Tryptophan Metabolism in Patients With Chronic Kidney Disease Secondary to Type 2 Diabetes: Relationship to Inflammatory Markers

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ABSTRACT

OBJECTIVE: Type 2 diabetes (T2D) is the primary case of chronic kidney disease (CKD). Inflammation is associated with metabolic dysregulation in patients with T2D and CKD. Tryptophan (TRP) metabolism may have relevance to the CKD outcomes and associated symptoms. We investigated the relationships of TRP metabolism with inflammatory markers in patients with T2D and CKD.

METHODS: Data were collected from a well-characterized cohort of type 2 diabetic individuals with all stages of CKD, including patients on hemodialysis. Key TRP metabolites (kynurenine [KYN], kynurenic acid [KYNA], and quinolinic acid [QA]), proinflammatory cytokines (tumor necrosis factor-α [TNF-α] and interleukin-6 [IL-6]), and C-reactive protein were measured in plasma. The KYN/TRP ratio was utilized as a surrogate marker for indoleamine 2,3-dioxygenase 1 (IDO1) enzyme activity.

RESULTS: There was a significant inverse association between circulating TRP level and stages of CKD (P < 0.0001). Downstream bioactive TRP metabolites KYN, KYNA, and QA were positively and robustly correlated with the severity of kidney disease (P < 0.0001). In multiple linear regression, neither TNF-α nor IL-6 was independently related to KYN/TRP ratio after adjusting for estimated glomerular filtration rate (eGFR). Only TNF-α was independently related to KYN after taking into account the effect of eGFR.

CONCLUSIONS: Chronic kidney disease secondary to T2D may be associated with accumulation of toxic TRP metabolites due to both inflammation and impaired kidney function. Future longitudinal studies to determine whether the accumulation of KYN directly contributes to CKD progression and associated symptoms in patients with T2D are warranted.

KEYWORDS: Chronic kidney disease, indoleamine 2,3-dioxygenase 1, inflammatory cytokines, kynurenine, tryptophan, type 2 diabetes

Introduction

Type 2 diabetes (T2D) is the single most important cause of chronic kidney disease (CKD), which often progresses to end-stage kidney disease (ESKD).¹ In patients with T2D, several factors play mechanistic roles in the initiation of CKD and progression to ESKD. Patients with advanced CKD endure myriad symptoms and dismal health outcomes. Inflammation has emerged as a novel risk marker in relation to the chronic worsening of kidney function and clinical complications in CKD.³ Metabolic aberrations, e.g. dysregulation in amino acid metabolism, are characteristics of CKD.³,⁴ Chronic inflammatory response is also associated with significant metabolic activity with consequent nutrient depletion in CKD.⁴

Essential amino acid l-tryptophan (TRP) contributes to the synthesis of nicotinamide adenine dinucleotide, a coenzyme important for energy metabolism in the mammalian tissues (Figure 1).⁵,⁶ The majority (~95%) of free TRP undergoes oxidative metabolism along the kynurenine (KYN) pathway yielding KYN involving 2 key enzymes: tryptophan 2,3-dioxygenase (TDO, highly expressed in the liver) and indoleamine 2,3-dioxygenase 1 (IDO1), which is expressed extrahepatically. Indoleamine 2,3-dioxygenase 1, potently induced by proinflammatory cytokines,⁷,⁹ acts locally to modulate TRP levels in response to inflammation. Kynurenine metabolism eventually generates kynurenic acid (KYNA) and other bioactive metabolites 3-hydroxykynurenine, quinolinic acid (QA), etc. collectively known as kynurenines. The regulation of TRP

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¹Deceased.
metabolism is sensitive to environmental stimuli (e.g. inflammation), and kynurenines are normally eliminated via urinary excretion. Because kynurenines play an important role in regulation of adaptive immunity and are implicated in comorbid atherosclerosis and neuropsychiatric symptoms, patients with CKD are at particularly high risk of KYN-associated pathophysiologies. For example, altered TRP metabolism may precipitate fatigue in patients with CKD. Likewise, TRP metabolites (e.g. KYN and QA) may promote atherosclerosis in ESKD by activating oxidative stress and leukocyte activation in endothelial and vascular smooth muscle cells.

The kidneys play an integral role in the metabolism of TRP. The rate-limiting enzyme IDO1 is overexpressed in kidney tissues. Interestingly, aberrations in TRP metabolism and specific enzyme activities in the KYN pathway could contribute to the pathogenesis of T2D. It should be emphasized that the molecular mechanisms of inflammatory pathways are also different in nondiabetic vs diabetic CKD, the latter group having an accelerated loss of kidney function than the former. Therefore, TRP metabolism may have potentially important clinical implications to CKD secondary to T2D. The association between inflammatory cytokines and TRP metabolism across the stages of CKD in patients with T2D is unknown. We aimed to study the associations between TRP metabolism and stages of CKD in presence of inflammatory markers in patients with T2D.

Materials and Methods

Participants

We analyzed data collected from 60 type 2 diabetic patients who participated in the Family Investigation of Nephropathy in Diabetes (FIND) study. The FIND study design, population, and phenotypic data have been published previously. Briefly, participants had clinical diagnosis of T2D and CKD, including ESKD (Supplemental Table). All participants were receiving standard medical treatment for diabetes, CKD, and ESKD and associated comorbidities as per guidelines. Individuals with chronic inflammatory conditions such as chronic hepatitis and rheumatoid arthritis were excluded. All participants provided written consent to the protocol approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio. Estimation of glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Kidney Disease equation. The KYN/TRP ratio was calculated to estimate IDO1 enzyme activity.

Laboratory measurements and inflammatory markers

Fasting blood was collected from each participant and stored at −80°C until analyzed. Creatinine and albumin were measured in stored serum using standard methods by the centralized laboratory. Albumin and creatinine in urine were quantified in all subjects except subjects with CKD stage 5 on ESKD. Plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured by high-sensitive enzyme-linked immunosorbent assay (Quantikine HS Human Immunoassay; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. The lower detection limits for IL-6 and TNF-α were 0.19 pg/mL and 0.11 pg/mL with intra-assay coefficients of variation (CV) 3.1% to 8.7% and 5.5% to 9.8% and inter-assay CV 7.4% to 10.4% and 5.5% to 11.2%, respectively. C-reactive protein (CRP) levels were measured by rate immunoturbidimetry (Beckman Coulter Inc., Brea, CA, USA) using a high-sensitivity CRP assay (lower detection limit of 0.2 mg/L).

TRP metabolites

Tryptophan and selective metabolites in the KYN pathway were measured in plasma by liquid chromatography/mass spectrometry (LC-MS) as reported previously. Briefly, 50µL plasma was diluted with 5 times of 0.2% acetic acid. Stable isotope–labeled standards, 2-picolinic-d4 acid, 2,3-pyridinedicarboxylic acid-d3, L-TRP-13C11,15N2, and KYN, were added at the time of extraction as internal standards for absolute quantification. The diluted samples were vortexed and transferred to 0.5-mL Millipore Amicon Ultra filter (3 kDa). The filter tubes were centrifuged at 13500g for 60 minutes at 4°C and the extracts were transferred to glass vials for LC-MS analyses. High-performance liquid chromatography/electrospray ionization mass spectrometry (HPLC-ESI-MS) analyses were conducted on a Thermo Fisher Q Exactive mass spectrometer with online separation by a Thermo Fisher/Dionex Ultimate 3000 HPLC. High-performance liquid chromatography conditions were as follows: column, YMC-Pack
CKD. The KYN/TRP ratio was robustly elevated \((P < 0.0001)\) and significantly higher levels of its metabolites (e.g., KYN, KYNA, and QA) showed strong negative correlations with eGFR, mL/min/1.73 m\(^2\)). In contrast, TRP metabolites KYN, KYNA, QA showed strong negative correlations with eGFR, mL/min/1.73 m\(^2\)). Table 1. Characteristics of the study participants \((n=60)\).

| VARIABLE                            | MEAN ± SD         |
|-------------------------------------|-------------------|
| Age, y                              | 57.17 ± 11.84     |
| Sex (female)                        | 33 (55%)          |
| Body mass index, kg/m\(^2\)         | 30.83 ± 7.18      |
| Serum albumin, g/dL                 | 3.61 ± 0.51       |
| eGFR, mL/min/1.73 m\(^2\)           | 51.26 ± 43        |
| CRP, mg/L                           | 0.75 ± 0.86       |
| TNF-\(\alpha\), pg/mL              | 5.71 ± 3.98       |
| IL-6, pg/mL                         | 3.20 ± 2.90       |
| Tryptophan, \(\mu\)M                | 49.17 ± 20.24     |
| Kynurenic acid, \(\mu\)M            | 3.80 ± 2.12       |
| Kynurenic acid, \(\mu\)M            | 12.36 ± 4.25      |
| 3-Hydroxykynurenic acid, \(\mu\)M   | 0.12 ± 0.14       |
| Quinolinic acid, \(\mu\)M           | 3.66 ± 4.10       |

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\).

Data are mean ± standard deviation or number (%).

\(<\)Excludes subjects \((n=21)\) with stage 5 CKD on hemodialysis.

The correlations between proinflammatory cytokines, eGFR, and TRP metabolites are shown in Table 3. The relationship between TRP and eGFR was remarkably robust and positive \((P < 0.0001)\). In contrast, TRP metabolites KYN, KYNA, QA showed strong negative correlations with eGFR \((P < 0.0001)\) for all. Among the measured parameters, TNF-\(\alpha\) had the strongest association with KYN metabolites \((P < 0.0001)\), followed by IL-6, whereas CRP did not show any significant correlation. Figure 2 shows a visual representation of the relationships between TRP metabolites and the stages of CKD modeled by a smooth function. Table 3 also shows significant associations between KYN/TRP ratio with BMI \((r=-.33, P<0.05)\), TNF-\(\alpha\) \((r=.73, P<0.0001)\) and IL-6 \((r=-.35, P<0.01)\), and eGFR \((r=-.93, P<0.0001)\). Using ANCOVA, we further examined the link between KYN/TRP ratio and the stages of CKD. Figure 3 depicts that each time the CKD stage progressed (except from CKD stages 3 to 4), there was a statistically significant increase in the KYN/TRP ratio independent of age, sex, BMI, and CRP.

Multiple linear regression analysis was used to examine the relation of inflammatory markers to KYN/TRP ratio and KYN taking into consideration the effect of important covariates such as age, sex, BMI, and eGFR (Table 4). Tumor necrosis factor-\(\alpha\) and IL-6 were directly associated with KYN/TRP ratio after the adjustment for age, sex, and BMI \((P < 0.001\) even
Table 2. Age- and sex-adjusted body mass index and biochemical parameters by stages of chronic kidney disease.

| VARIABLE | STAGE 1: eGFR ≥90 (N = 12) | STAGE 2: eGFR 60-89 (N = 15) | STAGE 3: eGFR 30-59 (N = 8) | STAGE 4: eGFR 15-29 (N = 4) | STAGE 5 ON ESKD: eGFR <15 (N = 21) |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| BMI, kg/m² | 32.14 (28.57–36.15) | 30.57 (27.72–32.72) | 29.08 (25.85–32.71) | 35.16 (29.46–41.95) | 27.66* (25.57–29.92) |
| Albumin, g/dL | 3.78 (3.43–4.17) | 3.74 (3.46–4.05) | 3.63 (3.23–4.09) | 3.16a (2.75–3.62) | 3.42 (3.23–3.63) |
| CRP, mg/L | 0.43 (0.21–0.87) | 0.30 (0.20–0.58) | 0.26 (0.10–0.87) | 0.29 (0.09–1.00) | 0.68 (0.43–1.06) |
| TNF-α, pg/mL | 3.16 (1.79–5.58) | 2.41 (1.54–3.78) | 3.74 (2.04–6.87) | 6.89 (2.91–16.3) | 7.69* (5.30–11.1) |
| IL-6, pg/mL | 2.39 (1.55–3.67) | 1.65 (1.18–2.30) | 1.03a (0.66–1.62) | 2.48 (1.30–4.74) | 4.01 (3.05–5.28) |
| TRP, µM | 69.4 (59.3–81.2) | 58.6 (51.0–67.2) | 50.4a (42.3–60.1) | 38.9 (30.1–50.1) | 28.2* (25.1–31.7) |
| KYN, µM | 2.08 (1.71–2.52) | 2.53 (2.17–2.96) | 3.32a (2.68–4.12) | 3.16* (2.31–4.32) | 5.58* (4.87–6.41) |
| KYN/TRP ratio | 0.03 (0.20–0.53) | 0.31 (0.21–0.46) | 0.43 (0.25–0.73) | 0.42 (0.20–0.88) | 4.30d (3.09–6.01) |
| 3-HKYN, µM | 1.05 (0.99–1.11) | 1.06 (1.02–1.10) | 1.06 (1.00–1.13) | 1.12 (1.03–1.21) | 1.22* (1.17–1.27) |
| QA, µM | 1.57 (1.24–1.98) | 1.63 (1.37–1.95) | 1.86 (1.47–2.35) | 2.32 (1.66–3.23) | 6.30d (5.38–7.37) |
| KYN/TRP ratio | 0.03 (0.03–0.04) | 0.05b (0.04–0.05) | 0.07d (0.05–0.08) | 0.08d (0.06–0.12) | 0.20d (0.18–0.22) |

Abbreviations: BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; IL-6, interleukin-6; KYN, kynurenine; KNYA, kynurenic acid; QA, quinolinic acid; TNF-α, tumor necrosis factor-α; TRP, tryptophan.

Data are geometric means and 95% confidence interval. Variables (except BMI and albumin) are log transformed.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Table 3. Spearman correlation coefficients of age, body mass index, and renal function with specific inflammatory markers and tryptophan metabolites.

| VARIABLE | AGE | BMI | eGFR | CRP | TNF-α | IL-6 | TRP | KYN | KNYA | QA |
|----------|-----|-----|------|-----|-------|------|-----|-----|------|-----|
| BMI | −0.33b | | | | | | | | | |
| eGFR | −0.2 | 0.35b | | | | | | | | |
| CRP | −0.17 | 0.26 | | | | | | | | |
| TNF-α | 0.08 | −0.17 | −0.72d | 0.06 | | | | | | |
| IL-6 | 0.17 | −0.13 | −0.41b | 0.33a | 0.33a | | | | | |
| TRP | −0.18 | 0.15 | 0.82a | −0.10 | −0.56d | −0.37b | | | | |
| KYN | 0.02 | −0.33a | −0.83d | 0.18 | 0.72d | 0.33a | −0.57d | | | |
| KNYA | 0.04 | −0.02 | −0.64d | 0.23 | 0.42b | 0.33a | 0.32a | 0.59a | | |
| QA | 0.10 | −0.29a | −0.85d | 0.24 | 0.65d | 0.49d | −0.67d | 0.84d | 0.72d | | |
| KYN/TRP ratio | 0.14 | −0.28a | −0.93d | 0.17 | 0.73a | 0.35a | −0.83d | 0.89a | 0.67a | 0.85d |

Abbreviations: BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; KYN, kynurenine; KNYA, kynurenic acid; QA, quinolinic acid; TNF-α, tumor necrosis factor-α; TRP, tryptophan.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
with the Bonferroni correction). Interleukin-6 was independently related to neither KYN/TRP ratio nor KYN after the additional adjustment for eGFR. Tumor necrosis factor-α was not related to KYN/TRP ratio after the additional adjustment for eGFR but was associated with KYN ($P = 0.010$). However, $P$ value was attenuated to marginal significance ($P = 0.060$) after the Bonferroni correction. Age, sex, BMI, and eGFR explained 66.9% and 90.4% of variability of KYN concentration and KYN/TRP ratio, respectively. Adding TNF-α to the model accounted for an additional 3.9% of the KYN variance and 0.0% of the KYN/TRP ratio variance.

The prevalence of albuminuria is highly variable in patients with CKD with T2D but is often considered as an important predictor of CKD progression—The higher the albuminuria, the greater the risk for kidney function.$^{25}$ We analyzed data according to the degree of albuminuria using the standard definition$^{21}$ and CKD stages 1 to 4; however, the number of subjects in each subgroup was extremely small, which precluded the generation of any meaningful result. Therefore, the analysis (age- and sex-adjusted) was limited only to degree of albuminuria, which revealed a significantly higher KYN/TRP ratio in subjects with macroalbuminuria ($−2.75 ± 0.09, P < 0.001$) and microalbuminuria ($−2.95 ± 0.18, P < 0.05$) than in patients with normoalbuminuria ($−3.34 ± 0.08$). The mean ± standard deviation eGFR in macroalbuminuria ($n = 15$), microalbuminuria ($n = 4$), and normoalbuminuria ($n = 21$) groups was $43.4 ± 6.1$.

![Figure 2. Relationship between kidney function and tryptophan metabolites.](image1)

Figure 2. Relationship between kidney function and tryptophan metabolites.$^a$

$^a$Penalized smoothing splines were used to assess the relationship between eGFR to tryptophan metabolites. eGFR indicates estimated glomerular filtration rate.

![Figure 3. Kynurenine/tryptophan ratio by stages of chronic kidney disease.](image2)

Data are geometric mean and 95% confidence interval. Statistical difference between stage 1 and stage 2: $P = 0.03$; stage 2 and stage 3: $P = 0.003$; stage 3 and stage 4: $P = 0.43$; stage 4 and stage 5: $P < 0.0001$. 

Figure 3. Kynurenine/tryptophan ratio by stages of chronic kidney disease.
Discussion

Our study reports the first data on TRP metabolism in relation to inflammatory markers in patients with T2D and varying stages of CKD including ESKD. The results demonstrate a proportional and significant depletion of circulating TRP with the loss of kidney function. Compared with stage 1 CKD, plasma TRP levels were nearly 60% lower at stage 5 CKD. Tryptophan reduction was accompanied with an increase in the levels of KYN, KYNA, and QA—this preliminary observation may indicate the inductions of TRP degrading enzymes. Circulating levels of TRP metabolites positively and robustly correlated not only with the severity of kidney function but also with proinflammatory cytokines TNF-\(\alpha\) and IL-6.

TRP metabolism in patients with moderate to severe CKD,\(^26\) including ESKD undergoing hemodialysis,\(^27,28\) has previously been reported. These studies included CKD patients of heterogeneous etiologies and excluded patients with early stages of CKD. The gradual TRP depletion that was proportional to the advancing CKD stages found in our study is in sharp contrast with Schefold et al.\(^26\) who reported unaltered TRP levels in CKD stages 3 to 5—an unconventional finding. Our data, however, regarding significant correlations of the TRP metabolites and KYN/TRP ratio with severity of kidney function, are consistent with the results by the aforementioned study\(^26\) and a recent study by Zhang et al (see Figure 2).\(^29\)

The overall findings, however, of the latter study\(^29\) that involved type 2 diabetic patients with impaired kidney function are difficult to interpret. Like Schefold et al.,\(^26\) the study did not find any difference in TRP levels between patients with eGFR greater than 60 and 31 to 60 mL/min/1.73 m\(^2\) (or CKD stage 3). It should be noted that eGFR greater than 60 mL/min/1.73 m\(^2\) includes both CKD stages 1 and 2. Importantly, Zhang et al\(^29\) did not measure any inflammatory marker or specify the number of subjects in each group of diabetic patients with eGFR of >60 and <60 ml/min/1.73 m\(^2\). Besides, the analytical method for TRP and KYN quantification and whether levels of TRP and KYN required log transformation remain unknown.

The underlying mechanism of accelerated TRP metabolism in CKD patients with T2D has not been investigated. Obviously, kidneys are the primary organs responsible for the elimination of TRP and its metabolites.\(^30\) TRP is effectively reabsorbed in the glomeruli, and reabsorption of KYN is significantly influenced by its plasma concentrations, ie, higher excretion fractions at increasing circulating levels.\(^30\) In CKD patients, this phenomenon is not observed,\(^31\) resulting in the accumulation of KYN in the circulation. Saito et al.\(^32\) demonstrated in nephrectomized animal model of CKD that in addition to diminished excretion, KYN accumulation could be due to enhanced synthesis and/or reduced metabolism.

We found enhanced IDO1 activity during the early stage of CKD (stage 2 vs stage 1, \(P<0.01\); Table 2). This novel observation suggests that upregulation of IDO1-mediated TRP

### Table 4. Multiple linear regression models of the relationships between KYN/TRP and KYN (dependent variables) and inflammatory markers (independent variables).

| CRP (independent variable) | KYN/TRP | KYN | CRP (independent variable) | KYN/TRP | KYN |
|----------------------------|---------|---------|---------------------------|---------|-----|
| Unadjusted 0.16 0.11 .163 0.11 0.07 .123 | Unadjusted 0.43 0.09 <.0001 0.30 0.05 <.0001 |
| Adjusted for age, sex, and BMI 0.24 0.10 .023 0.17 0.07 .014 | Adjusted for age, sex, and BMI 0.38 0.09 <.0001 0.27 0.05 <.0001 |
| Adjusted for age, sex, BMI, and eGFR 0.02 0.04 .700 0.04 0.05 .442 | Adjusted for age, sex, BMI, and eGFR −0.02 0.04 .550 0.12 0.04 .10 |

**Abbreviations:** BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; KYN, kynurenine; SE, standard error; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); TRP, tryptophan.

Dependent and independent variables are log transformed.
metabolism may be an intrinsic feature of CKD. During chronic inflammation, IDO1 is upregulated and oxidatively degrades the indole ring of TRP to formylkynurenine, which subsequently yields KYN; once synthesized, KYN undergoes further metabolism through 3 distinct pathways to form several downstream intermediary metabolites (Figure 1)—a prevailing concept. Consistent with this notion, our data show striking positive correlation between TRP metabolites and proinflammatory cytokines (Table 3). It is interesting to note that animal studies consistently showed remarkable upregulation of TDO causing TRP depletion with concomitant KYN elevation. In addition, these studies noted unchanged tissue IDO1 activity in rats with experimental CKD compared with control group. One recent study identified TDO-induced TRP metabolism in patients with CKD and T2D. Therefore, TRP degradation via TDO activation in this patient population is conceivable.

The relationship between proinflammatory cytokines and IDO1 activity (KYN/TRP ratio) in CKD seems complex and is not well investigated. First, circulating proinflammatory cytokines show substantial variability, which apparently depends on the kidney function as impaired kidney function affects the clearance of cytokines. Second, it should also be emphasized, as mentioned previously, that handling of TRP and KYN by the kidneys is altered in CKD setting. Third, the reported associations between TRP and its metabolites with eGFR in the literature are inconsistent. For example, Goek et al. did not find any correlation between eGFR change with either TRP or KYN. In contrast, Solini et al. reported positive and inverse relationship of eGFR with TRP and KYN, respectively. Fourth, it is important to consider that TNF-α alone is not sufficient to induce IDO1 as shown by in vivo experiments, but it is an important potentiator of IDO1 expression in several different contexts. Finally, emerging data convincingly show that TDO also mediates immunoregulatory effects. Taken together, it is perhaps the combination of TDO and IDO1, not IDO1 alone, which is responsible for the accelerated metabolism of TRP in CKD. In light of the above discussion, the validity of KYN/TRP as an index of accelerated metabolism may be an intrinsic feature of CKD compared with control group. One recent study identified TDO-induced TRP metabolism in patients with CKD and T2D. Therefore, TRP degradation via TDO activation in this patient population is conceivable.

The stimulating preliminary data, nevertheless, presented here suggest that accelerated TRP metabolism via the KYN pathway may have significant contributions to CKD initiation, progression, and other symptomatology in patients with T2D. Of note, accelerated TRP metabolism is often implicated to fatigue, depression, and other psychiatric disorders, which are prevalent in advanced CKD. Moreover, our regression analysis suggests that primarily two distinct mechanisms (inflammation and impaired kidney function) contribute to the accumulation of KYN. This observation is potentially important because targeting inflammation alone may not be effective in reducing KYN levels and explains why hemodialysis alone is inefficient in removing KYN from the circulation of ESKD patients. Therefore, the KYN pathway may offer a viable new avenue for therapeutic intervention to address CKD-associated comorbidities in patients with T2D.

The present study is limited by the cross-sectional analysis of data from a relatively small sample size, particularly in stage 4CD owing to extremely low prevalence and extraordinary mortality rate. We did not measure TDO or glucagon, and neurobehavioral phenotypes were not ascertained. In the parent study, plasma HbA1c was not available for all subjects. Recommended modest dietary animal protein restriction in patients with CKD with eGFR less than 60 mL/min/1.73 m² may contribute to the lower level of TRP; however, it seems unlikely that dietary intake would affect circulating levels of TRP and or KYN/TRP ratio. The validity of the results in our study, however, is strengthened with the consistent findings of the positive relationship of TRP with eGFR and an inverse association between KYN and eGFR reported in a patient population similar to ours. Moreover, results of the current study are in agreement with the findings from a prospective study that showed significant association of KYN with eGFR progression.

In the aggregate, the study findings demonstrate the robust association between TRP metabolism with concomitant rise in several bioactive kynurenines in type 2 diabetic patients with impaired kidney function. The key metabolite KYN correlated significantly with circulating TNF-α independent of eGFR, although the strength of correlation attenuated to marginal significance (P=0.060) after Bonferroni correction. These preliminary data provide a strong rationale for additional studies to determine the influence of TRP metabolites on the...
progression of diabetic kidney disease and overwhelming symptoms burden in this population. Future prospective studies should determine any temporal relationship of TRP metabolites with the progression of CKD stages and clarify the true cause of TRP depleting and kynurenines accumulation by measuring simultaneous direct assessment of TDO2 and both rate-limiting enzymes IDO1 and TDO.

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Author Contributions

SD, HEA, and JCO conceived and designed the experiments. SD, CV, LR, FT, BK, CL, HEA, and JCO contributed to the writing of the manuscript. All authors agree with manuscript results and conclusions, jointly developed the structure and arguments for the article, made critical revisions, and approved the final version.

Disclosures

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