

Research Article**Skeletal Muscle–Liver Crosstalk and Its Association With Insulin Resistance and Glycemic Control in Patients With Type 2 Diabetes Mellitus: A Cross-Sectional Study****Nadeem Abbas¹, Asad Mehmood², Khudija Bibi³, Jahanzaib⁴, Baseera Imran⁵, Ambreen Zeeshan⁶**

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insulin resistance, and glycemic control among adults with T2DM.

Abstract**Background:**

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, progressive pancreatic β -cell dysfunction, and impaired regulation of hepatic glucose production. Skeletal muscle and the liver serve as primary organs governing peripheral glucose clearance and endogenous glucose output, respectively. Their physiological interplay—termed skeletal muscle–liver crosstalk—plays a decisive role in systemic metabolic control. However, the exact physiological associations between markers of this inter-organ axis, systemic insulin resistance, and long-term glycemic outcomes remain complex and are modulated by adiposity, disease duration, renal function, and therapeutic regimens.

Objective:

To investigate the association between markers of skeletal muscle–liver crosstalk, indirect indicators of hepatic and peripheral

Methods:

This cross-sectional observational study included 160 adults with established T2DM attending an outpatient clinic at University College of Medicine and Dentistry. Demographic characteristics, diabetes duration, body mass index (BMI), waist circumference, treatment history, fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), fasting insulin, fasting C-peptide, liver enzymes, and renal function were systematically evaluated. The fasting C-peptide-to-insulin molar ratio was determined as a surrogate index reflecting insulin metabolism and hepatic extraction within the muscle–liver axis. Glycemic control was evaluated using HbA1c, while FPG was used as an indicator of fasting hepatic glucose regulation. Associations were examined using Pearson/Spearman correlation coefficients and multivariable linear regression models.

Results:

Among the 160 participants, the mean age was 52.4 ± 10.6 years and the mean diabetes duration was 7.8 ± 5.1 years. Mean HbA1c was $8.1 \pm 1.4\%$, and mean FPG was 164.7 ± 42.6 mg/dL. The fasting C-peptide-to-insulin molar ratio demonstrated a significant inverse correlation with HbA1c ($r = -0.31$, $p < 0.001$) and FPG ($r = -0.28$, $p < 0.001$). Higher HbA1c was independently associated with longer diabetes duration, elevated waist circumference, and lower C-peptide-to-insulin ratios in multivariable linear regression analysis (adjusted $\beta = -0.24$, 95% CI: -0.43 to -0.05 , $p = 0.014$).

Conclusion:

Skeletal muscle–liver crosstalk and insulin clearance kinetics are key physiological determinants of glycemic regulation in patients with T2DM. A lower C-peptide-to-insulin ratio is significantly associated with poorer glycemic control and elevated fasting glycemia. Integrating surrogate markers of the muscle–liver axis into clinical research provides valuable insights into phenotypic variations in glycemic control and insulin resistance.

Keywords: Type 2 diabetes mellitus; Skeletal muscle–liver crosstalk; Insulin resistance; Glycemic control; C-peptide; Fasting plasma glucose; HbA1c; Hepatic glucose production.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease defined by persistent hyperglycemia resulting from a variable combination of systemic insulin resistance, impaired pancreatic β -cell insulin secretion, and unsuppressed hepatic glucose release [1, 2]. Human metabolic homeostasis

relies on tightly orchestrated inter-organ communication, particularly between skeletal muscle and the liver [3]. Skeletal muscle represents the primary site for insulin-stimulated glucose uptake, disposing of approximately 75% to 80% of postprandial glucose loads [3, 4]. Concurrently, the liver serves as the main hub for endogenous glucose output via glycogenolysis and gluconeogenesis, while also acting as the principal site for first-pass insulin clearance [4, 5].

Under physiological conditions, postprandial insulin surges stimulate skeletal muscle uptake via GLUT4 transporter translocation while simultaneously suppressing hepatic glucose production [1, 3]. In states of insulin resistance, this metabolic coordination is severely disrupted. Ectopic lipid accumulation in both skeletal muscle (myosteatosis) and hepatic tissue (hepatic steatosis) triggers cell-autonomous signal transduction blunting via myokines, hepatokines, and circulating non-esterified fatty acids [3, 6]. This pathogenic inter-organ signaling, recognized as skeletal muscle–liver crosstalk, perpetuates both fasting hyperglycemia and postprandial glycemic volatility [3, 7].

In addition to cellular insulin signaling, circulating insulin levels are governed by the balance between pancreatic insulin secretion and metabolic clearance [5]. Pancreatic β -cells secrete insulin into the portal circulation, where the liver extracts between 50% and 70% during its initial passage before the remainder enters systemic circulation to act on peripheral tissues like skeletal muscle [5, 8]. Reductions in hepatic insulin extraction or altered extrahepatic clearance prolong circulating insulin exposure, altering systemic peripheral insulin sensitivity and receptor regulation [5, 6].

Because C-peptide is equimolarly secreted with insulin but escapes substantial first-pass hepatic extraction and exhibits primarily renal clearance, the fasting C-peptide-to-insulin molar ratio serves as an established clinical surrogate for evaluating hepatic insulin extraction and metabolic clearance dynamics within the skeletal muscle–liver pathway [5, 8]. Despite its clinical utility, the exact relationships linking muscle–liver crosstalk markers, estimated insulin clearance, and sustained glycemic control (measured by HbA1c) remain to be fully elucidated across diverse clinical populations with T2DM.

Therefore, this study was designed to investigate the association between markers of skeletal muscle–liver crosstalk, estimated insulin clearance indices, and glycemic control in patients with T2DM while adjusting for pertinent clinical, anthropometric, and biochemical confounders.

2. Rationale of the Study

While insulin resistance and β -cell failure are widely recognized as primary drivers of T2DM progression, the specific contribution of skeletal muscle–liver crosstalk and hepatic insulin clearance to glycemic deterioration is less frequently evaluated in routine clinical settings. Unraveling how parameters of inter-organ insulin dynamics align with clinical markers such as fasting plasma glucose and long-term HbA1c levels is critical for identifying distinct metabolic sub-phenotypes in T2DM. Evaluating fasting insulin, C-peptide, glycemic indicators, hepatic enzymes, and renal markers in a structured observational framework allows for a comprehensive physiological

assessment of the muscle–liver metabolic axis.

3. Objectives

Primary Objective

- To evaluate the association between the fasting C-peptide-to-insulin molar ratio—a surrogate index of skeletal muscle–liver insulin dynamics—and overall glycemic control (HbA1c) in adults with T2DM.

Secondary Objectives

1. To determine the relationship between the C-peptide-to-insulin molar ratio and fasting plasma glucose levels.
2. To examine the correlations between HbA1c, diabetes duration, body mass index (BMI), and waist circumference.
3. To assess whether the C-peptide-to-insulin ratio remains independently associated with HbA1c after adjusting for major clinical and biochemical confounders in multivariable analysis.
4. To evaluate the relationship of liver biochemical markers (ALT, AST) and renal parameters with glycemic variables.

4. Materials and Methods

4.1 Study Design

A hospital-based cross-sectional observational study was conducted to examine the associations between skeletal muscle–liver crosstalk markers, insulin clearance indices, and glycemic control.

4.2 Study Setting

The study was conducted at University College of Medicine and Dentistry.

4.3 Study Duration

The study was conducted over a six-month period, encompassing participant screening, enrollment, standardized clinical evaluations, laboratory testing, data compilation, and statistical analysis.

4.4 Study Population

The target population comprised adult men and women aged 30 years or older with an established clinical diagnosis of T2DM attending outpatient services.

4.5 Sample Size

The sample size consisted of 160 adults with T2DM. The sample size was calculated based on detecting a moderate correlation coefficient ($r = 0.25$ to 0.30) between the fasting C-peptide-to-insulin ratio and HbA1c, maintaining an alpha error level of 0.05 and a statistical power of 80%, while accounting for potential missing data or uncollectible variables.

4.6 Inclusion Criteria

1. Adults aged 30 years or older.
2. Confirmed diagnosis of T2DM based on standard clinical diagnostic criteria.
3. Minimum diabetes duration of 6 months.
4. Capacity to provide written informed consent.
5. Complete medical record and availability of required laboratory parameters.

4.7 Exclusion Criteria

1. Diagnosis of Type 1 diabetes mellitus or secondary forms of diabetes.
2. Pregnancy or active lactation.
3. Acute infectious illnesses, major surgical procedures, or acute metabolic complications (e.g., diabetic ketoacidosis or hyperosmolar hyperglycemic state) within the preceding 3 months.
4. Advanced chronic kidney disease ($\text{eGFR} < 30 \text{ mL/min/1.73 m}^2$) or renal replacement therapy.
5. Decompensated liver disease or active hepatitis.
6. Current systemic corticosteroid therapy or use of medications known to markedly perturb carbohydrate metabolism.

4.8 Data Collection

A standardized structured data collection sheet was utilized to record demographic information, clinical history, disease duration, current treatment regimens, anthropometric indices, and key biochemical variables.

Recorded parameters included:

- Age and biological sex.
- Duration of diabetes (years).
- Current antidiabetic medications (oral agents and insulin therapy).

- Comorbidities including hypertension and dyslipidemia.
- Height, weight, BMI, and waist circumference.
- Systolic and diastolic blood pressure.
- Fasting plasma glucose (FPG) and HbA1c.
- Fasting serum insulin and C-peptide levels.
- Serum creatinine and estimated glomerular filtration rate (eGFR).
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST).
- Serum lipid profile (Triglycerides, HDL cholesterol, LDL cholesterol).
- **HbA1c:** Measured using high-performance liquid chromatography (HPLC) standardized according to the National Glycohemoglobin Standardization Program (NGSP).
- **Fasting Insulin and C-peptide:** Determined using chemiluminescent immunoassay (CLIA) techniques.
- **Renal and Liver Function:** Serum creatinine, ALT, and AST were measured using automated enzymatic and spectrophotometric biochemistry analyzers. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

4.9 Anthropometric Measurements

Weight was measured to the nearest 0.1 kg using a calibrated digital balance, and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest using a non-stretchable measuring tape at the end of normal respiration.

4.10 Laboratory Investigations

Venous blood samples were collected after an 8- to 12-hour overnight fast following standard phlebotomy protocols.

- **Fasting Plasma Glucose:** Quantified via the hexokinase method.

4.11 Assessment of Muscle–Liver Crosstalk and Insulin Clearance

To evaluate insulin clearance kinetics within the skeletal muscle–liver axis, the fasting C-peptide-to-insulin molar ratio was calculated. Fasting C-peptide values (ng/mL) were converted to pmol/L (multiplied by 331.1), and fasting insulin values ($\mu\text{IU}/\text{mL}$) were converted to pmol/L (multiplied by 6.945). The ratio was calculated as:

$$\text{C-peptide-to-insulin ratio} = \frac{\text{Fasting C-peptide (pmol/L)}}{\text{Fasting Insulin (pmol/L)}}$$

A higher molar ratio reflects greater first-pass hepatic insulin extraction and relative insulin clearance, whereas a lower molar ratio signifies impaired hepatic clearance and altered peripheral insulin availability.

4.12 Statistical Analysis

Statistical analysis was conducted using SPSS version 26.0. Continuous variables were assessed for normality using the

Kolmogorov-Smirnov test and expressed as mean $\pm$ standard deviation (SD). Categorical variables were presented as absolute counts and percentages (n, %). Pearson correlation coefficients (r) were calculated to examine linear relationships between continuous metabolic parameters.

Multivariable linear regression analysis was performed with HbA1c as the continuous dependent variable to evaluate the independent predictive capacity of the C-peptide-to-insulin molar ratio while controlling for potential confounders including age, sex, diabetes duration, waist circumference, and renal function (eGFR).

Statistical significance was set at a two-sided p-value < 0.05 .

5. Results

5.1 Demographic and Clinical Characteristics

The study included 160 participants (88 females, 55.0%; 72 males, 45.0%). The mean age was 52.4 ± 10.6 years, with a mean diabetes duration of 7.8 ± 5.1 years. Hypertension was present in 56.9% (n = 91) and dyslipidemia in 64.4% (n = 103) of the cohort. Current insulin therapy was utilized by 20.0% (n = 32) of patients.

Variable	Result
Age, years	52.4 ± 10.6
Female sex, n (%)	88 (55.0)
Male sex, n (%)	72 (45.0)
Diabetes duration, years	7.8 ± 5.1
BMI, kg/m ²	29.1 ± 4.8
Waist circumference, cm	99.4 ± 11.2
Systolic blood pressure, mmHg	132.6 ± 15.4
Diastolic blood pressure, mmHg	82.1 ± 9.6
Hypertension, n (%)	91 (56.9)

Variable	Result
Dyslipidemia, n (%)	103 (64.4)
Current insulin treatment, n (%)	32 (20.0)

Table 1: Demographic and clinical characteristics of participants (n = 160).

5.2 Biochemical Profile and Glycemic Parameters

The mean HbA1c was $8.1 \pm 1.4\%$, and mean FPG was 164.7 ± 42.6 mg/dL. Fasting insulin averaged 14.8 ± 8.2 μ IU/mL, while fasting C-peptide averaged 2.9 ± 1.4 ng/mL.

Parameter	Result
Fasting plasma glucose, mg/dL	164.7 ± 42.6
HbA1c, %	8.1 ± 1.4
Fasting insulin, μ IU/mL	14.8 ± 8.2
Fasting C-peptide, ng/mL	2.9 ± 1.4
Serum creatinine, mg/dL	0.92 ± 0.24
ALT, U/L	31.6 ± 17.2
AST, U/L	27.8 ± 12.9
Triglycerides, mg/dL	183.5 ± 78.6

Parameter	Result
HDL cholesterol, mg/dL	42.7 ± 10.1

Table 2: Biochemical characteristics of the study cohort.

5.3 Relationship Between C-Peptide-to-Insulin Ratio and Metabolic Parameters

Correlation analysis demonstrated a significant inverse correlation between the fasting C-peptide-to-insulin molar ratio and HbA1c ($r = -0.31$, $p < 0.001$) as well as FPG ($r = -0.28$, $p < 0.001$). Inverse correlations were also observed with waist circumference ($r = -0.21$, $p = 0.008$) and ALT levels ($r = -0.16$, $p = 0.043$).

Variable	Correlation Coefficient (r)	p-value
HbA1c	-0.31	<0.001
Fasting plasma glucose	-0.28	<0.001
Diabetes duration	-0.19	0.016
BMI	-0.14	0.078
Waist circumference	-0.21	0.008
ALT	-0.16	0.043

Table 3: Correlations between the fasting C-peptide-to-insulin molar ratio and clinical variables.

5.4 Multivariable Linear Regression for Factors Associated with HbA1c

In multivariable linear regression analysis, the C-peptide-to-insulin molar ratio remained an independent inverse predictor of HbA1c (adjusted $\beta = -0.24$, 95% CI: -0.43 to -0.05 , $p = 0.014$). Diabetes duration (adjusted $\beta = 0.19$, $p = 0.001$) and waist circumference (adjusted $\beta = 0.02$, $p = 0.041$) were significantly associated with higher HbA1c.

Predictor	Adjusted β	95% CI	p-value
C-peptide-to-insulin ratio	-0.24	-0.43 to -0.05	0.014
Diabetes duration, years	0.19	0.08 to 0.30	0.001
Waist circumference, cm	0.02	0.00 to 0.04	0.041
Age, years	-0.01	-0.03 to 0.01	0.280
eGFR, mL/min/1.73 m ²	-0.01	-0.02 to 0.00	0.063

Table 4: Multivariable linear regression analysis with HbA1c as the dependent variable.

5.5 Glycemic Stratification by C-Peptide-to-Insulin Ratio Tertiles

Participants were categorized into tertiles based on their fasting C-peptide-to-insulin molar ratio. Participants in the lowest ratio tertile exhibited the highest HbA1c ($8.6 \pm 1.4\%$) and highest FPG (178 ± 44 mg/dL), whereas those in the upper tertile demonstrated better glycemic metrics (HbA1c $7.7 \pm 1.3\%$, FPG 153 ± 40 mg/dL).

Variable	Lower Tertile	Middle Tertile	Upper Tertile
Participants, n	53	54	53
HbA1c, %	8.6 ± 1.4	8.0 ± 1.3	7.7 ± 1.3

Variable	Lower Tertile	Middle Tertile	Upper Tertile
Fasting glucose, mg/dL	178 ± 44	163 ± 40	153 ± 40
Diabetes duration, years	8.4 ± 5.2	7.8 ± 5.0	7.1 ± 5.1

Table 5: Glycemic characteristics stratified by C-peptide-to-insulin ratio tertiles.

6. Discussion

This study evaluated the interplay between skeletal muscle–liver insulin dynamics, hepatic clearance surrogates, and glycemic outcomes in patients with T2DM. The findings demonstrate that a decreased fasting C-peptide-to-insulin molar ratio is independently associated with poorer glycemic control (higher HbA1c) and elevated fasting plasma glucose levels. These results highlight the importance of hepatic insulin handling and inter-organ crosstalk in modulating long-term metabolic health [3, 5].

6.1 Insulin Clearance and Skeletal Muscle–Liver Crosstalk

The liver clearance of endogenous insulin during portal passage represents a primary regulatory step shaping peripheral insulin availability [5, 8]. Skeletal muscle, as the principal site of insulin-stimulated glucose uptake, is directly influenced by systemic insulin dynamics [3, 4]. When hepatic insulin clearance declines—often secondary to hepatic steatosis or intracellular lipid accumulation—peripheral hyperinsulinemia ensues [5, 6]. Prolonged peripheral hyperinsulinemia downregulates muscle insulin receptor expression and impairs post-receptor signaling pathways, exacerbating

insulin resistance within skeletal muscle tissue [3, 7].

The inverse correlation observed between the C-peptide-to-insulin ratio and HbA1c indicates that altered insulin extraction is closely linked to overall glycemic dysregulation. Reduced clearance may reflect an adaptive response to β -cell exhaustion or, conversely, a primary driver of systemic receptor desensitization [5, 8].

6.2 Hepatic Glucose Production and Glycemic Control

The liver controls fasting glucose levels through glycogenolysis and gluconeogenesis [2, 4]. In healthy physiology, portal insulin suppresses hepatic glucose release [1, 2]. However, in T2DM, selective hepatic insulin resistance blocks insulin’s inhibitory action on gluconeogenesis while promoting lipogenesis [2, 7]. The elevated FPG observed among participants in the lower C-peptide-to-insulin ratio tertile reinforces the physiological coupling between impaired hepatic insulin handling and unsuppressed hepatic glucose production [2, 5].

6.3 Influence of Adiposity and Diabetes Duration

In agreement with established pathophysiological frameworks, central adiposity (waist circumference) and longer disease duration were independent predictors of higher HbA1c levels [1, 3]. Visceral adiposity promotes the flow of free fatty acids directly into the portal vein, driving hepatic lipid deposition and disrupting hepatic insulin clearance mechanisms [3, 6]. Additionally, progressive β -cell failure over time reduces total endogenous insulin output, impairing muscle-mediated glucose disposal and hepatic glucose suppression [1, 8].

6.4 Clinical Implications

Evaluating surrogates of hepatic insulin extraction and muscle–liver crosstalk provides clinical value beyond simple static insulin measurements. Identifying patients with impaired hepatic clearance and profound inter-organ insulin resistance can assist clinicians in tailoring therapeutic strategies. Pharmacological agents that reduce hepatic fat deposition (such as pioglitazone or GLP-1 receptor agonists) or enhance skeletal muscle insulin sensitivity (such as metformin and SGLT2 inhibitors) may be particularly beneficial in mitigating crosstalk disruption [1, 7].

6.5 Limitations

Several study limitations should be noted. First, the cross-sectional design precludes establishing direct causality between altered insulin clearance and glycemic deterioration. Second, while the C-peptide-to-insulin molar ratio is an established surrogate marker, it does not replace tracer-based clamp techniques or kinetic modeling for absolute quantitative measurement of hepatic glucose production or clearance rates [5, 8]. Third, renal clearance dynamics affect circulating C-peptide levels, though patients with

advanced renal dysfunction were excluded to minimize this confounder.

7. Conclusion

Skeletal muscle–liver crosstalk and hepatic insulin clearance kinetics play an essential role in systemic glycemic regulation in T2DM. A reduced fasting C-peptide-to-insulin molar ratio is significantly associated with higher HbA1c and elevated fasting plasma glucose levels, independent of body mass index, diabetes duration, and renal function. Incorporating surrogate markers of inter-organ insulin metabolism into clinical research enhances our understanding of glycemic heterogeneity and supports targeted metabolic management strategies.

8. Recommendations

1. Utilize standardized morning fasting samples for paired insulin, C-peptide, and glucose measurements to ensure consistency in calculating metabolic ratios.
2. Account for renal function (eGFR) and liver enzyme profiles when interpreting systemic insulin and C-peptide levels.
3. Incorporate visceral adiposity metrics (waist circumference) alongside BMI when assessing inter-organ insulin resistance in clinical settings.
4. Conduct prospective longitudinal studies to determine whether baseline changes in the C-peptide-to-insulin ratio predict therapeutic responsiveness or glycemic progression over time.
5. Consider tracer-based kinetic studies in specialized clinical settings to further quantify the direct contributions of skeletal muscle glucose uptake and hepatic glucose

production across different T2DM phenotypes.

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9. References

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