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THE ARGINASE-NO AXIS IN TYPE 2 DIABETES AND DIABETIC NEPHROPATHY: A CROSS-SECTIONAL STUDY

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ABSTRACT

The aim of this study was to characterize the arginase-NO axis in type 2 diabetes mellitus (T2DM) with and without nephropathy versus control subjects. We performed a cross-sectional comparative study including 181 individuals, divided into three groups: 60 patients with Diabetic nephropathy (DN), 60 T2DM patients without nephropathy (DM2), and 61 healthy controls. The serum activity of arginase was measured by a colorimetric assay and the levels of nitrites and nitrates by the Griess reaction method. The estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI 2021 equation. In diabetic patients, the level of arginase was higher than in the control group (DN: 9.69 ± 2.16 U/L; DM2: 11.06 ± 2.35 U/L; Control: 7.84 ± 1.07 U/L; $p < 0.001$). Both nitrite and nitrate levels were lower in diabetic patients ($p < 0.001$). The L-arginine metabolic pathway plays an important role in vascular dysfunction in diabetes via competing actions of two enzymes: nitric oxide synthase (NOS) and arginase. DN is one of the main causes of end-stage renal disease around the world. Strong negative correlations of arginase with nitrate ($r = -0.517$, $p < 0.001$) and nitrite ($r = -0.519$, $p < 0.001$) were found in the DN group. All groups exhibited strong positive correlations between nitrite and nitrate ($r = 0.884$ in DN, $p < 0.001$). Serum creatinine levels had a strong negative correlation with arginase activity in the DN group ($r = -0.844$, $p < 0.001$). Conclusions: Elevated arginase is not unique to DN but a feature of diabetes. Inverse correlations of arginase with NO metabolites further validate the hypothesis of arginase being involved in decreasing NO availability. Strong correlation of nitrite with nitrate indicates an internal connectivity of the NO pathway, although it is perturbed by diabetes. Arginase is a potential therapeutic target and NO metabolites are potential biomarkers of endothelial dysfunction in diabetes.

Keywords: Arginase, Nitric Oxide, Diabetic Nephropathy, Type 2 Diabetes, Nitrite, Nitrate, Endothelial Dysfunction.

1 INTRODUCTION

Diabetes mellitus (DM) is one of the most common chronic metabolic diseases in the world, considering life-threatening, disabling, and costly complications which can affect life expectancy (1). Diabetic nephropathy is a progressive kidney disease caused by specific structural and functional changes that occur with long-term high blood sugar levels (2). Pathological characteristics of DN include thickening of the glomerular basement membrane, mesangial expansion, nodular glomerulosclerosis (Kimmelstiel-Wilson lesions), podocyte damage and death, and tubulointerstitial fibrosis (3, 4). L-arginine is a semi-essential amino acid that serves as the sole substrate for two competing enzyme families: nitric oxide synthase (NOS) and arginase. The competition for a common substrate positions L-arginine metabolism as a critical regulatory node in vascular homeostasis and pathophysiology (5). Arginase Upregulation in Diabetes: Arginase (ARG) 1 is a detoxification enzyme in the urea cycle to remove ammonia; upregulation of ARG 1 contributes to vascular dysfunction by reducing l-arginine availability to nitric oxide (NO) synthase (6).

In T2DM, plasma ARG activity was increased by 50% compared to the control group (7). There are multiple factors: hyperglycemia, oxidative stress, and pro-inflammatory cytokines upregulate the ARG (8). Arginase Switch in DN: The role of ARG in DN is believed to be related to its function in the regulation of pancreatic β-cell function leading to reduced NO bioavailability and increased production of downstream metabolites that promote fibrosis (9). The chronic inflammatory state in DN results in upregulation of arginase via pro-inflammatory cytokines such as IL-6 and TNF-α stimulating arginase expression in renal cells (10). Serum arginase-1 activity was measured and compared between diabetic patients with nephropathy, diabetic patients without nephropathy, and healthy controls. Serum levels of stable nitric oxide metabolites (nitrite/nitrate) were quantified and compared among the study groups. The measured biochemical parameters (arginine, NO, cytokines) were correlated with clinical indices of renal function (eGFR and UACR).

2 MATERIALS AND METHODS

2.1 Study Design and Setting

This cross-sectional comparative study was conducted at Al-Sadiq Teaching Hospital, Hilla City, between October 2025 and March 2026. The study protocol was approved by the Research Ethical Committee at the University of Babylon/Hammurabi College of Medicine (Approval Number: 45, Date: 18/12/2025) and Shahid Beheshti University of Medical Sciences (Approval Number: IR.SBMU.MSP.REC.1405.174, Date: 2/6/2026). All participants provided written informed consent.

2.2 Study Population

A total of 181 participants were enrolled and divided into three groups:

- **DN Group (n=60):** Patients with T2DM and diabetic nephropathy, defined by persistent macroalbuminuria (UACR ≥ 300 mg/g) and/or eGFR < 60 mL/min/1.73m^2.
- **DM2 Group (n=60):** Patients with T2DM without nephropathy, defined by UACR < 30 mg/g and eGFR ≥ 60 mL/min/1.73m^2.
- **Control Group (n=61):** Healthy individuals with no history of diabetes, normal fasting glucose, and normal renal function.

Exclusion criteria included: age < 18 years, pregnancy, type 1 diabetes, acute diabetic complications, end-stage renal disease, malignancy, autoimmune disorders, acute infections, and use of immunosuppressive medications or nitrate supplements.

2.3 Sample Collection and Biochemical Analysis

Blood samples were collected after 8-12 hours of overnight fasting. Serum was separated by centrifugation at 3000 rpm for 10 minutes at 4°C and stored at -80°C until analysis.

Arginase Activity Assay: Serum arginase activity was measured using a colorimetric assay kit (Elabscience® E-BC-K848-M). Arginase hydrolyzes L-arginine to L-ornithine and urea; the produced urea reacts with a chromogenic agent forming a colored product measured at 520 nm. Results were expressed in U/L, with one unit defined as the amount of enzyme producing 1 μmol of urea per minute at 37°C.

Nitric Oxide Metabolites: Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were measured using the Griess reaction method after enzymatic reduction of nitrate to nitrite. Nitrite reacts with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride (NED) under acidic conditions to form a stable pink azo chromophore

measured at 540 nm. Nitrate concentration was calculated as (Total nitrite/nitrate) - (Nitrite alone). Results were expressed in µmol/L.

Renal Function Markers: Serum creatinine and urea were measured using the Fujifilm DRI-CHEM 7000i automated analyzer. eGFR was calculated using the CKD-EPI 2021 equation.

2.4 Statistical Analysis

Data were analyzed using JASP (version 0.17.1). Normality was assessed using the Shapiro-Wilk test. Analysis of Covariance (ANCOVA) was performed with age as a covariate to compare means among groups, followed by Tukey's HSD post-hoc test. Pearson correlation coefficients were calculated to examine relationships between variables. A p-value < 0.05 was considered statistically significant.

3 RESULTS

3.1 Baseline Characteristics

The mean ages of participants were: Control 47.43 ± 7.38 years, DM2 55.10 ± 7.87 years, and DN 57.75 ± 11.67 years. Gender distribution was balanced across groups (Control: 30M:31F; DM2: 30M:30F; DN: 32M:28F). Age was included as a covariate in all analyses, as age showed significant correlations with HbA1c in DN ($r = 0.329$, $p = 0.010$), IL-6 in DM2 ($r = 0.235$, $p = 0.040$), and nitrite in controls ($r = -0.239$, $p = 0.043$).

No significant sex differences were observed for any biochemical parameters across all three groups ($p > 0.05$ for all comparisons).

3.2 Arginase Activity

Table 1 summarizes arginase activity across the three groups.

Table 1: Serum Arginase Activity Across Study Groups

Group	N	Mean (U/L)	SD	SE	p-value (ANCOVA)
Control	61	7.84	1.07	0.137	
DM2	60	11.06	2.35	0.304	< 0.001 vs Control
DN	60	9.69	2.16	0.279	< 0.001 vs Control

ANCOVA revealed a significant overall difference in arginase activity among the three groups ($F(2,177) = 35.61$, $p < 0.001$). Post-hoc comparisons demonstrated that both DM2 and DN groups exhibited significantly higher arginase activity compared to healthy controls ($p < 0.001$ for each). Interestingly, DM2 patients showed higher arginase activity than DN patients ($p = 0.003$), suggesting that arginase elevation is primarily a diabetes-associated phenomenon rather than DN-specific.

3.3 Nitric Oxide Metabolites

Table 2: Serum Nitrite and Nitrate Concentrations Across Study Groups

Group	Nitrite (µmol/L)	Nitrate (µmol/L)
Control	19.77 ± 1.20	30.01 ± 1.66
DM2	7.43 ± 1.42	14.52 ± 2.47
DN	6.53 ± 1.90	24.43 ± 4.07

Both nitrite and nitrate levels were significantly lower in diabetic patients compared to controls ($p < 0.001$). A significant difference was also observed between DM2 and DN for both metabolites (nitrite: $p = 0.032$; nitrate: $p < 0.001$). Notably, DN patients demonstrated substantially higher nitrate levels than DM2 patients, suggesting stage-specific differences in NO metabolism.

3.4 Correlation Analysis

Table 3: Correlation Matrix for the Arginase-NO Axis in the DN Group

Parameter 1	Parameter 2	Pearson's r	p-value
Arginase	Nitrate	-0.517	< 0.001
Arginase	Nitrite	-0.519	< 0.001
Nitrite	Nitrate	0.884	< 0.001
Arginase	Creatinine	-0.844	< 0.001
Arginase	Urea	-0.262	0.043

In the DN group, arginase activity showed moderate inverse correlations with both nitrate ($r = -0.517$, $p < 0.001$) and nitrite ($r = -0.519$, $p < 0.001$), supporting arginase-mediated competition for L-arginine. Nitrite and nitrate were strongly positively intercorrelated ($r = 0.884$, $p < 0.001$), consistent with their linked roles as sequential oxidative products in NO metabolism. Arginase demonstrated a strong inverse correlation with creatinine ($r = -0.844$, $p < 0.001$) and a weaker inverse correlation with urea ($r = -0.262$, $p = 0.043$).

Similar inverse correlations between arginase and NO metabolites were observed in DM2 and control groups ($p < 0.001$ for all), confirming that the arginase-NO relationship is a fundamental physiological pathway that becomes pathologically relevant in diabetes.

4 DISCUSSION

Both T2DM and DN show higher arginase activity than controls, while T2DM vs DN appears similar. The result also supports the interpretation that arginase upregulation is a diabetes-associated alteration present in both uncomplicated T2DM and diabetic nephropathy, rather than a marker that cleanly separates DN from T2DM alone. This is consistent with recent findings of increased arginase activity or expression in T2DM, lowered nitric oxide bioavailability, and arginase-associated oxidative and endothelial stress in diabetic tissues (11).

The simplest explanation is that diabetes directly causes the primary change in circulating arginase activity. Competition for L-arginine between arginase and NOS lowers NO production in T2DM, leading to eNOS uncoupling and oxidative stress, a process consistent with the observed consistent control versus diabetes separation in current dataset (12).

The absence of a significant difference between DM2 and DN does not imply that nephropathy lacks arginase involvement. There is an evidence that arginase activation accompanies both T2DM and DN, but serum activity alone may have limited discriminatory value for staging nephropathy (13).

The main signal is a diabetes-associated NO deficit: both nitrite and nitrate are lower in DM2 than in controls, which matches evidence that T2D reduces nitric oxide bioavailability and disrupts both the NOS-dependent and nitrate-nitrite-NO pathways (14). Experimental T2D also shows lower nitrite or nitrate across kidney, aorta, heart, adipose tissue, and other organs, supporting a system-wide reduction in NO metabolites rather than an isolated vascular change (15).

Hyperglycaemia reduces NO via eNOS uncoupling and oxidative stress. NO gets consumed by superoxide to form peroxynitrite, contributing to NO deficiency. Endothelial dysfunction and insulin resistance are also represented by lower nitrite/nitrate in dysglycaemia (16).

The DM2-DN comparison is more a matter of stages than a conflict: DN is lower than controls but higher than DM2, specifically for nitrate. Reviewing DN finds that NO production and its metabolites may increase or decrease with renal stage, compartment, and relative balance between endothelial dysfunction and compensatory intrarenal NOS activity. Early DN can include raised renal eNOS expression and hyperfiltration-related NO signaling, while chronic renal damage shows impaired NOS function and NO depletion (17).

The urea finding is consistent with studies showing higher blood urea in T2DM than in healthy controls. Several papers also interpret rising urea as a marker of kidney impairment or ineffective renal function in diabetes (18).

The much higher DN mean is also plausible because nephropathy is characterized by abnormal renal function with rising urea as kidney disease progresses. Studies comparing nephropathy severity report that worsening renal disease is accompanied by higher filtration markers and lower GFR, which matches the large separation between DM2 and DN in this study (19).

Creatinine is higher in diabetes than in healthy controls across multiple case-control studies. DN patients have higher serum creatinine and lower eGFR than T2DM patients without nephropathy. Longer diabetes duration and albuminuria are associated with elevated creatinine, supporting creatinine as a progression marker (20).

The inverse correlations between arginase and both nitrite and nitrate are biologically consistent with a broad diabetes literature showing that arginase activation is associated with lower NO bioavailability and endothelial dysfunction (21). Diabetes and high glucose repeatedly increase arginase activity while reducing NO production in endothelial and plasma compartments (22). DM2 fits prior reports of elevated plasma arginase and reduced endothelial NO in diabetes (23). This may suggest a pathway with some degree of physiology but one that becomes more pathologically important in diabetes (24). The strong positive correlations between nitrite and nitrate are expected given that nitrite and nitrate are related stable end products of NO metabolism that are frequently assayed in tandem as NOx (25).

This strong correlation of nitrite and nitrate in controls and their still strong correlation in DM2 and DN suggest that while diabetes disturbs the overall activity of the NO pathway, it does not break the internal connection between these two metabolites (26). Overall, the group results support that higher arginase is consistently associated with lower nitrite and nitrate, while nitrite and nitrate remain tightly linked as shared NO oxidation products. In summary, the DN, DM2, and control correlations all support disturbance of the L-arginine–NO pathway, with DN showing the same direction of effect but more renal-pathophysiologic complexity.

Study Limitations

This is a cross-sectional study, which prevents drawing causal conclusions. Only macroalbuminuric DN patients (UACR ≥ 300 mg/g) were studied; inclusion of microalbuminuric patients could yield further information.

5 CONCLUSIONS

Arginase increase is largely a diabetes-induced change observed in both uncomplicated T2DM and diabetic nephropathy. The negative correlations between arginase and nitrite/nitrate imply the role of arginase in decreasing NO bioavailability. The high correlation between nitrite and nitrate seems to suggest that the NO pathway is still internally linked even in the presence of diabetes-associated disturbances. Our results validate the arginase-NO axis as an important axis in diabetic vascular dysfunction and also highlight arginase as a possible target for intervention.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

This study was approved by the Research Ethical Committee at University of Babylon/Hammurabi College of Medicine (Approval Number: 45, Date: 18/12/2025) and Shahid Beheshti University of Medical Sciences (Approval Number: IR.SBMU.MSP.REC.1405.174).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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