Research Article

# Alterations and Correlation of Metabolic Hormones (Irisin, Amylin, SIRT1), In lammatory Marker, and Vitamins D3 and B3 in Type 2 Diabetes Patients with or without Diabetic Nephropathy

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**Article info:**
**Received:** 5 May 2026
**Revised:** 12 June 2026
**Accepted:** 20 June 2026

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## ABSTRACT

**Objectives:** Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in patients with type 2 diabetes mellitus (T2DM), involving complex metabolic, inflammatory, and hormonal dysregulation. This study investigated the circulating levels and interrelationships between Irisin, Amylin, SIRT1, vitamin D3 (Vit-D3), vitamin B3 (Vit-B3), and serum amyloid A (SAA) among healthy controls, patients withT2DM, and DN patients.

**Methods:** In this case-control study, 150 male participants (50 per group; aged 40–65 years) were enrolled and matched for age and BMI. Fasting samples were used to measure routine biochemical parameters, HOMA-IR, HbA1c, and specific markers (Irisin, Amylin, SIRT1, SAA, Vit-B3 via ELISA; Vit-D3 via CLIA).

**Results:** Both diabetic groups had elevated FBS, HbA1c, insulin, HOMA-IR, and BMI versus controls. DN group exhibited marked renal impairment (higher urea, creatinine, microalbumin; lower eGFR). Vit-D3 and Vit-B3 were significantly lower in patients with T2DM and DN, with a further reduction in Vit-B3 in DN. Serum amyloid A levels were unexpectedly lower in diabetic groups. The order of amylin levels was: control > T2DM > DN, while Irisin and SIRT1 were in the opposite order. The association between Irisin and SIRT1 was weak in DN group. Amylin-SIRT1 was negative in T2DM and positive in DN groups. Vit-B3 correlated positively with SIRT1 only in DN group. Serum amyloid A was negatively correlated with SIRT1 in diabetic groups. This parameter correlated with HbA1c in DN group.

**Conclusion:** These findings highlight potential compensatory roles of Irisin-SIRT1, niacin-NAD+ dependency, and atypical serum amyloid A regulation, suggesting novel biomarkers and therapeutic avenues (e.g., NAD+ precursors, SIRT1 modulation) for diabetic kidney disease.

**Keywords:** Amylin, Diabetic Nephropathy, Irisin, SIRT1, Type 2 diabetes, Vitamins

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**Citation:** Sabih Wajdi S, Mohyadini M, Abdlwhab Hasanain M, Jassim W, Bathaie S. Alterations and Correlation of Metabolic Hormones (Irisin, Amylin, SIRT1), Inflammatory Marker, and Vitamins D3 and B3 in Type 2 Diabetes patients with or without Diabetic Nephropathy. Acta Biochimica Iranica. 2026;4(2):71-79.

https://doi.org/10.18502/abi.v4i2.23035

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## Introduction

Type 2 diabetes mellitus (T2DM) represents a global pandemic and a major public health challenge, with its prevalence projected to rise persistently (1). It is a leading cause of morbidity and mortality, primarily due to the development of debilitating microvascular and macrovascular complications (2). Among these, diabetic nephropathy (DN) stands as the foremost cause of end-stage renal disease (ESRD) worldwide, affecting approximately 30-40% of diabetic patients. The progression of DN is characterized by a complex interplay of metabolic derangements, hemodynamic changes, chronic low-grade inflammation, and fibrotic pathways, ultimately leading to a decline in glomerular filtration rate (GFR) and albuminuria. Despite advances in management, DN continues to impose a significant clinical and economic burden, underscoring the urgent need for a deeper understanding of its pathophysiology and the identification of novel biomarkers and therapeutic targets (3).

Inflammation is a recognized cornerstone in the pathogenesis of T2DM and its complications (4). Serum Amyloid A (SAA) is a major acute-phase reactant primarily produced by the liver in response to inflammatory cytokines like interleukin-6 (IL-6) and IL-1β (5). While traditionally associated with acute inflammation, chronically elevated SAA levels are observed in obesity, metabolic syndrome, and T2DM, where it may contribute to endothelial dysfunction and insulin resistance. In the context of DN, SAA has been localized within renal tubules and is postulated to promote pro-inflammatory and pro-fibrotic responses, potentially accelerating renal injury (6). However, the dynamic relationship between SAA, metabolic hormones, and vitamins across the spectrum of diabetes to overt nephropathy is not well-defined.

Beyond classical hormones, emerging metabolic regulators play crucial roles in energy homeostasis and may be implicated in diabetic complications. Amylin (or islet amyloid polypeptide) is a peptide hormone co-secreted with insulin from pancreatic β-cells. It contributes to glycemic control by suppressing glucagon secretion, delaying gastric emptying, and promoting satiety (7). In T2DM, amylin aggregation causes deficiency, and amyloid deposition may contribute to β-cell dysfunction. The exact role of Amylin in diabetes is still under question (8).

Irisin is a myokine cleaved from fibronectin type III domain-containing protein 5 (FNDC5) in response to exercise. It mediates some exercise benefits by promoting the browning of white adipose tissue, enhancing energy expenditure, and improving insulin sensitivity (9).

Sirtuin 1 (SIRT1), a NAD+-dependent deacetylase, is a master metabolic sensor that regulates glucose and lipid metabolism, mitochondrial function, oxidative stress response, and inflammation by deacetylating key transcription factors and co-factors (10).

Vitamins are essential cofactors in metabolic processes, and their status is often compromised in chronic diseases. Vitamin D3 (cholecalciferol) exerts pleiotropic effects, including modulation of immune function, suppression of the renin-angiotensin system, and potential protective roles against insulin resistance and endothelial dysfunction. Vitamin D deficiency is highly prevalent in diabetes and is associated with an increased risk of albuminuria and faster decline in renal function (11). Vitamin B3 (niacin) is a precursor for nicotinamide adenine dinucleotide (NAD+), a critical coenzyme for redox reactions and the substrate for SIRT1. Niacin deficiency or altered $NAD^+$ metabolism can impair sirtuin activity, potentially disrupting cellular stress resistance and metabolic adaptation, which may be particularly relevant in the energy-depleted environment of the diabetic kidney (12) .

All the above-mentioned molecules are interconnected in metabolic regulation, and their dysregulation is increasingly observed in insulin-resistant states. However, their precise roles in the progression to DN remain unclear. This study aims to investigate the circulating levels and interrelationships among key metabolic hormones (Irisin and Amylin), SIRT1, vitamins (D3 and B3), and the inflammatory marker SAA across three distinct groups: healthy controls, T2DM patients with and without nephropathy.

## Materials and Methods

### Study Design and Participants

This case-control study included three groups of 50 participants each: healthy controls, patients with T2DM, and patients DN. Groups were matched for age and body mass index (BMI). Participants were recruited from the Al Sadiq Pathology Laboratory in Balad District, Salah Aldeen Governorate, Iraq. Routine blood, serum, and urine analyses were performed at this laboratory, while selected biomarker measurements by enzyme-linked immunosorbent assay (ELISA) were conducted at the International Research Centre (Dr. Ali Abdul Hussein) in Baghdad, Iraq. T2DM was diagnosed according to the American Diabetes Association criteria. DN was diagnosed clinically based on the physician's assessment.

### Inclusion and Exclusion Criteria

Inclusion criteria were: male participants aged 40–65 years; T2DM patients receiving treatment with metformin and/or dipeptidyl peptidase-4 (DPP-4) inhibitors; and normal liver function, cardiac function, protein status, and hematological profiles in all participants. The DN group included T2DM patients with stage 2 or 3 chronic kidney disease (CKD), confirmed by eGFR, serum creatinine, and microalbuminuria. Exclusion criteria included: type 1 diabetes mellitus, malignant neoplasms, age <40 or >65 years, female sex, insulin

therapy, multiple diabetic complications (including cardiovascular disease or coronary artery disease), and CKD stages 1, 4, or 5.

### Ethical Considerations

The study adhered to the principles of the Declaration of Helsinki. The protocol was approved by the Ethical Committee of Tarbiat Modares University (approval code: IR.MODARES.REC.1403.098). Written informed consent was obtained from all participants before enrollment.

### Sample Collection and Biochemical Analyses

Venous blood samples were collected after an overnight fast of 10–12 hours. Serum and plasma were separated by centrifugation and processed or stored at appropriate conditions until analysis. Routine biochemical parameters were measured using enzymatic methods on an automated spectrophotometer (BioSystems S.A., Barcelona, Spain): fasting blood glucose (glucose oxidase/peroxidase method), total cholesterol (cholesterol oxidase/peroxidase method), triglycerides (glycerol-3-phosphate oxidase/peroxidase method), high-density lipoprotein cholesterol (HDL-C; phosphotungstate/magnesium method), urea (urease/salicylate method), and creatinine (Jaffé method). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: LDL-C = total cholesterol − (triglycerides/5) − HDL-C. Plasma insulin level was quantified by chemiluminescence immunoassay (BioSystems S.A., Barcelona, Spain). Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR): HOMA-IR = [fasting insulin (μU/mL) × fasting glucose (mg/dL)] / 405.

Glycated hemoglobin (HbA1c) level was measured using the CLOVER A1c Plus Analyzer (OSANG Healthcare Co., Ltd., South Korea). Microalbuminuria was assessed in first-morning urine samples using a commercial immunoturbidimetric kit (Boditech Med Inc., South Korea) per the manufacturer's instructions. Serum levels of amylin, irisin, and SIRT1 were determined by ELISA using kits from Elabscience Biotechnology Inc. (Houston, TX, USA), according to the manufacturer's protocols. Serum vitamin B3 (niacin) and serum amyloid A (SAA) were measured by ELISA using kits from ELK Biotechnology Co., Ltd. (Sugar Land, TX, USA). Serum 25-hydroxyvitamin D (vitamin D3) was quantified using a chemiluminescence immunoassay (CLIA) kit (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation (2021 version):

eGFR = 142 × min (SCR/κ, 1) ^ α × max (SCR/κ, 1) ^ −1.200 × 0.9938^age × (1.012 if female),

where SCR is standardized serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, and α is −0.241 for females and −0.302 for males (all participants were male; results expressed in mL/min/1.73 $m^2$). BMI was calculated as weight (kg) / [height (m)] $^2$.

### Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Continuous variables are presented as mean ± standard deviation (SD) for normally distributed data or as median (25th–75th percentiles) for non-normally distributed data. Normality was assessed with the Kolmogorov–Smirnov test. For comparisons across the three groups: one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for normally distributed variables; the Kruskal–Wallis's test followed by Dunn's post-hoc test was applied for non-normally distributed variables. Pairwise comparisons (when needed) used independent t-tests (parametric) or Mann–Whitney U tests (non-parametric). Correlations between variables were assessed using Pearson's correlation coefficient (normally distributed data) or Spearman's rank correlation coefficient (non-normally distributed data). Results were visualized as heatmaps (one per group). A p-value $< 0.05$ was considered statistically significant.

## Results

The clinical and biochemical characteristics of the study in control, T2DM, and DN subjects are presented in Table 1.

### Metabolic and Glycemic Parameters

In Table 1, FBS, HbA1c, insulin levels, and HOMA-IR were all significantly higher in the T2DM and DN groups compared to the control group ($p < 0.0001$ for all). While FBS and insulin were significantly higher in the T2DM group compared to the DN group ($p < 0.0001$), HbA1c and HOMA-IR did not differ significantly between the two diabetic groups. Additionally, BMI was significantly higher in both the T2DM and DN groups compared to the control group ($p = 0.0026$ and $p = 0.0013$, respectively), with no significant difference between the two diabetic groups.

### Kidney Function Parameters

Table 1 indicates that serum urea, creatinine, and urine microalbumin levels were significantly higher in the DN group than in both the control and T2DM groups ($p < 0.0001$ for all). Conversely, the eGFR was significantly lower in the DN group than in both the control and T2DM groups (both $p < 0.0001$). No significant differences in these kidney parameters were found between the control and T2DM groups.

### Lipid Profile

Total cholesterol and LDL were significantly higher in the T2DM group compared to controls ($p = 0.0108$ and $p < 0.0001$, respectively). In the DN

group, total cholesterol was not significantly different from controls but was significantly lower than in the T2DM group (p = 0.0081). LDL levels in the DN group showed no significant difference from controls but were significantly lower than in the T2DM group (p = 0.0098). Triglycerides were lower in the T2DM group compared to controls (p = 0.0006), while HDL levels remained unchanged across all three groups.

## Vitamins and Specific Markers

The levels of Vit-D3 and Vit-B3 were significantly lower in both the T2DM and DN groups compared to controls (p < 0.0001 for all comparisons). Vitamin B3 was also significantly lower in the DN group compared to the T2DM group (p < 0.0001). The inflammatory marker, SAA, was significantly lower in both diabetic groups compared to controls (p < 0.0001). Amylin levels were significantly lower in both diabetic groups compared to controls (p < 0.0001) and were further reduced in the DN group compared to the T2DM group (p = 0.0022). In contrast, Irisin and SIRT1 levels were significantly and progressively higher from control to T2DM and DN groups (p < 0.0001 for all).

## Correlations in the Control Group

In healthy controls, Spearman's correlation analysis revealed significant associations among the markers (Figure 1A). Notably, Vit-D3 showed a negative correlation with BMI (r = -0.587, p = 0.000008) and a positive correlation with amylin (r = 0.397, p = 0.00431). Irisin exhibited significant negative correlations with HDL (r = -0.378, p = 0.006798), BMI (r = -0.509, p = 0.000161), and a positive correlation with SIRT1 (r = 0.444, p = 0.001237). SIRT1 was positively correlated with Vit B3 (r = 0.282, p = 0.04692) and amylin (r = 0.255, p = 0.073984; approaching significance), but negatively with BMI (r = -0.378, p = 0.006799). SAA displayed a negative correlation with serum urea (r = -0.365, p = 0.009148) and a negative association with FBS (r = -0.305, p = 0.031296). Vit B3 was positively correlated with amylin (r = 0.291, p = 0.040103).

Regarding glycemic parameters, FBS was positively correlated with HOMA-IR (r = 0.395, p = 0.00457) and HbA1c (r = 0.633, p = 0.000001), and negatively with HDL (r = -0.377, p = 0.006959). HbA1c showed positive correlations with insulin (r = 0.318, p = 0.025764) and HOMA-IR (r = 0.452, p = 0.001124). Among the key markers, SIRT1 showed a negative association with insulin (r = -0.330, p = 0.019336).

## Correlations in the T2DM Group

In the T2DM group, correlation patterns differed

**Table1.** comparisons of each parameter in three groups.

| Parameters | Groups (mean ± SD) | | | p-value | | |
|---|---|---|---|---|---|---|
| | Control (n=50) | DM (n=50) | DN (n=50) | (DM vs Control) | (DN vs Control) | (DM vs DN) |
| **FBS (mg/dL)** | 91.9 ± 13.2 | 164 ± 65.5 | 142 ± 48.8 | < 0.0001 | < 0.0001 | < 0.0001 |
| **HbA1c (%)** | 5.38 ± 0.42 | 10.1 ± 1.21 | 8.19 ± 1.61 | < 0.0001 | < 0.0001 | < 0.0001 |
| **Insulin (µIU/mL)** | 10.4 ± 6.3 | 21.2 ± 24.4 | 17.9 ± 11.0 | < 0.0001 | < 0.0001 | < 0.0001 |
| **HOMA-IR** | 2.42 ± 1.52 | 9.37 ± 15.1 | 6.31 ± 4.25 | < 0.0001 | < 0.0001 | < 0.0001 |
| **Urea (mg/dL)** | 30.62 ± 5.53 | 31.7 ± 7.05 | 45.7 ± 15.4 | ns | < 0.0001 | < 0.0001 |
| **Creatinine (mg/dL)** | 0.88 ± 0.09 | 0.91 ± 0.08 | 1.38 ± 0.21 | ns | < 0.0001 | < 0.0001 |
| **eGFR (mL/min/1.73m2)** | 101 ± 6.43 | 100 ± 8.27 | 61.1 ± 10.1 | ns | < 0.0001 | < 0.0001 |
| **Microalbumin (mg/L)** | 9.78 ± 2.42 | 9.50 ± 2.31 | 17.5 ± 12.8 | ns | < 0.0001 | < 0.0001 |
| **Cholesterol (mg/dL)** | 174 ± 27.9 | 197 ± 42.1 | 173 ± 44.7 | 0.0108 | ns | 0.0081 |
| **Triglyceride (mg/dL)** | 94.6 ± 31.5 | 158 ± 45.9 | 135 ± 58.2 | 0.0006 | ns | ns |
| **HDL (mg/dL)** | 43.2 ± 6.62 | 42.6 ± 8.98 | 42.4 ± 8.97 | ns | ns | ns |
| **LDL (mg/dL)** | 87.5 ± 11.8 | 123 ± 41.3 | 102 ± 42.6 | <0.0001 | ns | 0.0098 |
| **Vit-D3 (ng/mL)** | 34.8 ± 5.79 | 23.4 ± 17.43 | 23.1 ± 14.3 | <0.0001 | <0.0001 | ns |
| **Vit-B3** | 53.4 ± 4.63 | 33.9 ± 8.85 | 25.4 ± 4.55 | <0.0001 | <0.0001 | <0.0001 |
| **Amyloid A** | 40.9 ± 8.29 | 11.0 ± 1.89 | 11.5 ± 2.88 | <0.0001 | <0.0001 | ns |
| **Amylin (pg/mL)** | 184 ± 14.2 | 112 ± 17.9 | 100 ± 16.3 | <0.0001 | <0.0001 | 0.0022 |
| **Irisin (pg/mL)** | 66.9 ± 20.9 | 215 ± 113 | 370 ± 102 | <0.0001 | <0.0001 | <0.0001 |
| **SIRT1 (ng/mL)** | 3.32 ± 0.67 | 6.19 ± 2.31 | 8.28 ± 1.86 | <0.0001 | <0.0001 | <0.0001 |
| **BMI (Kg/m2)** | 26.9 ± 2.93 | 29.9 ± 4.60 | 30.1 ± 5.34 | 0.0026 | 0.0013 | ns |

(Figure 1B). Vit-D3 was positively correlated with HDL (r = 0.296, p = 0.036854) and LDL (r = 0.280, p = 0.048935). Irisin showed a negative correlation with Vit B3 (r = -0.333, p = 0.018032) and a positive correlation with SIRT1 (r = 0.356, p = 0.011146). SIRT1 was negatively correlated with SAA (r = -0.359, p = 0.01053) and positively with irisin. SAA exhibited a positive correlation with Vit B3 (r = 0.368, p = 0.008523).

Glycemic markers showed FBS positively correlated with HbA1c (r = 0.729, p < 0.000001) and HOMA-IR (r = 0.444, p = 0.001243). HbA1c was positively associated with HOMA-IR (r = 0.320, p = 0.023453). Among key markers, irisin positively correlated with BMI (r = 0.270, p = 0.057783; approaching significance).

### Correlations in the Diabetic Nephropathy Group

In the DN group, distinct correlations emerged (Figure 1C). Vit-D3 positively correlated with SAA (r = 0.296, p = 0.037159). SIRT1 was positively correlated with amylin (r = 0.342, p = 0.015196) and Vit B3 (r = 0.271, p = 0.057381; approaching significance). SAA negatively correlated with HbA1c (r = -0.526, p = 0.000089) and positively with creatinine (r = 0.291, p = 0.040207). Amylin positively correlated with creatinine (r = 0.277, p = 0.051256; approaching significance).

Glycemic parameters included positive correlations between FBS and HbA1c (r = 0.606, p = 0.000003) and HOMA-IR (r = 0.371, p = 0.008063). Insulin correlated with BMI (r = 0.645, p < 0.000001) and HOMA-IR (r = 0.866, p < 0.000001). SAA was negatively associated with GFR (r = -0.308, p = 0.029769).

Table 2 summarizes significant correlations among the primary markers and with selected metabolic parameters in three groups

## Discussion

This study elucidates distinct alterations in key metabolic hormones, vitamins, and an inflammatory marker in healthy individuals and in patients with T2DM with or without nephropathy. Our findings reveal not only quantitative changes in circulating levels of Irisin, Amylin, SIRT1, Vit-D3, Vit-B3, SAA, and other related

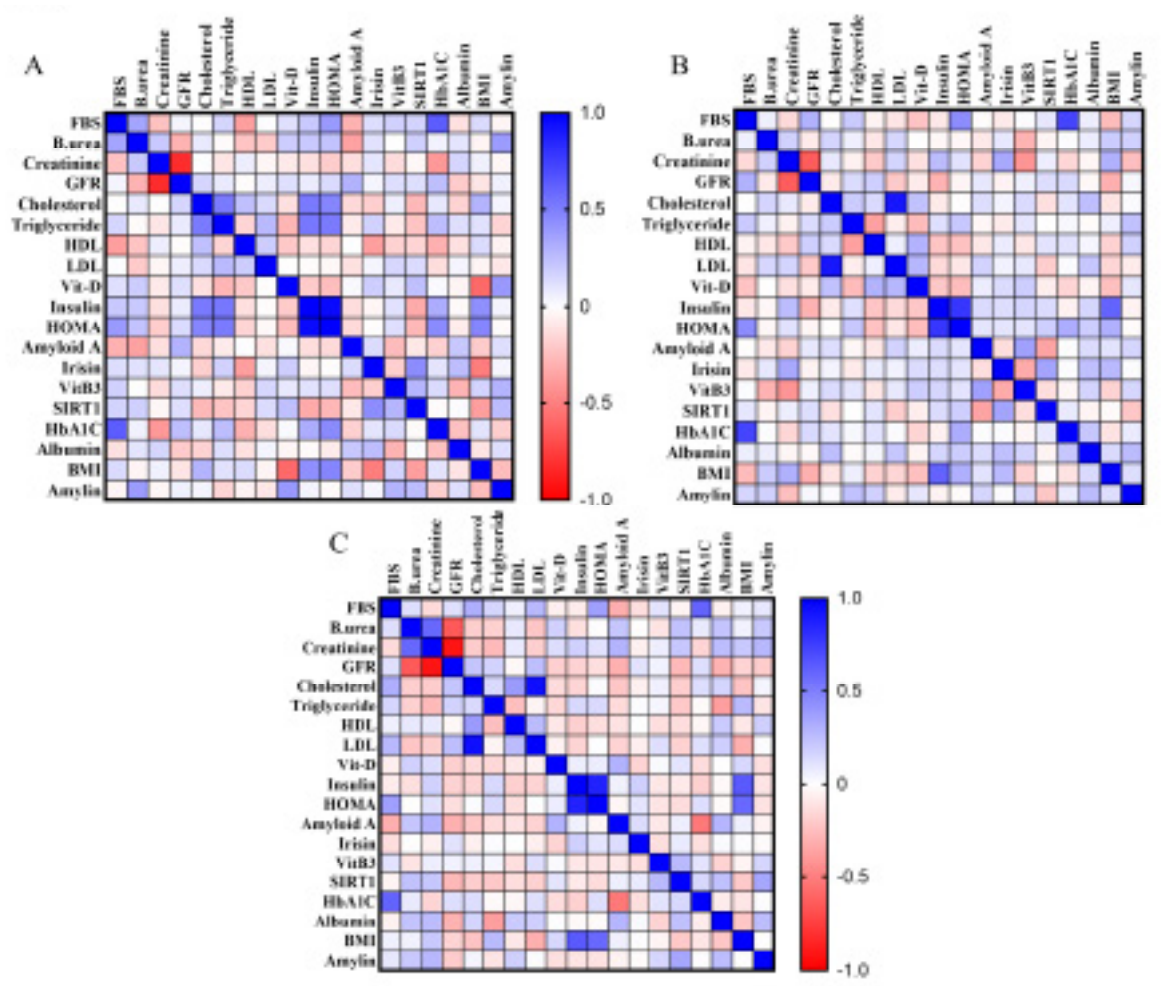



**Figure 1.** Comparative Analysis of Metabolic, Renal, and Inflammatory Biomarkers in control, DM, and DN groups. This figure presents heatmaps depicting correlation matrices for (A) control (n = 50), (B) patients with T2DM (DM group, n = 50), and (C) patients with DN (n = 50), where values represent Spearman's rank correlation coefficients ranging from -1.0 (dark red, negative correlation) to +1.0 (dark blue, positive correlation) with white indicating no correlation (0.0).

metabolic parameters but also a profound reorganization of their correlations in three different groups. These findings suggest a complex interplay between energy metabolism, inflammation, and renal function in the pathogenesis of DN.

This study observed fluctuations in Irisin levels across control, T2DM, and DN groups. Irisin, as a myokine, has been known to promote browning of white adipose tissue and improve glucose homeostasis (13). Also, Irisin showed a protective correlation in the control group (e.g., positive with SIRT1, negative with BMI), consistent with its role in promoting browning of adipose tissue and insulin sensitivity (14). SIRT1, a $NAD^+$-dependent deacetylase, is a key regulator of cellular metabolism, stress resistance, and inflammation (10). Our findings showed SIRT1 positively correlated with Vit B3 and irisin in controls and T2DM, but with amylin in DN. This supports SIRT1's renoprotective effects, including anti-apoptotic and anti-fibrotic actions in diabetic kidneys (15). Downregulation of SIRT1 in DN, as seen in our data through negative associations with SAA, may promote epithelial-mesenchymal transition and fibrosis via p53/NRF2 pathways (16). Studies indicate SIRT1 deficiency exacerbates oxidative stress and inflammation in DN models, and its activation ameliorates podocyte injury (17). One study reported high Irisin levels as a potential risk factor for DN progression in normoalbuminuric T2DM patients (18). Also, in another study, resveratrol treatment has been shown to promote SIRT1 expression in diabetic kidneys, suggesting a potential compensatory role (19). Although their initial increase in T2DM might represent a compensatory mechanism to counteract insulin resistance and metabolic stress (20). Their further rise in DN, coupled with a marked attenuation of their previously strong positive correlation, suggests a potential shift in their biological roles or regulatory circuits in advanced disease. Additionally, this elevation may reflect an adaptive response to hypoxia and oxidative stress in renal tissues, where Irisin could enhance mitochondrial biogenesis via peroxisome proliferator activated receptor gamma coactivator 1 α (PGC-1α) activation, and SIRT1 could deacetylate key transcription factors such as forkhead box O (FOXO) to mitigate cellular damage (21).

In contrast, amylin (islet amyloid polypeptide) levels were lower in T2DM and DN compared to the control group. This aligns with findings of lower plasma amylin in subjects with impaired glucose tolerance and established T2DM (22), particularly in insulin-treated T2DM patients, where levels are reduced compared to non-insulin-treated counterparts (23). Amylin is co-secreted with insulin and contributes to glycemic control by suppressing glucagon secretion and gastric emptying (7). Its reduction aligns with β-cell dysfunction in diabetes. The further significant decrease in DN, alongside the emergence of significant positive correlations with creatinine and negative correlations with eGFR, strongly implicates renal function in amylin clearance. The kidneys are a major site for amylin catabolism (24). Thus, declining eGFR in DN likely contributes to its reduced clearance. Yet the observed lower circulating levels suggest that the rate of production/secretion is further diminished. Moreover, amyloid deposition from amylin aggregates in renal tissues could exacerbate podocyte injury and fibrosis, contributing to a vicious cycle of beta-cell exhaustion

**Table 2.** Summarized significant correlations.

| Control | | | T2DM | | | DN | | |
|---|---|---|---|---|---|---|---|---|
| **Marker Pair** | **r** | **p-value** | **Marker Pair** | **r** | **p-value** | **Marker Pair** | **r** | **p-value** |
| **Vit-D3 - BMI** | -0.587 | 0.000008 | Vit-D3 - HDL | 0.296 | 0.036854 | Vit-D3 - SAA | 0.296 | 0.037159 |
| **Vit-D3 - Amylin** | 0.397 | 0.00431 | Irisin - Vit B3 | -0.333 | 0.018032 | SAA - HbA1c | -0.526 | 0.000089 |
| **Irisin - HDL** | -0.378 | 0.006798 | Irisin - SIRT1 | 0.356 | 0.011146 | SAA - Creatinine | 0.291 | 0.040207 |
| **Irisin - SIRT1** | 0.444 | 0.001237 | SAA - Vit B3 | 0.368 | 0.008523 | SAA - GFR | -0.308 | 0.029769 |
| **Irisin - BMI** | -0.509 | 0.000161 | SIRT1 - SAA | -0.359 | 0.01053 | SIRT1 - Amylin | 0.342 | 0.015196 |
| **SIRT1 - Vit B3** | 0.282 | 0.04692 | FBS - HbA1c | 0.729 | <0.000001 | FBS - HbA1c | 0.606 | 0.000003 |
| **SIRT1 - BMI** | -0.378 | 0.006799 | FBS - HOMA-IR | 0.444 | 0.001243 | FBS - HOMA-IR | 0.371 | 0.008063 |
| **SAA - B.urea** | -0.365 | 0.009148 | HbA1c - HOMA-IR | 0.320 | 0.023453 | Insulin - BMI | 0.645 | <0.000001 |
| **SAA - FBS** | -0.305 | 0.031296 | Creatinine - Vit B3 | -0.420 | 0.00242 | HOMA-IR - BMI | 0.585 | 0.000008 |
| **Vit B3 - Amylin** | 0.291 | 0.040103 | Creatinine - Irisin | 0.334 | 0.017598 | SAA - Albumin | 0.282 | 0.046918 |
| **SIRT1 - Insulin** | -0.330 | 0.019336 | | | | | | |
| **HbA1c - Insulin** | 0.318 | 0.025764 | | | | | | |
| **HbA1c - HOMA-IR** | 0.452 | 0.001124 | | | | | | |

and renal decline (25). This highlights a potentially severe deficiency in amylin activity in DN, which may exacerbate postprandial hyperglycemia (8).

The significant depletion of Vit-D3 in both diabetic groups corroborates extensive literature on Vit-D3 insufficiency in diabetes and its link to insulin resistance and inflammation (26). Similar depletions have been reported in T2DM patients with poor glycemic control and microalbuminuria (11), with lower serum Vit-D3 levels associated with increased risk of diabetic nephropathy and progression in large pooled analyses (27). However, some reports indicated no significant correlation between Vit-D3 and glycemic control or insulin resistance (28). In T2DM, the positive correlation between Vit-D3 and HDL supports Vit-D3's lipid-modulating effects, potentially mitigating dyslipidemia. Meta-analyses confirmed lower Vit-D3 levels in DN patients, associating deficiency with reduced eGFR and increased mortality (29). Our findings extend this by showing that Vit-D3 is associated with other markers, such as SAA, suggesting that it modulates inflammatory pathways in advanced disease. Supplementation trials have demonstrated that Vit-D3 improves SIRT1 and irisin levels in T2DM, reducing HbA1c and enhancing renal protection (30). Thus, Vit-D3 status may serve as a modifiable risk factor for DN progression.

Further reduction of Vit-B3 in DN is also noTable. While direct studies on serum niacin levels are limited, dysregulation in the niacin-derived $NAD^+$ salvage pathway has been observed, with markers like NAMPT increasing and SIRT1 declining across albuminuria stages in T2DM (31). Higher niacin intake is linked to reduced CKD risk in diabetic populations (32). Niacin is a precursor for $NAD^+$, the essential cofactor for SIRT1 activity. The strong positive correlation between Vit-B3 and SIRT1, especially in the DN group, which is absent in controls and T2DM, may indicate a heightened dependency of the compromised SIRT1 signaling pathway on niacin/$NAD^+$ bioavailability in the context of renal disease. This finding suggests a potential mechanistic link between niacin status and the dysregulated cellular energy sensing and stress response in DN. Furthermore, niacin deficiency could impair $NAD^+$-dependent PARP activation, leading to unchecked DNA damage and accelerated tubular atrophy in DN (33).

The behavior of the inflammatory marker SAA offers critical insights. Its significant reduction in diabetic groups versus controls was unexpected, as SAA is an acute-phase reactant typically elevated in inflammation (34). However, it shows strong negative correlations with SIRT1 in both T2DM and DN groups. In contrast, SIRT1 is known for its anti-inflammatory actions, including the deacetylation and inhibition of NF-κB signaling (35). This inverse relationship suggests that higher SIRT1 activity may suppress SAA production. The novel, strong negative correlation between SAA and HbA1c exclusively in the DN group is particularly compelling. It implies that in established nephropathy, poorer long-term glycemic control is associated with lower levels of this inflammatory marker, which may reflect a state of "immune exhaustion" or altered hepatic synthetic function in advanced diabetes complications, moving beyond the classic acute-phase response paradigm.

## Conclusion

In conclusion, the findings suggest that early disruptions in these interconnected pathways contribute to disease progression. Vitamin D, irisin, and SIRT1 appear to play protective roles that weaken as disease progresses, while changes in amylin clearance and SAA dynamics highlight the impact of renal impairment. These results emphasize the complex molecular interplay in diabetic kidney disease and support the need for future longitudinal and interventional studies to explore these markers as potential therapeutic targets or indicators of progression.

## Funding

This project was supported and funded by Tarbiat Modares University (Grant number #29660072).

## Authors contributions

S. S. Wajdi performed the research and analyzed the data. M. Mohyadini analyzed and interpreted the data. M.A. Hasanain, an Endocrinologist, checked for the patients' inclusion and exclusion criteria and sample collection. W.E. Jassim, Advisor, conceived and designed the research. S.Z. Bathaie, Supervisor, conceived and designed the research, administered the projects, and finally edited the manuscript. All authors were involved in drafting and revising the manuscript.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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