (Model 3: OR = 2.883, 95% CI 1.173–7.084; P = 0.021), similar to the results of the above studies.TSK was significantly elevated in GDM, and the liver may regulate metabolism through the secretion of more TSK during pregnancy, when the metabolic burden of the mother increases. However, this compensatory mechanism may be dysregulated in patients with GDM, resulting in abnormally elevated TSK levels, while GDM is usually accompanied by low-grade chronic inflammation, which may up-regulate TSK expression through pro-inflammatory cytokines, which have been previously shown to regulate immune cell migration and adhesion and influence cell aggregation at sites of inflammation through interactions with extracellular matrix and cell surface receptors, and TSK is able to mediate multiple signalling pathways, such as TGF-β and Wnt signalling pathways, which have a key role in the inflammatory response. In summary, we hypothesize that serum TSK levels are associated with the onset and development of GDM, however, the exact role of TSK needs to be further investigated.

After FDR correction, only the TSK–total cholesterol link remained significant. This pattern is consistent with reports that hepatic TSK responds to steatotic stress and interfaces with cholesterol–bile acid homeostasis (eg, SR-BI /LDLR uptake; CYP7A1-mediated bile acid synthesis). In pregnancy, cholesterol-centric remodeling may therefore yield a higher signal-to-noise ratio than triglyceride handling, which could explain the FDR-robust association with TC while TG did not survive correction. Abnormal lipid metabolism is one of the most important factors contributing to insulin resistance in GDM patients. During normal pregnancy, increased intestinal fat absorption, increased levels of progesterone and placental lactogen with increasing gestational weeks, and insulin resistance factors lead to hyperlipidemic state during pregnancy. Studies have shown that TC, TG, VLDL-C, LDL-C and HDL-C are all higher than those of non-pregnant women and increase with gestational weeks, with a particular predominance of TG elevation. This hyperlipidemic state helps the mother to use fat to provide energy for the foetus during fasting or starvation to maintain its normal growth and development. Compared to normal pregnancies, patients with GDM exhibit more significant elevations of TG and VLDL in late pregnancy, along with a significant decrease in HDL. This is due to more severe insulin resistance and further reduction in insulin sensitivity in GDM patients,[16] resulting in impaired TG degradation in celiac microsomes (CM) and VLDL, increased free fatty acids (FFA), and increased VLDL synthesis. In addition, enhanced fat β-oxidation and large amounts of acetyl coenzyme A production contribute to elevated TC levels. Meanwhile, increased secretion of estrogen, progesterone, placental lactogen, glucagon, thyroid hormone and adrenaline during pregnancy exacerbates insulin resistance, inhibits lipoprotein lipase activity and enhances adipose tissue hormone-sensitive lipase activity, further aggravating lipid metabolism disorders in GDM patients. Several studies have shown that TSK plays multiple roles in lipid metabolism, and its overexpression is tangentially associated with lipid metabolism disorders,[17] non-alcoholic fatty liver disease (NAFLD)[7,18] and obesity dense.[19] TSK expression levels were found to be significantly elevated in ob/ob and db/db mice as well as in a high-fat diet (HFD)-induced mouse model of NASH.TSK reduces cholesterol uptake by inhibiting the expression of hepatic scavenger receptor B1 (Srb1) and low-density lipoprotein receptor (LDLR) and reduces cholesterol uptake through the down-regulation of cholesterol 7α-hydroxylase (Cyp7α1) and other expression of key enzymes, inhibiting the conversion of cholesterol to bile acids, thus affecting cholesterol homeostasis and bile acid metabolism.[7] In addition, TSK deficiency significantly ameliorated the pathological features of NAFLD, including attenuating hepatic steatosis, inflammation and fibrosis, while reducing plasma ALT and AST levels. In terms of energy metabolism, TSK-deficient mice exhibited higher rates of oxygen consumption (VO2) and energy expenditure, reduced lipid droplet volume and fat content in brown adipose tissue, and enhanced thermogenesis, which resisted high-fat diet-induced obesity.[19] Similar results were verified in the GDM population in the present study, where LDL-C and TC gradually increased with increasing serum TSK levels, while correlation analysis showed significant positive correlation between serum TSK levels and ALT, conjugated bilirubin, FT3, LDL-C, and TC, Based on this, we speculate that TSK may indirectly affect ALT levels by influencing liver lipid metabolism and regulating LDL-C synthesis and

clearance. Existing literature indicates that TSK is involved in fatty acid metabolism and cholesterol transport processes, which may serve as potential mechanisms linking TSK to LDL-C. However, some findings showed divergent effects of TSK knockdown on adipose tissue thermogenic function, weight gain and glucose homeostasis,[20] which may be related to differences in experimental conditions, model selection and genetic background. Future studies can further reveal the specific regulatory effect of TSK on liver lipid metabolism through animal models or in vitro experiments.

Beyond regulating blood glucose levels, TSK is also associated with renal and hepatic phenotypes. For instance, in type 2 diabetes patients, elevated serum TSK levels correlate with the urine albumin-to-creatinine ratio (UACR), suggesting that the glomerular endothelial stress pathway may extend beyond blood glucose control.[21] Another study revealed a positive correlation between liver fibrosis severity and TSK levels, independent of diabetes status,[22] further confirming the potential link between the liver-TSK-fibrosis axis and pregnancy-associated lipid remodeling. Population-based data indicate TSK's association with metabolic syndrome and its components,[23] while genetic studies have linked TSK gene variants to obesity-related metabolic traits.[24] Based on these findings, we propose that TSK could be positioned as a biomarker with functional roles encompassing hepatic lipid metabolism, renal microvascular stress, and systemic metabolic risk management.

While this study highlights the critical role of TSK in GDM, several limitations remain. As a single-center cross-sectional study with a relatively small sample size, its findings may lack generalizability. Crucially, the absence of placental TSK expression analysis—where placental tissue serves as a vital organ for metabolic regulation during pregnancy—remains unexplored, necessitating further investigation into placental TSK levels and their maternal correlates. Given variations in race, region, climate, socioeconomic status, and fertility attitudes, these findings require rigorous validation and expanded application of TSK's potential. Future research topics include longitudinal tracking of TSK levels during pregnancy and postpartum, panel predictive models combining hepatocyte factors and lipidomics, genetic methods for causal inference, and exploration of their association with placental biology and perinatal outcomes.

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Disclosure

The authors report no conflicts of interest in this work.

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