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AVALIAÇÃO DA RELAÇÃO ENTRE A ATIVIDADE DA TIOSULFATO SULFOTRANSFERASE E O ÍNDICE TRIGLICERÍDEOS-GLICOSE/ÍNDICE DE MASSA CORPORAL NO DIABETES MELLITUS TIPO 2

EVALUATION OF THE RELATIONSHIP BETWEEN THIOSULFATE SULFURTRANSFERASE ACTIVITY AND TRIGLYCERIDE GLUCOSE-BODY MASS INDEX IN TYPE 2 DIABETES MELLITUS

تقييم العلاقة بين فعالية ثيوسلفات سلفور ترانسفيراز ومؤشر الدهون الثلاثية للكلوكوز -كتلة الجسم لدى مرضى السكري من النوع الثاني

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RESUMO

Introdução: A prevalência global do diabetes mellitus tipo 2 (DM2) continua a aumentar de forma dramática, exigindo uma reavaliação dos mecanismos fisiopatológicos subjacentes. A tiossulfato sulfurtransferase (TST), uma enzima envolvida na função mitocondrial, surgiu como um possível fator na regulação metabólica, embora sua relação com a resistência à insulina no DM2 ainda não esteja suficientemente caracterizada. **Objetivos**: Este estudo teve como objetivo investigar a relação entre a atividade da TST e a resistência à insulina, avaliada por meio do índice triglicerídeos-glicose (TyG) e do índice triglicerídeos-glicose/índice de massa corporal (TyG-BMI) em pacientes com DM2, buscando identificar potenciais novas vias para intervenções diagnósticas e terapêuticas. **Métodos**: Neste estudo caso-controle, foram incluídos 300 participantes (com idades entre 33 e 65 anos), sendo 150 pacientes com DM2 atendidos no Centro Al-Wafa de Endocrinologia e Diabetes em Mossul, Iraque, e 150 controles saudáveis pareados por idade. A atividade sérica da TST foi determinada por espectrofotometria, enquanto os níveis de insulina e glicose foram medidos por técnicas padronizadas. Os índices TyG e TyG-BMI foram calculados utilizando fórmulas validadas. As análises estatísticas incluíram testes t independentes e correlação de Pearson, com significância estabelecida em $p \leq 0,05$. **Resultados**: A atividade da TST foi significativamente reduzida em 46% nos pacientes com DM2 em comparação aos controles (11,3±5,3 vs. 20,2±4,8 U/ml, $p<0,01$). A atividade da TST diminuiu progressivamente com o aumento do IMC, apresentando reduções de 36%, 50% e 56% em indivíduos com peso normal, sobrepeso e obesidade, respectivamente. Os pacientes com DM2 apresentaram índices TyG (4,56±1,3 vs. 3,82±1,1, $p<0,01$) e TyG-BMI (154,56±18,8 vs. 107,16±11,2, $p<0,01$) significativamente elevados. Foram observadas fortes correlações negativas entre a atividade da TST e tanto o TyG ($r=-0,75$, $p<0,01$) quanto o TyG-BMI ($r=-0,81$, $p<0,01$). **Discussão**: Nossos achados sugerem que o DM2 está associado à atividade prejudicada da TST, possivelmente como resultado de disfunção mitocondrial. A forte correlação inversa entre a atividade da TST e os marcadores de resistência à insulina indica que a função reduzida da TST pode contribuir para a desregulação metabólica no DM2, ao comprometer a captação e o metabolismo da glicose nos tecidos sensíveis à insulina. **Conclusão**: A atividade da TST demonstra associações significativas com a resistência à insulina e a obesidade em pacientes com DM2. Esses achados destacam a TST como um possível alvo terapêutico para melhorar a função metabólica e reduzir as complicações do diabetes, justificando investigações adicionais sobre intervenções que modulam a TST no manejo do DM2.

Palavras-chave: *Tiosulfato sulfurtransferase; TyG; TyG-IMC; Resistência à insulina; Diabetes tipo 2.*

ABSTRACT

Introduction: The global prevalence of type 2 diabetes mellitus (T2DM) continues to rise dramatically, necessitating reconsideration of underlying pathophysiological mechanisms. Thiosulfate sulfurtransferase (TST), an enzyme involved in mitochondrial function, has emerged as a potential factor in metabolic regulation; however, its relationship with insulin resistance in type 2 diabetes mellitus (T2DM) remains inadequately characterized.

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Objectives: This study aimed to investigate the relationship between TST activity and insulin resistance, as assessed by the triglyceride glucose index (TyG) and the triglyceride glucose-body mass index (TyG-BMI), in patients with T2DM, potentially identifying new pathways for diagnostic and therapeutic interventions. **Methods:** In this case-control study, 300 participants (aged 33-65 years) were enrolled, comprising 150 patients with type 2 diabetes mellitus (T2DM) from Al-Wafa Centre for Endocrinology and Diabetes in Mosul, Iraq, and 150 age-matched healthy controls. Serum TST activity was determined spectrophotometrically, while insulin and glucose levels were measured using standardized techniques. TyG and TyG-BMI were calculated using validated formulas. Statistical analyses included independent t-tests and Pearson correlation, with significance set at $p \leq 0.05$. **Results:** TST activity was significantly decreased by 46% in T2DM patients compared to controls (11.3 ± 5.3 vs. 20.2 ± 4.8 U/mL, $p < 0.01$). TST activity progressively declined with increasing BMI, showing reductions of 36%, 50%, and 56% in normal weight, overweight, and obese subjects, respectively. T2DM patients exhibited significantly elevated TyG index (4.56±1.3 vs. 3.82±1.1, $p<0.01$) and TyG-BMI (154.56±18.8 vs. 107.16±11.2, $p<0.01$). Strong negative correlations were observed between TST activity and both TyG ($r=-0.75$, $p<0.01$) and TyG-BMI ($r=-0.81$, $p<0.01$). **Discussion:** Our findings suggest that T2DM is associated with impaired TST activity, potentially resulting from mitochondrial dysfunction. The strong inverse correlation between TST activity and insulin resistance markers indicates that diminished TST function may contribute to metabolic dysregulation in T2DM by impairing glucose uptake and metabolism in insulin-sensitive tissues. **Conclusion:** TST activity demonstrates significant associations with insulin resistance and obesity in T2DM patients. These findings highlight TST as a potential therapeutic target for improving metabolic function and reducing diabetes complications, warranting further investigation into TST-modulating interventions for the management of type 2 diabetes mellitus (T2DM).

Keywords: *Thiosulfate sulfurtransferase; TyG; TyG-BMI; Insulin Resistance; type 2 diabetes.*

الملخص

الخلفية: يشهد الانتشار العالمي لداء السكري من النوع الثاني (T2DM) ارتفاعًا حادًا، مما يستدعي إعادة النظر في الآليات المرضية الكامنة وراءه. برز إنزيم ثيوكبريتات سلفور ترانسفيراز (TST)، وهو إنزيم يشارك في وظيفة الميتوكوندريا، كعامل محتمل في تنظيم عملية الأيض؛ إلا أن علاقته بمقاومة الأنسولين في داء السكري من النوع الثاني (T2DM) لا تزال غير واضحة المعالم. **الأهداف:** هدفت هذه الدراسة إلى دراسة العلاقة بين نشاط إنزيم ثيوكبريتات سلفور ترانسفيراز ومقاومة الأنسولين، كما تم تقييمها من خلال مؤشر الكلوكوز ثلاثي الكليسريد (TyG) ومؤشر كتلة الجسم للكلوكوز ثلاثي الكليسريد (TyG-BMI)، لدى مرضى داء السكري من النوع الثاني، مما قد يُسهم في تحديد مسارات جديدة للتدخلات التشخيصية والعلاجية. **طرق العمل**: في هذه الدراسة المقارنة، تم تسجيل 300 مشارك (تتراوح أعمارهم بين 33 و65 عامًا)، منهم 150 مريضًا مصابًا بداء السكري من النوع الثاني (T2DM) من مركز الوفاء للغدد الصماء والسكري في الموصل، العراق، و150 شخصًا سليمًا من نفس الفئة العمرية. حُدد نشاط TST في المصل باستخدام مطيافية ضوئية، بينما قيست مستويات الأنسولين والكلوكوز باستخدام عدة تحليل جاهزة. حُسب مؤشر TyG ومؤشر TyG-BMI باستخدام صيغ رياضية مُعتمدة. وشملت التحليلات الإحصائية اختبارات t المستقلة ومعامل ارتباط بيرسون، مع قيمة دلالة إحصائية عند $p \leq 0.05$. **النتائج**: انخفض نشاط TST بشكل ملحوظ بنسبة 46% لدى مرضى داء السكري من النوع الثاني مقارنةً بالمجموعة الضابطة (11.3 ± 5.3 مقابل 20.2 ± 4.8 وحدة/مل، $p < 0.01$). انخفض نشاط TST تدريجيًا مع زيادة مؤشر كتلة الجسم، حيث أظهر انخفاضًا بنسبة 36% و50% و56% لدى الأشخاص ذوي الوزن الطبيعي، وزيادة الوزن، والسمنة على التوالي. أظهر مرضى السكري من النوع الثاني ارتفاعًا ملحوظًا في مؤشر TyG (4.56±1.3 مقابل 3.82±1.1، قيمة $P<0.01$) ومؤشر TyG-BMI (154.56±18.8 مقابل 107.16±11.2، قيمة $P<0.01$). ولوحظت ارتباطات سلبية قوية بين نشاط TST وكلٍّ من TyG ($r=-0.75$، قيمة $P<0.01$) ومؤشر TyG-BMI ($r=-0.81$، قيمة $P<0.01$). **المناقشة:** تشير نتائجنا إلى أن مرض السكري من النوع الثاني يرتبط بضعف نشاط TST، والذي قد يكون ناتجًا عن خلل في الميتوكوندريا. يشير الارتباط العكسي القوي بين نشاط TST وعلامات مقاومة الأنسولين إلى أن انخفاض وظيفة TST قد يُسهم في اختلال التمثيل الغذائي لدى مرضى السكري من النوع الثاني، وذلك من خلال إضعاف امتصاص الكلوكوز واستقلابه في الأنسجة الحساسة للأنسولين. **الاستنتاج:** يُظهر نشاط TST ارتباطًا وثيقًا بمقاومة الأنسولين والسمنة لدى مرضى السكري من النوع الثاني. تُبرز هذه النتائج أهمية TST كهدف علاجي محتمل لتحسين الوظيفة الأيضية وتقليل مضاعفات السكري، مما يستدعي إجراء المزيد من الدراسات حول التدخلات المُعدّلة لـ TST لإدارة داء السكري من النوع الثاني.

الكلمات المفتاحية. *انزيم ثيوسلفات سلفور ترانسفيراز؛ الدهون الثلاثية كلوكوز؛ الدهون الثلاثية كلوكوز- كتلة الجسم؛ مقاومة الأنسولين؛ داء السكري من النوع الثاني*

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most common and accounts for the majority of cases. It is a global health problem of great importance, as it was found in 2021 to affect approximately 529 million individuals aged 20 to 79 years. By 2045, the number of cases is expected to rise to 783 million (International Diabetes Federation, 2023). Type 2 diabetes mellitus (T2DM) occurs when the pancreas fails to produce enough insulin to control glucose levels within the normal range, or the body's cells become resistant to insulin. This is associated with physical inactivity, overweight, and poor eating habits (Galicia *et al.*, 2020). Obese individuals have hyperinsulinemia, which correlates with insulin resistance to control glucose tolerance in normoglycemia and hyperglycemia (Luo *et al.*, 2024). The progressive metabolic deterioration after a certain period causes hyperinsulinemia to

fail to adequately compensate for insulin resistance, resulting in impaired glucose tolerance that leads to diabetes (Janssen, 2024). Therefore, insulin resistance is a prominent feature of metabolic disorders and is recognised as a major causative factor in type 2 diabetes mellitus (T2DM), playing an important role in diabetes-related complications, including cardiovascular disease (Charlton *et al.*, 2020). Therefore, monitoring insulin resistance is crucial and is associated with complications in T2DM (Hong *et al.*, 2016).

Triglyceride-glucose (TyG) index is a reliable metabolic marker of insulin resistance (Saadon & Allwsh, 2024; Randrianarisoa *et al.*, 2020). TyG is positively associated with increased atherosclerosis and renal microvascular damage, and TyG indicates a risk of vascular diabetic complications Sánchez *et al.*, 2020), while triglyceride glucose-body mass index (TyG-BMI) reflects insulin resistance by integrating glucose and lipid metabolism markers with anthropometric indices (Xiao *et al.*, 2024; Liu *et al.*, 2021).TyG-BMI is associated with heart disease, non-alcoholic fatty liver disease, and hyperuricemia (Xiao *et al.*, 2024; Yang *et al.*, 2023). Previous analyses have shown a strong correlation between the triglyceride-glucose (TyG) index and insulin resistance, suggesting that it may be a reliable indicator of insulin resistance (Saadon & Allwsh, 2024; Sánchez-García *et al*., 2020). Furthermore, a value of the TyG index is strongly linked to an increased likelihood of arterial stiffness and nephric microvascular damage, suggesting the potential use of the TyG index to assess the vascular risk of diabetic complications (Charlton *et al.*, 2020).

The triglyceride glucose-body mass index (TyG-BMI index) reflects insulin resistance by integrating glucose and lipid metabolic markers with anthropometric indicators (Xiao *et al.*, 2024; Liu *et al.*, 2021). Recent studies have established the connection between cardiac disease, NAFLD, hyperuricemia, and the TyG-BMI index (Xiao *et al.*, 2024; Yang *et al.*, 2023).

Thiosulfate sulfurtransferase (TST) or rhodanase (TST, EC 2.8.1.1) was first identified as an enzyme that utilizes thiosulfate to detoxify cyanide, forming thiocyanate (Chaudhary & Gupta, 2012). The study by Morton *et al*. (2016) showed that thiosulfate could be a genetic marker for type 2 diabetes, as it modulated glucose levels and insulin resistance in diabetic mice by enhancing the release of adiponectin from fat cells, thereby increasing insulin sensitivity (Kruithof *et al.*, 2020). One possible explanation for this phenomenon is the association between TST and adiponectin expression (Yanai *et al.*, 2019). Similar evidence supporting the beneficial metabolic effects of TST activity in human adipose tissue was found (Dahmani *et al.*, 2022). The activity of TST has been linked to metabolic diseases through its function of stimulating mitochondrial activity and increasing the level of antioxidants. It also plays a significant role in reducing the level of H2S (Mutar *et al*., 2024).

TST is also involved in the formation and repair of iron-sulfur proteins, which are critical for the functioning of various mitochondrial enzymes, thereby indirectly supporting mitochondrial respiration, cellular energy production, and regulating cellular metabolism (Alsohaibani *et al.*, 2023).

1.1. Objectives

The association of thiosulfate transferase (TST) with type 2 diabetes, based on reliable metabolic markers, the TyG-BMI index and TyG, was aimed to be explored. Several variables related to diabetes and insulin resistance were included in the study for estimation. The study suggested that TST, as it relates to insulin resistance, plays a crucial role in determining the severity and progression of the disease and could serve as a potential therapeutic target.

2. MATERIALS AND METHODS

2.1. Materials

High-purity analytical-grade chemicals, including sodium thiosulfate, potassium dihydrogen phosphate, formaldehyde, potassium cyanide, and ferric nitrate (Sigma-Aldrich, St. Louis, MO, USA), were utilized for the precise determination of thiosulfate sulfurtransferase (TST) enzyme activity.

2.2. Study design

This prospective case-control study was conducted on 300 participants aged 33-65 years, comprising 150 type 2 diabetes (T2D) patients (85 males and 65 females) from Al-Wafa Center for Endocrinology and Diabetes in Mosul, Iraq, and 150 healthy controls (83 males and 67 females). Comprehensive participant data were documented, including demographic information (age, gender), lifestyle factors (physical activity), and current medications. Anthropometric measurements (body weight, height, waist, and hip circumference) and clinical parameters (blood pressure) were systematically recorded for all

subjects according to standardized protocols.

2.3. Exclusion criteria

Individuals with hypertension, cardiovascular disease, renal disorders, or liver disease were excluded from participation in this study, as these conditions constituted the predetermined exclusion criteria.

2.4. Collection of blood samples

Venous blood samples (5 mL) were collected from all participants following a minimum 8-hour overnight fasting period. Blood was drawn into clot activator gel tubes (Arzer Grande, Italy) and subsequently centrifuged at 3000 rpm (approximately 1000 × g) for 10 minutes at room temperature to obtain serum. The separated serum was aliquoted and either analyzed immediately or stored at -80°C until biochemical analysis to ensure sample stability and integrity.

2.5. Laboratory parameter assessments

This research was administered at the Chemistry Department, College of Science, University of Mosul. The blood biomarker assessments in the serum of patients and control groups included:

2.5.1. Estimating TST activity

Thiosulfate sulfurtransferase (TST; EC 2.8.1.1) activity was quantified using an optimized spectrophotometric method adapted from Chuang *et al*. (2021). This assay specifically measures the enzyme's catalytic capacity to transfer a sulfur atom from thiosulfate to cyanide, resulting in the generation of thiocyanate as the reaction product. All reagents used were analytical grade (≥99.5% purity; Sigma-Aldrich, St. Louis, MO, USA).

The reaction mixture (500 µL total volume) was prepared with precise stoichiometry: 200 µL of 0.125 M sodium thiosulfate, 100 µL of 0.2 M potassium dihydrogen phosphate buffer (pH 7.4 ± 0.05, adjusted with 1 M NaOH), 100 µL of freshly prepared serum homogenates, and 100 µL of 0.25 M potassium cyanide solution. All solutions were prepared using deionized water (resistance >18.2 MΩ·cm at 25 °C) and filtered through 0.22 µm membrane filters prior to use.

For each analytical batch, blank samples were prepared by adding 100 µL of 38% formaldehyde before adding potassium cyanide to inhibit enzymatic activity. All reaction components were equilibrated to assay temperature before combining. Following gentle vortex mixing (10 seconds at medium speed), the reaction mixtures were incubated precisely for 5 minutes (±5 seconds) at stringently controlled room temperature (22 ± 2 °C) in polypropylene microcentrifuge tubes.

The enzymatic reaction in the test samples was terminated by the rapid addition of 100 µL of 38% formaldehyde, followed by immediate mixing. The resulting thiocyanate product was quantified by adding 500 µL of freshly prepared 0.2 M ferric nitrate reagent in 1 N HNO_3, which formed a stable chromogenic ferric-thiocyanate complex with maximum absorbance at 460 nm. After 2 minutes of color development, absorbance measurements were performed using a double-beam UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) against reagent blanks.

Standard curves were generated using potassium thiocyanate (0-500 µmol/L) with linear response ($r^2 > 0.998$). Enzyme activity was expressed as units per milliliter (U/mL), where one unit represents the amount of enzyme catalyzing the formation of 1 µmol of thiocyanate per minute under the specified assay conditions. Intra-assay and inter-assay coefficients of variation were maintained below 5.2% and 7.4%, respectively, demonstrating robust analytical precision.

2.5.2. Estimating insulin levels

Serum insulin concentrations were quantified using an electrochemiluminescence immunoassay (ECLIA) based on the sandwich principle, performed on a Cobas e 411 automated analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA) with commercial reagent kits (Material Number: 12017547122). This highly sensitive method utilizes two monoclonal antibodies specifically directed against human insulin to form sandwich complexes, which generate measurable electrochemiluminescent signals. Rigorous quality control protocols were implemented throughout the analytical process, with each assay batch including both low- and high-concentration controls. Assay performance was validated through a comprehensive reproducibility assessment, yielding intra-assay coefficients of variation (CV) of 1.9% at 6.36 µU/mL and 1.2% at 20.9 µU/mL, and inter-assay CV of 2.6% at 6.36 µU/mL and 2.8% at 20.9 µU/mL (all with 95% confidence intervals). The analytical measurement range was 0.2-1000 µU/mL with a

functional sensitivity of 0.2 µU/mL.

2.5.3. Clinical Evaluations

Multiple biochemical parameters were systematically analysed using standardised enzymatic colourimetric methods with commercial kits (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's validated protocols. All assays were performed on a fully automated clinical chemistry analyzer (model specifications) with appropriate calibration and quality control measures.

Serum glucose was determined using the glucose oxidase-peroxidase method based on Trinder's reaction (Catalog Number: STA-680), where glucose is oxidized to gluconic acid and hydrogen peroxide, which reacts with phenol and 4-aminoantipyrine to form a quinonimine dye measured at 505 nm.

Lipid profile components were quantified as follows: triglycerides were measured after enzymatic hydrolysis by lipase into glycerol and fatty acids, followed by glycerol phosphorylation and oxidation to produce a chromogenic complex (Catalog Number: STA-396); total cholesterol was assessed after enzymatic hydrolysis of cholesterol esters by cholesterol esterase to free cholesterol, which was then oxidized by cholesterol oxidase (Catalog Number: STA-390); HDL-cholesterol was determined using a selective precipitation method that isolates HDL particles before quantification (Catalog Number: STA-391).

Renal function parameters were assessed through specific methodologies: albumin concentration was determined by bromocresol green binding method at pH 4.2 (Catalog Number: STA-383); creatinine was measured using the kinetic Jaffe reaction, where creatinine reacts with alkaline picrate to form an orange-red complex (Catalog Number: AT-80107); urea was quantified via the urease method with subsequent glutamate dehydrogenase reaction (Catalog Number: STA-382); uric acid was determined through the uricase method generating hydrogen peroxide that forms a chromogen (Catalog Number: STA-375).

Liver enzyme activities were measured kinetically: alanine aminotransferase (ALT/GPT) activity was assessed through monitoring of NADH oxidation during the coupled enzymatic reaction catalyzed by ALT and lactate dehydrogenase (Catalog Number: MET-5123); aspartate aminotransferase (AST/GOT) was similarly determined through a coupled reaction with malate dehydrogenase (Catalog Number: MET-5127).

All assays demonstrated analytical coefficients of variation <3.5% within the clinically relevant ranges.

2.5.4. Calculation of Derived Metabolic Indices

Secondary metabolic parameters were calculated using validated mathematical equations as described in recent literature. All calculations were performed using calibrated laboratory data with appropriate unit conversions to ensure accuracy.

Lipid-related parameters were calculated according to Gao *et al*. (2022):

- **Very low-density lipoprotein cholesterol (VLDL-C)** = Triglycerides / 2.5
- **Low-density lipoprotein cholesterol (LDL-C)** = Total cholesterol (TC) − High-density lipoprotein cholesterol (HDL-C) − (Triglycerides/2.5)
- **Non-HDL cholesterol (Non-HDL-C)** = Total cholesterol (TC) - HDL-C

Insulin resistance and β-cell function indices were determined using established formulas from Anmar *et al*. (2024) and Abdallha & Allwsh (2023):

- **Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)** = Fasting insulin (µU/mL) × Fasting glucose (mmol/L) / 22.5
- **Homeostatic Model Assessment for β-cell function (HOMA-β)** = [Fasting insulin (µU/mL) × 20] / [Fasting glucose (mmol/L) - 3.5]
- **Homeostatic Model Assessment for insulin sensitivity (HOMA1-%S)** = [1 / HOMA-IR] × 100%

Triglyceride-glucose indices were calculated as described by Xiao *et al*. (2024):

- **Triglyceride-Glucose Index (TyG)** = ln[Fasting triglycerides (mg/dL) × Fasting plasma glucose (mg/dL)]/2
- **Triglyceride-Glucose-Body Mass Index (TyG-BMI)** = ln[Fasting triglycerides (mg/dL) × Fasting plasma glucose (mg/dL)/2] × BMI (kg/m²)

All calculated indices were validated for internal consistency, and appropriate statistical analyses were performed to examine their distributions and relationships with measured parameters.

2.6. Data analysis

Comprehensive statistical analyses were

conducted using IBM SPSS Statistics software (Version 29.0, 2022, IBM Corporation, Armonk, NY, USA). A priori power analysis was performed using G*Power software (Version 3.1) to determine the appropriate sample size, achieving 90% power to detect medium effect sizes (Cohen's d = 0.5) at an alpha level of 0.05.

Descriptive statistics were calculated for all variables, with results expressed as mean ± standard error of the mean (SEM) after confirmation of normal distribution using the Shapiro-Wilk test. For non-normally distributed variables, data transformation was applied when necessary to meet the assumptions of parametric tests.

Between-group comparisons (T2DM patients versus healthy controls) were performed using independent samples t-tests with Levene's test for equality of variances. When the assumption of homogeneity of variance was not met, Welch's t-test was applied as a robust alternative. For categorical variables, chi-square tests were employed to assess between-group differences.

Associations between continuous variables were evaluated using the Pearson correlation coefficient (r) after confirming linear relationships through scatter plot examination. Correlation strength was interpreted as follows: $|r| < 0.3$ (weak), $0.3 \leq |r| < 0.7$ (moderate), and $|r| \geq 0.7$ (strong).

For all statistical analyses, a two-tailed p-value ≤ 0.05 was considered statistically significant. Confidence intervals (95% CI) were calculated for all key parameters to indicate the precision. Multiple comparison adjustments were applied when necessary using the Bonferroni method to control the family-wise error rate.

3. RESULTS AND DISCUSSION:

3.1. Results

The study results showed the activity of TST and the TyG index in relation to the TyG BMI in patients with T2D, focusing on the relationship between enzymes and insulin resistance in relation to BMI. All measured index values will be represented as mean ± standard error (SE). The details are summarised as follows:

3.1.1. The demographic, anthropometric, and clinical features

Table 1 presents the comprehensive baseline characteristics of participants in this study. Demographic variables, including age, sex distribution, and smoking habits, showed no significant differences between T2DM patients and healthy controls, confirming the appropriate matching of the study cohorts. In contrast, anthropometric parameters exhibited marked differences, with T2DM patients demonstrating significantly elevated blood pressure, waist circumference, body mass index (BMI), and hip circumference compared to control subjects ($p \leq 0.01$).

Glycemic and lipid metabolism markers revealed substantial metabolic dysregulation in the T2DM group. Fasting glucose, insulin, HOMA-IR, total cholesterol (TC), triglycerides (TG), triglyceride glucose index (TyG), triglyceride glucose-BMI index (TyG-BMI), and non-HDL cholesterol were significantly higher in T2DM patients compared to healthy controls ($p \leq 0.01$). Conversely, HDL cholesterol levels and insulin sensitivity indicators (HOMA-β and HOMA-%S) were significantly lower in the T2DM group, reflecting the characteristic impairments in glucose homeostasis and lipid metabolism associated with this condition.

Regarding organ function parameters, renal biomarkers (urea, creatinine, uric acid, and albumin) and hepatic enzymes (aspartate aminotransferase and alanine aminotransferase) remained comparable between groups, with no statistically significant differences, suggesting that kidney and liver function were preserved in the study population despite the metabolic abnormalities observed in T2DM subjects.

3.1.2. Assessment of TST activity

Table 2 illustrates the significant differences in serum thiosulfate sulfurtransferase (TST) activity between patients with type 2 diabetes mellitus (T2DM) and healthy controls, with further stratification by body mass index (BMI) categories. Overall, T2DM patients exhibited a marked 46% reduction in TST activity compared to the control group (11.3 ± 3.65 vs. 20.2 ± 3.17 U/ml, $p \leq 0.01$), indicating substantial impairment of this enzyme's function in diabetes.

.***Table 1:*** *The demographic, anthropometric, and clinical features of T2DM patients and control groups*

fundamental features	Controls group Mean± S. E	T2DM group Mean± S. E
Age (year)	49.95 ± 11.8	53.72 ±12.9
Sex (F / M)	83/67	85/65
blood pressure (mmHg)	126.84±13.15/ 75.42 ± 7.66	141.26±12.1 /86.29± 9.93*
Waist Circumference (cm)	96.3±10.7	118.2±13.8*
BMI (Kg/m^2)	28.42±6.25	34.14±5.62*
Hip Circumference (cm)	100.6 ± 8.4	115.3± 11.1*
Smoking/ non-Smoking	80/70	82/68
TC (mmol/L)	1.30 ± 0.06	2.9 ± 0.08 *
TG (mmol/L)	0.91 ± 0.05	1.45 ± 0.02*
HDL (mmol/L)	7.32 ± 0.27	2.67 ± 0.18*
Non HDL (mmol/L)	0.047 ± 0.004	0.50± 0.011*
Glucose (mmol/L)	5.03 ± 0.11	9.69 ± 0.13 *
Insulin (µU/mL)	7.8 ± 0.2	9.4 ± 0.1 *
TyG Index	3.82 ± 1.1	4.56 ± 1.3*
TyG-BMI	107.16 ± 11.2	154.56± 18.8 *
HOMA-β	122.87 ± 13.2	95.21 ± 9.8*
HOMA-IR	2.12 ± 0.08	3.39 ± 0.61 *
HOMA-%S	0.47± 0.06	0.29 ± 0.09*
Urea (mg/dL)	28.7 ± 5.3	26.8 ± 6.8
Creatinine (mg/dL)	0.68 ± 0.18	0.71 ± 0.19
Albumin(g/L)	40.13 ± 2.11	38.46 ± 2.62
Uricacid(mg/dl)	4.5 ± 1.7	4.8 ± 1.5
AST (U/L)	20.3 ± 6.7	23.4 ± 8.5
ALT (U/L)	22.5 ± 9.3	26.1 ± 10.2

***Significant at the level P≤0.01**

Note: TG: Triglyceride; HDL: High Density Lipoprotein; Non-HDL: non-High Density Lipoprotein; TC: Total cholesterol; TyG Index: Triglyceride Glucose Index; TyG-BMI: triglyceride glucose-body mass index; HOMA-β: Homeostasis Model Assessment of β-Cell Function; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; HOMA-%S: homeostasis model assessment for insulin sensitivity; AST: Aspartate Transaminase; ALT: Alanine Transaminase.

A pronounced BMI-dependent gradient in TST activity was observed in both groups. Among control subjects, TST activity progressively decreased from normal weight (26.1 ± 3.98 U/ml) to overweight (19.5 ± 2.61 U/ml) to obese (14.7 ± 2.37 U/ml) categories. This trend was mirrored but more pronounced in T2DM patients, with respective values of 16.5 ± 3.73, 11.1 ± 2.89, and 6.1 ± 2.48 U/ml across the same BMI categories.

The magnitude of TST activity reduction in T2DM compared to controls intensified with increasing BMI: 36% reduction in normal-weight individuals, 50% reduction in overweight individuals, and 56% reduction in obese individuals (all p ≤ 0.01), as shown in Figure 1. This progressive diminution suggests a synergistic negative effect of diabetes and adiposity on TST enzymatic function, potentially contributing to the metabolic dysregulation characteristic of T2DM, particularly in patients with concurrent obesity.

Table 2: *Thiosulfate sulfurtransferase activity*

TST (U/ml)			
BMI-Kg/m2	**Controls group Mean±S. E**	**T2DM group Mean± S. E**	**%**
Normal weight (18-24.9)	26.1 ±3.98	16 .5± 3.73*	36 -
Overweight (25-29.9)	19.5 ±2.61	11.1± 2.89*	-50
Obese ≥ 30	14.7 ±2.37	6.1± 2.48*	-56
TOTAL	20.2± 3 17	11.3 ± 3.65	-46

***Significant at the level p ≤ 0.01**

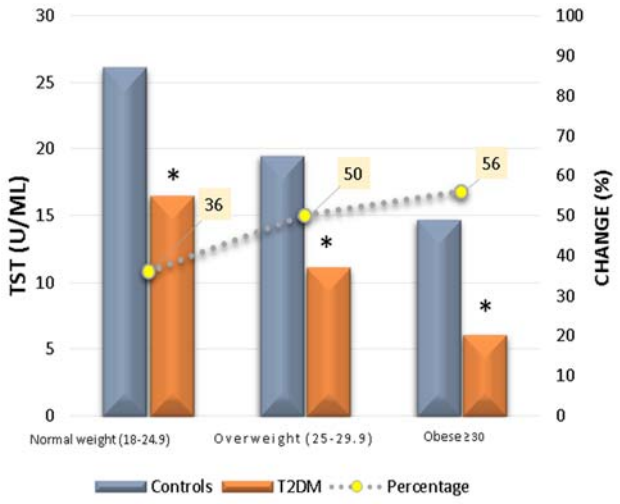


Figure 1: *The % activity of TST*
****Significant at the level p ≤ 0.01***

3.1.3. Assessment of TyG Index

Table 3 presents a comprehensive analysis of Triglyceride Glucose (TyG) Index values stratified by BMI categories, revealing distinct patterns of insulin resistance between patients with type 2 diabetes mellitus (T2DM) and control subjects. Across all BMI categories, T2DM patients consistently demonstrated significantly elevated TyG Index values compared to their BMI-matched counterparts in the control group ($p \leq 0.01$).

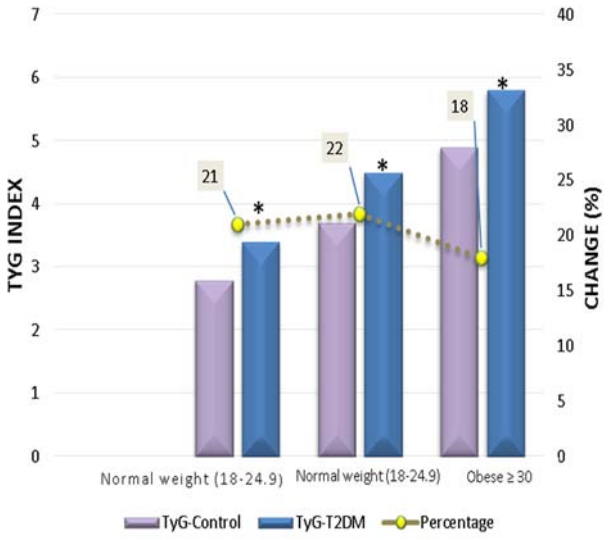
In the normal weight category (BMI 18-24.9 kg/m²), T2DM patients exhibited a TyG Index of 3.4 ± 0.8 compared to 2.8 ± 0.9 in controls, representing a 21% increase. This differential was maintained in the overweight category (BMI 25-29.9 kg/m²), where T2DM patients showed a TyG Index of 4.5 ± 1.4, compared to 3.7 ± 1.2 in controls, corresponding to a 22% increase. Even in the obese category (BMI ≥ 30 kg/m²), T2DM patients had higher TyG values (5.8 ± 1.2 vs. 4.9 ± 1.1), reflecting an 18% increase compared to controls. As shown in Figure 2.

Notably, a consistent upward trend in TyG Index values was observed with increasing BMI in both groups, indicating that adiposity amplifies insulin resistance independently of diabetes status. However, the diabetes-associated increment in TyG Index remained relatively consistent across all BMI categories (18-22%), suggesting that T2DM confers additional insulin resistance beyond that attributable to increased adiposity alone. These findings demonstrate that while obesity contributes substantially to insulin resistance in both diabetic and non-diabetic individuals, T2DM is associated with further metabolic dysregulation as quantified by the TyG Index, a well-established surrogate marker of insulin resistance.

Table 3: *The level of TyG Index*

TyG			
BMI-Kg/m²	**Controls group Mean± S. E**	**T2DM group Mean± S. E**	**%**
Normal weight (18-24.9)	2.8± 0.9	3.4± 0.8	21
Overweight (25-29.9)	3.7± 1.2	4.5± 1.4	22
Obese ≥ 30	4.9± 1.1	5.8± 1.2	18

***Significant at the level p ≤ 0.01**



****Significant at the level p ≤ 0.01***

Figure 2: *The % of TyG Index*

3.1.4. Assessment of TyG-BMI

Analysis of the Triglyceride Glucose-Body Mass Index (TyG-BMI) revealed pronounced metabolic differences between T2DM patients and controls across all BMI categories (Table 4). T2DM patients consistently demonstrated significantly elevated TyG-BMI values compared to BMI-matched controls ($p \leq 0.01$), with the magnitude of this difference amplifying dramatically with increasing adiposity.

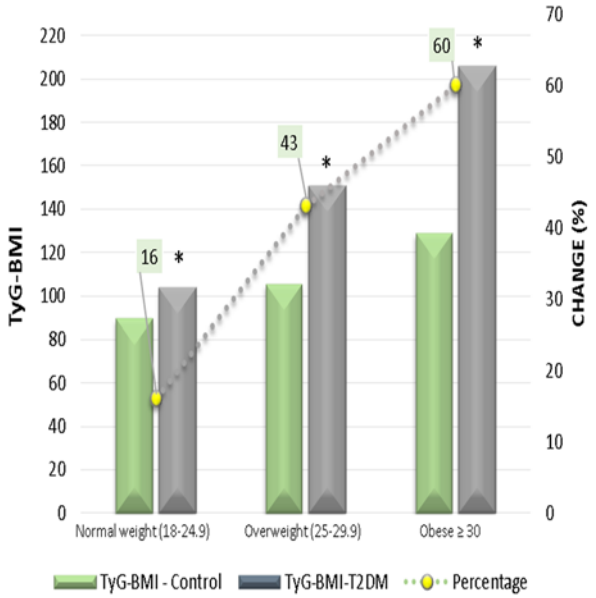
In normal weight individuals (BMI 18-24.9 kg/m²), T2DM patients exhibited moderately elevated TyG-BMI values compared to controls (103.7 ± 13.6 vs. 89.5 ± 9.6), representing a 16% increase. This differential expanded substantially in the overweight category (BMI 25-29.9 kg/m²), where T2DM patients demonstrated TyG-BMI values of 150.9 ± 11.6 compared to 105.6 ± 12.8 in controls, corresponding to a 43% elevation. The disparity reached its maximum in obese subjects (BMI ≥ 30 kg/m²), with T2DM patients showing markedly higher TyG-BMI values (206.1 ± 9.2 vs. 128.9 ± 16.5), reflecting a striking 60% increase over non-diabetic obese controls. As shown in Figure 3.

This progressive pattern of TyG-BMI elevation demonstrates a synergistic interaction between diabetes status and adiposity. While both parameters independently contribute to metabolic dysregulation, their combined effect appears to be multiplicative rather than merely additive, particularly in obese individuals with type 2 diabetes mellitus (T2DM). The amplified TyG-BMI differential in higher BMI categories suggests that insulin resistance mechanisms may be fundamentally altered in the presence of both diabetes and obesity, potentially explaining the accelerated metabolic deterioration frequently observed in obese patients with type 2 diabetes mellitus (T2DM).

Table 4: *Level of TyG-BMI*

TyG-BMI			
BMI-Kg/m²	**Controls group Mean± S. E**	**T2DM group Mean± S. E**	**%**
Normal weight (18-24.9)	89.5± 9.6	103.7± 13.6	16
Overweight (25-29.9)	105.6± 12.8	150.9± 11.6	43
Obese ≥ 30	128.9± 16.5	206.1± 9.2	60

***Significant at the level p ≤ 0.01**



***Significant at the level p ≤ 0.01**

Figure 3: *The % of TyG-BMI*

3.1.5. Correlation BETWEEN TST with TyG Index, TyG-BMI

Table 5 presents the correlation analysis between thiosulfate sulfurtransferase (TST) activity and key metabolic parameters in patients with type 2 diabetes mellitus (T2DM). Statistical evaluation revealed robust negative correlations between TST activity and multiple indicators of metabolic dysfunction.

TST activity demonstrated a strong inverse relationship with triglyceride glucose index (TyG Index) ($r = -0.75$, $p \leq 0.01$), indicating that diminished TST function is significantly associated with increased insulin resistance as measured by this established surrogate marker. An even stronger negative correlation was observed between TST activity and the triglyceride glucose-body mass index (TyG-BMI) ($r = -0.81$, $p \leq 0.01$), highlighting the particularly strong relationship between TST activity and this composite metabolic parameter that incorporates both glycolipid metabolism and adiposity. Additionally, a substantial negative correlation was documented between TST activity and body mass index (BMI) ($r = -0.71$, $p \leq 0.01$), confirming that decreased TST function is significantly associated with increased adiposity in T2DM patients.

The strength and consistency of these negative correlations provide compelling evidence for the integral involvement of TST in the pathophysiological mechanisms underlying insulin

resistance and metabolic dysregulation in type 2 diabetes mellitus (T2DM). The particularly robust correlation with TyG-BMI suggests that TST activity may be simultaneously influenced by, or exert influence upon, both glycolipid metabolism and adiposity-related pathways, positioning this enzyme at a critical intersection of metabolic regulation in diabetes.

Table 5: *Correlation between TST with TyG Index, TyG-BMI, and BMI*

TST	
Variable	**T2DM group (R-value)**
TyG Index	- 0.75*
TyG-BMI	- 0.81*
BMI	-0.71*

Significant at the level p ≤ 0.01

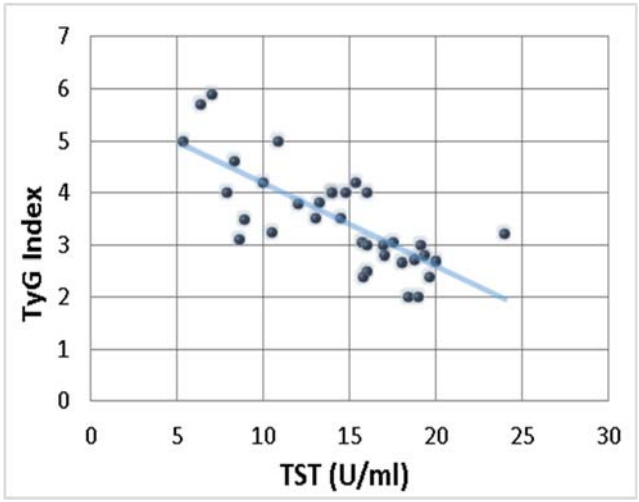


Figure 4: *The Correlation between TST and TyG Index*

Figures 4, 5, and 6 provide visual confirmation of the significant negative correlations between thiosulfate sulfurtransferase (TST) activity and key metabolic parameters in T2DM patients, supporting the statistical findings presented in Table 5.

Figure 5 illustrates the robust inverse relationship between TST activity (U/ml) and TyG-BMI values. The scatter plot shows a clear, negative linear trend, with TST activity decreasing as TyG-BMI increases. Data points are distributed across a range of TST values (approximately 5-25 U/ml) and TyG-BMI values (approximately 90-240), with the regression line indicating a strong negative correlation (r = -0.81, p ≤ 0.01) as previously reported in the statistical analysis.

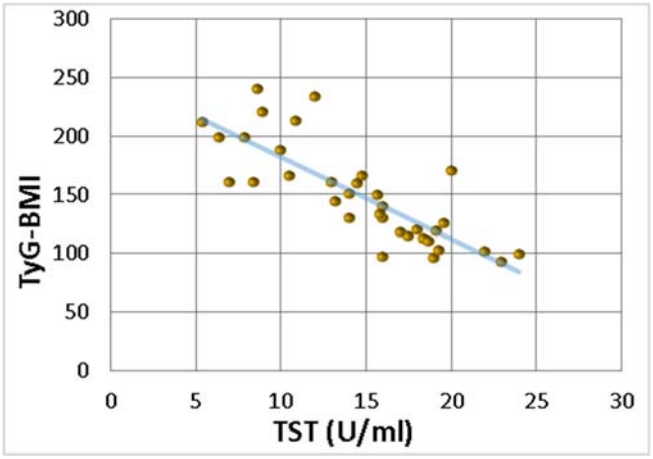


Figure 5: *The Correlation between TST with TyG-BMI*

Similarly, Figure 6 depicts the negative association between TST activity and BMI. Although this correlation appears less steep than that with TyG-BMI, it nonetheless confirms a significant inverse relationship between TST function and adiposity. The scatter plot shows BMI values ranging from approximately 30 to 40 kg/m² plotted against TST activity (5-23 U/ml), with the regression line corroborating the reported correlation coefficient (r = -0.71, p ≤ 0.01).

These graphical representations provide compelling visual evidence that reduced TST activity is consistently associated with increased metabolic dysregulation across multiple parameters in patients with T2DM. The linearity of these relationships suggests a dose-dependent association between declining TST function and worsening metabolic indices, further supporting the potential mechanistic role of TST in the pathophysiology of diabetes. The consistency of these correlations across different metabolic parameters (TyG Index, TyG-BMI, and BMI) underscores the potential significance of TST as an integrative factor in glycemic control, lipid metabolism, and adiposity regulation.

3.2. Discussion

The incidence of diabetes is higher in obese individuals, due to the accumulation of fat in the tissues and its subsequent conversion into

sugar, which leads to high blood pressure (Hussein *et al.*, 2021). Dyslipidemia is attributed to metabolic disorders leading to an increase in the concentration of lipoprotein, TG, with a decrease in HDL, and insulin resistance causes an increase in lipolysis and apo B activity (Mutar *et al.*, 2024).

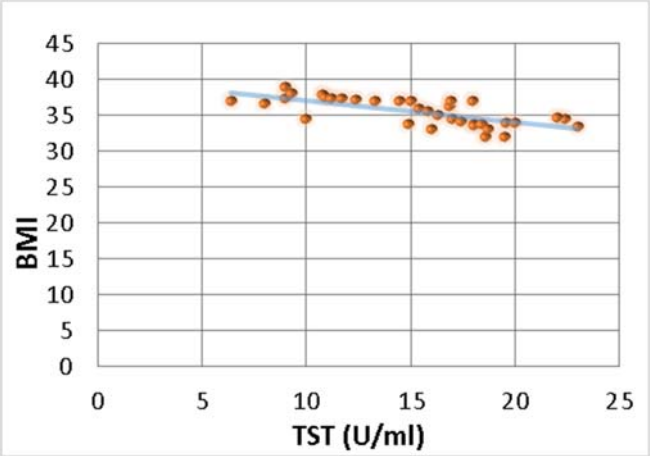


Figure 6: *The Correlation between TST and BMI*

The non-HDL cholesterol index is more important in cardiovascular diseases because it is closely related to atherogenic lipoproteins. Also, in the follow-up of drugs used to reduce blood lipid levels (Allwsh & Mohammad, 2013). The high glucose concentration in the blood results from its secretion from the liver, which leads to an excessive increase in insulin secretion to restore the glucose concentration to the standard limit (Gaggini *et al.*, 2023).

The TYG index is a modern and reliable measure for determining insulin resistance and inflammation (Randrianarisoa *et al.*, 2020). Researchers have recently introduced the (TyG-BMI) index, which integrates glucose metabolism indices, lipid metabolism, and anthropometric factors. They found that TyG-BMI has a significant diagnostic significance in determining insulin resistance (Liu *et al.*, 2021; Sharabi *et al.*, 2015). It was also observed that patients with high fasting glucose and insulin levels had higher HOMA-IR values (Yan *et al.*, 2023) due to increased insulin secretion, which leads to decreased tissue sensitivity to insulin action (White *et al.*, 2021), which may explain the decreased beta function through HOMA-β and HOMA-%S values in the patient group (Berbudi *et al.*, 2020).

The T2D Patients group showed a significant decrease in the activity of (TST), and this result was in agreement with that obtained by (Al-Dahmania *et al.*, 2022). High oxidative stress, characterized by increased reactive oxygen species (ROS) in individuals with diabetes, along with metabolic changes associated with insulin resistance and disturbances in the electron transport chain, can affect mitochondrial function and reduce TST activity (Al-Dahmani *et al.*, 2023). Genetic changes, like those found in the TCF7L2 gene and the rs7903146 variant, can disrupt the normal function of beta cells in the pancreas (del Bosque-Plata *et al.*, 2021). These changes make it more difficult for the cells to respond to incretin hormones, which play a crucial role in stimulating insulin production (Lyssenko & Vaag, 2023). Furthermore, familial dyslipidemia, often genetically inherited in individuals with type 2 diabetes, can worsen lipid metabolism and insulin resistance, and may result in reduced expression of TST mRNA in their fat tissue (Galderisi *et al.*, 2020).

It was also found that TST activity decreased with increasing body mass index, indicating an inverse relationship between enzyme activity and increasing body mass index and this result was in agreement with (Zhang *et al.*, 2024) This is because TST is crucial for maintaining normal lipid metabolism, and its absence leads to a shift toward fat accumulation and impaired oxidative processes, contributing to metabolic disorders such as hypertriglyceridemia and fatty liver. These results demonstrate that infection with diabetes predisposes individuals to lower TST Activity, which may partly explain why the enzyme activity is affected by disease, overweight, and obesity. TST is involved in sulfur metabolism, and disturbances in this pathway, which are prevalent in diabetes, can lead to reduced enzyme expression and functionality (Kruithof *et al.*, 2020). The negative correlation in the TST activity in the group T2DM with (TyG) Index, TyG-BMI, is because TST protects against hyperglycemia-induced damage, which is close to what he showed (Kruithof *et al.*, 2020) that TST had a strong negative correlation with fat mass and plasma glucose levels, while having a positive

correlation with adiponectin expression. The TST is linked to improved mitochondrial function, which facilitates glucose uptake and metabolism in insulin-sensitive tissues. Also, TST enzyme influences insulin sensitivity by enhancing adiponectin secretion, a hormone that promotes insulin sensitivity. This suggests that TST helps to improve insulin signaling and reduce insulin resistance, which is critical in managing glucose homeostasis in diabetic conditions (Buonvino *et al.*, 2022).

4. CONCLUSIONS

This study sheds light on important results linking the activity of the enzyme thiosulfate transferase (TST) in the blood serum to type 2 diabetes (T2DM) through the main axes:

The most significant is the decrease in TST activity in the blood serum of patients with type 2 diabetes compared to healthy individuals. Additionally, individuals with a high body mass index experience a decrease in TST activity compared to those with a normal weight.

The third important axis revealed by the results is the presence of significant and negative correlations between TST activity and insulin resistance indicators (TyG index and TyG-BMI). It is concluded that the TST enzyme plays a crucial role in type 2 diabetes, being linked to it through mechanisms of insulin resistance and obesity. The results also have important clinical implications, as indicated by the inverse correlation between TST activity and insulin resistance indicators, which in turn sheds light on the potential importance of TST as a future therapeutic target for sugar level disorders, especially in patients with type 2 diabetes. Another clinical implication is the inverse association between TST activity and BMI, which opens up new horizons for weight management strategies through TST activity, especially for patients with type 2 diabetes.

Therefore, our research contributes to the pathophysiological understanding of type 2 diabetes by highlighting the important role of TST in metabolic regulation. We hope that future studies will shed light on the molecular and genetic mechanisms that investigate its metabolic and regulatory role, as well as its relationship with insulin resistance in the long term, so that the results will be of greater value and potentially lead to the discovery of potential therapeutic interventions for type 2 diabetes.

The final conclusion of the study reveals the relationship between TST activity and type 2 diabetes indicators identified in this study, which opens up new potential horizons for diagnostic and therapeutic methods in the management of type 2 diabetes, potentially leading to more effective treatment strategies for patients with metabolic disorders.

5. DECLARATIONS

5.1. Study Limitations

Since the study was conducted in a single medical centre, the results may have been influenced by the patients' lifestyle factors. Therefore, we suggest conducting the study in multiple medical centres and with a larger sample size.

5.2. Acknowledgements

We would like to express our gratitude and appreciation to the Al-Wafa Center for Endocrinology and Diabetes in Mosul for their assistance in collecting data and blood samples from patients with type 2 diabetes, as well as to the University of Mosul for their support in completing the study and preparing the necessary laboratories and devices for measurement.

5.3. Funding source

The authors funded this research. In accordance with the ethical guidelines of Periódico Tchê Química, which do not allow donations from authors with manuscripts under review (even when research funds are available), or in cases of financial constraints on the part of the authors, the publication costs were fully covered by the journal under our Platinum Open Access policy, through the support of the Associação Científica Araucária (https://acaria.org/). This policy aims to ensure complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equity in knowledge dissemination.

5.4. Competing Interests

The authors declare that there are no

conflicts of interest related to this publication.

5.5. Open Access

5.6. Use of AI

Sections of this manuscript were initially drafted in Arabic and subsequently translated to English using artificial intelligence language tools, primarily Claude by Anthropic. The translation process involved converting technical terminology, methodological descriptions, and research findings from Arabic to English while maintaining scientific accuracy and precision. Following AI-assisted translation, all content was thoroughly reviewed and edited by the authors to ensure technical correctness, appropriate use of scientific terminology, and clarity of expression. The authors take full responsibility for the accuracy and integrity of all translated content. This AI-assisted translation approach was employed to facilitate international dissemination of research conducted in an Arabic-speaking context while maintaining high standards of scientific communication.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and received formal ethical approval from the Medical Research Ethics Committee of the Iraqi Ministry of Health - Nineveh Health Directorate (Research Protocol No. 2023228, Approval No. 55197, dated December 12, 2023). The study protocol underwent a comprehensive ethical review, which included an assessment of scientific merit, a risk-benefit analysis, participant selection criteria, and data protection measures.

Prior to enrollment, all potential participants were provided with detailed information about the study's purpose, procedures, potential risks and benefits, confidentiality protections, and the voluntary nature of participation in both verbal and written formats. Sufficient time was allowed for questions and consideration before obtaining written informed consent from each participant. For participants with limited literacy, the consent process was conducted in the presence of an impartial witness who attested to the accurate explanation of study information and the participant's apparent understanding and voluntary agreement.

The study design incorporated appropriate measures to protect participant privacy and confidentiality, including the use of coded identifiers rather than personal information in all study documentation and datasets. All investigators and research personnel completed the required training in research ethics and good clinical practice prior to study initiation.

6.2. Informed Consent

A comprehensive, two-tiered informed consent process was implemented for this investigation, in compliance with international ethical guidelines and local regulatory requirements. All participants provided formal written informed consent through institutionally approved documents that clearly delineated two distinct consent components: participation in the research study and permission for the subsequent publication of anonymized research findings.

The consent documents were prepared in both English and Arabic to ensure complete comprehension by all participants, with certified

translations verified for accuracy and cultural appropriateness. Each consent form explicitly detailed: (1) the study's objectives, methodology, and duration; (2) potential risks, benefits, and alternatives; (3) confidentiality protections and data management procedures; (4) the voluntary nature of participation with freedom to withdraw at any time without penalty; (5) specific permission for blood sampling and biomarker analysis; and (6) authorization for publication of aggregated, de-identified results in scientific journals and presentations.

Special attention was given to explaining how participants' data would be anonymized in publications, with assurance that no personally identifiable information would be disclosed. For participants with limited literacy, the consent process was conducted verbally in the presence of an independent witness who confirmed that information was presented comprehensibly and that consent was provided voluntarily. All participants received signed copies of their consent forms, and the original documents are securely maintained in locked cabinets with restricted access at the research institution.

7. AUTHORS' CONTRIBUTIONS

All authors developed and performed the experiments, calculations, and simulations, analyzed the data, and wrote the manuscript. Additionally, all authors contributed to the final version of the manuscript.

7. REFERENCES:

1. Abdallha, M. S., & Allwsh, T. A. (2023). ASPROSIN AND ITS RELATIONSHIP TO INSULIN RESISTANCE IN METABOLIC SYNDROME. *MMSL*, 92(4), 376-384. https://doi.org/10.31482/mmsl.2023.008
2. Al-Dahmani, Z. M., Hadian, M., Ruiz-Moreno, A. J., Maria, S.-G. A., Batista, F. A., Zhang, R., ... & Groves, M. R. (2023). Identification and characterization of a small molecule that activates thiosulfate sulfurtransferase and stimulates mitochondrial respiration. *Protein Science*, 32(11), e4794. https://doi.org/10.1002/pro.4794
3. Al-Dahmani, Z. M., Li, X., Wiggenhauser, L. M., Zhang, R., Ruiz-Moreno, A. J., Maria, S.-G. A., & Batista, F. A. (2022). Thiosulfate sulfurtransferase prevents hyperglycemic damage to the zebrafish pronephros in an experimental model for diabetes. *Scientific Reports*, 12(1), 12077. https://doi.org/10.1038/s41598-022-16320-1
4. Allwsh, T. A., & Mohammad, J. A. (2013). Clinical study of obestatin hormone and its relation to diabetes mellitus. *Rafidain Journal of Science,* 24(4E), 74-87. https://doi.org/10.33899/rjs.2013.77818
5. Alsohaibani, R., Claudel, A.-L., Perchat-Varlet, R., Boutserin, S., Talfournier, F., Boschi-Muller, S., & Selles, B. (2023). Rhodanese-Fold Containing Proteins in Humans: Not Just Key Players in Sulfur Trafficking. Antioxidants, 12(4), 843. https://doi.org/10.3390/antiox12040843
6. Berbudi, A., Rahmadika, N., Tjahjadi, A. I., & Ruslami, R. (2020). Type 2 Diabetes and its Impact on the Immune System. *Current Diabetes Reviews*, 16(5), 442–449. https://doi.org/10.2174/1573399815666191024085838
7. Buonvino, S., Arciero, I., & Melino, S. (2022). Thiosulfate-Cyanide Sulfurtransferase a Mitochondrial Essential Enzyme: From Cell Metabolism to the Biotechnological Applications. *International Journal of Molecular Sciences,* 23(15), 8452. https://doi.org/10.3390/ijms23158452
8. Charlton, A., Garzarella, J., Jandeleit-Dahm, K. A. M., & Jha, J. C. (2020). Oxidative Stress and Inflammation in Renal and Cardiovascular Complications of Diabetes. Biology, *10*(1), 18. https://doi.org/10.3390/biology10010018
9. Chaudhary M, Gupta R. (2012). Cyanide detoxifying enzyme: Rhodanese. Current Biotechnology, 1, 327–335. DOI:10.2174/2211550111201040327
10. Chuang, S. Y., Chang, H. Y., Tsai, T. Y., Cheng, H. M., Pan, W. H., & Chen, C. H. (2021). Isolated systolic hypertension and central blood pressure: Implications from the national nutrition and health survey in Taiwan. *Journal of Clinical Hypertension*, 23(3), 656-664. https://doi.org/10.1111/jch.14105
11. Del Bosque-Plata, L., Martínez-Martínez, E., Espinoza-Camacho, M. Á., & Gragnoli,

C. (2021). The Role of TCF7L2 in Type 2 Diabetes. *Diabetes*, 70(6), 1220–1228. https://doi.org/10.2337/db20-0573
12. Gaggini, M., Michelucci, E., Ndreu, R., Rocchiccioli, S., Chatzianagnostou, K., Berti, S., & Vassalle, C. (2023). Lipidomic Analysis to Assess the Correlation between Ceramides, Stress Hyperglycemia, and HbA1c in Acute Myocardial Infarction. *Molecules (Basel, Switzerland),* 28(2), 716. https://doi.org/10.3390/molecules28020716
13. Galderisi, A., Tricò, D., Pierpont, B., Shabanova, V., Samuels, S., Dalla Man, C., Galuppo, B., Santoro, N., & Caprio, S. (2020). A Reduced Incretin Effect Mediated by the rs7903146 Variant in the TCF7L2 Gene Is an Early Marker of β-Cell Dysfunction in Obese Youth. *Diabetes care*, 43(10), 2553–2563. https://doi.org/10.2337/dc20-0445
14. Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *International journal of molecular sciences*, *21*(17), 6275. https://doi.org/10.3390/ijms21176275
15. Gao, P., Wen, X., Ou, Q., & Zhang, J. (2022). Which one of LDL-C /HDL-C ratio and non-HDL-C can better predict the severity of coronary artery disease in STEMI patients. *BMC cardiovascular disorders*, 22(1), 318. https://doi.org/10.1186/s12872-022-02760-0
16. Hong, S. B., Lee, J. J., Kim, S. H., Park, K. H., & Yoo, K. H. (2016). The effects of adiponectin and inflammatory cytokines on diabetic vascular complications in obese and non-obese patients with type 2 diabetes mellitus. *Diabetes Research and Clinical Practice*, 111, 58-65. https://doi.org/10.1016/j.diabres.2015.10.017
17. Hussein, A. A., Abid, Z. S., & Abid, S. A. (2021). Atherogenic indices in clinical practice and biomedical research: A short review. *Baghdad Journal of Biochemistry and Applied Biological Sciences*, 2(2), 60-70.https://doi.org/10.47419/bjbabs.v2i02.52
18. International Diabetes Federation. (2023). IDF Diabetes Atlas (10th ed.). International Diabetes Federation. https://diabetesatlas.org
19. JalilianKabiri, K., & Baghishani, H. (2015). Evaluation of the activity of cyanide-metabolizing sulfurtransferase enzymes in different tissues of turkey (Meleagris gallopavo). *Iranian Journal of Veterinary Science and Technology*, 7(1), 48-55. https://doi.org/10.22067/veterinary.v7i1.45646
20. Janssen, J. A. M. J. L. (2024). Overnutrition, Hyperinsulinemia and Ectopic Fat: It Is Time for A Paradigm Shift in the Management of Type 2 Diabetes. *International Journal of Molecular Sciences*, 25(10), 5488. https://doi.org/10.3390/ijms25105488
21. Kruithof, P. D., Lunev, S., Aguilar Lozano, S. P., Dolga, A., Groves, M. R., & van Goor, H. (2020). Unraveling the role of thiosulfate sulfurtransferase in metabolic diseases. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1866(6), 165716. https://doi.org/10.1016/j.bbadis.2020.165716
22. Liu, L., Xia, R., Song, X., Zhang, B., & Zhang, Y. (2021). Association between the triglyceride-glucose index and diabetic nephropathy in patients with type 2 diabetes: A cross-sectional study. *Journal of Diabetes Investigation*, 12(4), 557-565. https://doi.org/10.1111/jdi.13371
23. Luo, Y., Chatre, L., Melhem, S., Al-Dahmani, Z. M., Homer, N. Z. M., Miedema, A., ... & van Goor, H. (2023). Thiosulfate sulfurtransferase deficiency promotes oxidative distress and aberrant NRF2 function in the brain. *Redox Biology*, 68, 102965. https://doi.org/10.1016/j.redox.2023.102965
24. Luo, Yu, Luo, Dan, Li, Maojun, Tang, Binzhi . (2024) . Insulin Resistance in Pediatric Obesity: From Mechanisms to Treatment Strategies. *Pediatric Diabetes*, 2298306, 22. https://doi.org/10.1155/2024/2298306
25. Lyssenko, V., & Vaag, A. (2023). Genetics of diabetes-associated microvascular complications. *Diabetologia*, 66(9), 1601–1613. https://doi.org/10.1007/s00125-023-05964-x
26. Morton, N., Beltram, J., Carter, R., Pospisilik, J. A., & Tschöp, M. (2016). Genetic identification of thiosulfate sulfurtransferase as an adipocyte-expressed antidiabetic target in mice selected for leanness. *Nature Medicine*, 22(6), 771–779. https://doi.org/10.1038/nm.4115

27. Mutar, B. A. K., Al-Tu'ma, F. J., & Allwsh, T. A. (2024). Association between serum concentration of HSP-70, C-peptide, and VDBP with the pathogenesis of type 2 diabetes mellitus. *International Journal of Diabetes in Developing Countries*. https://doi.org/10.1007/s13410-024-01375-9
28. Randrianarisoa, E., Lehn-Stefan, A., Hieronimus, A., Artati, A., & Adamski, J. (2020). Reduced insulin clearance is linked to subclinical atherosclerosis in individuals at risk for type 2 diabetes mellitus. *Scientific Reports*, 10(1), 22453. https://doi.org/10.1038/s41598-020-80581-x
29. Saadon, S. R., & Allwsh, T. A. (2024). Lipocalin-2 as a marker of inflammation, bone density, and triglyceride-glucose index for new-onset arthritis patients in Mosul, Iraq. *Qatar Medical Journal*, (3), 23. https://doi.org/10.5339/qmj.2024.23
30. Sánchez-García, A., Rodríguez-Gutiérrez, R., Mancillas-Adame, L., González-Nava, V., Díaz González-Colmenero, A., Solis, R. C., ... & González-González, J. G. (2020). Diagnostic Accuracy of the Triglyceride and Glucose Index for Insulin Resistance: A Systematic Review. *International Journal of Endocrinology*, 4678526. https://doi.org/10.1155/2020/4678526
31. Sharabi, K., Tavares, C. D., Rines, A. K., & Puigserver, P. (2015). Molecular pathophysiology of hepatic glucose production. *Molecular Aspects of Medicine*, 46, 21–33. https://doi.org/10.1016/j.mam.2015.09.003
32. Taki, A.G., Mohammed, S.A. and Allwsh, T.A., 2024. ASPROSIN: RELATION TO OXIDATIVE STRESS FOR METABOLIC SYNDROME PATIENTS IN MOSUL/IRAQ. *Periódico Tchê Química*, 21(48). DOI:10.52571/PTQ.v21.n48.2024_11_TAKI_pgs_132_139.pdf
33. White, P. J., McGarrah, R. W., Herman, M. A., Bain, J. R., Shah, S. H., & Newgard, C. B. (2021). Insulin action, type 2 diabetes, and branched-chain amino acids: A two-way street. *Molecular Metabolism*, 52, 101261. https://doi.org/10.1016/j.molmet.2021.101261
34. Xiao, S., Zhang, Q., Yang, H. Y., Tong, J. Y., & Yang, R. Q. (2024). The association between triglyceride glucose-body mass index and all-cause and cardiovascular mortality in diabetes patients: A retrospective study from NHANES database. *Scientific Reports*, 14(1), 13884. https://doi.org/10.1038/s41598-024-63886-z
35. Yan, S., Wang, D., & Jia, Y. (2023). Comparison of insulin resistance-associated parameters in US adults: A cross-sectional study. Hormones (Athens, Greece), 22(2), 331–341. https://doi.org/10.1007/s42000-023-00448-4
36. Yanai, H., & Yoshida, H. (2019). Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression: Mechanisms and perspectives. *International Journal of Molecular Sciences*, 20(5), 1–25. https://doi.org/10.3390/ijms20051190
37. Yang, S., Shi, X., Liu, W., Wang, Z., Li, R., Xu, X., Wang, C., Li, L., Wang, R., & Xu, T. (2023). Association between triglyceride glucose-body mass index and heart failure in subjects with diabetes mellitus or prediabetes mellitus: a cross-sectional study. *Frontiers in endocrinology*, *14*, 1294909. https://doi.org/10.3389/fendo.2023.1294909
38. Zhang, J., Gong, S., Liu, Y., Zheng, Z., & Feng, G. (2024). Simultaneous detection of peroxynitrite and viscosity in diabetes by a dual response fluorescent probe. *Sensors and Actuators B: Chemical*, 406, 135432. https://doi.org/10.1016/j.snb.2024.135432