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Increased indoleamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms

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Abstract

Background. Tryptophan (Trp) is catabolized by indoleamine 2,3-dioxygenase (IDO). Changes in Trp metabolism and IDO activity in chronic kidney disease (CKD) have not been widely studied, and the impact of haemodialysis is uncertain. Here we investigate Trp catabolism, IDO activity and the role of inflammation in moderate to very severe CKD and haemodialysis.

Methods. Eighty individuals were included in a prospective blinded endpoint analysis. Using tandem mass spectrometry, serum levels of Trp, kynurenine (Kyn), kynurenic-acid (Kyna), quinolinic-acid (Quin), 5-hydroxytryptophan (OH-Trp), serotonin (5-HT), estimated IDO activity and inflammatory markers were assessed in 40 CKD patients (age 57 $\pm$ 14 years, 21 male, creatinine 4.5 $\pm$ 2.7, $n = 17$ receiving haemodialysis), and in 40 healthy controls (age 34 $\pm$ 9 years, 26 male).

Results. Trp levels were unchanged in CKD ($P = 0.78$ versus controls). Serum levels of Kyn, Kyna and Quin increased with CKD severity (stages 4, 5 versus controls all $P \leq 0.01$). IDO activity was significantly induced in CKD and correlated with disease severity (stages 3–5 versus controls, all $P \leq 0.01$) and inflammatory markers [high-sensitivity C-reactive protein (hsCRP), soluble TNF-receptor-1 (sTNFR-I); both $P \leq 0.03$]. IDO products (Kyn, Kyna, Quin) correlated also with hsCRP and sTNFR-I (all $P \leq 0.04$). Haemodialysis did not influence IDO activity ($P = 0.26$) and incompletely removed Kyn, Kyna, Quin, OH-Trp

and 5-HT by 22, 26, 50, 44 and 34%, respectively. In multiple regression, IDO activity correlated with hsCRP and sTNFR-I (both $P \leq 0.03$) independent of serum creatinine, age and body weight.

Conclusions. IDO activity and serum levels of tryptophan catabolites of the kynurenine pathway increase with CKD severity. In CKD, induction of IDO may primarily be a consequence of chronic inflammation.

Keywords: IDO; immune system; infection; kynurenine; renal failure

Introduction

Catabolism of the essential amino acid tryptophan (Trp) is tightly regulated in healthy individuals, occurs via two distinct pathways (Figure 1), and is controlled to ∼99% by the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO). Induced IDO activity leads to the formation of kynurenine (Kyn), kynurenic acid (Kyna) and quinolinic acid (Quin) [1, 2]. This may contribute to the restoration of energy supplies via formation of acetyl-CoA (glutarate pathway) and nicotinamide adenine dinucleotide (NAD pathway) (Figure 1).

Trp catabolites have been demonstrated to be involved in the development of key uraemic symptoms. This includes, among others, neurotoxicity [3–5], lipid metabolism disorders and atherosclerosis [6] and increased susceptibility to infection [7,8]. IDO itself has been demonstrated as a key player in immunologic processes including infection, autoimmunity, allergic reaction, chronic inflammation and renal injury [9–17]. IDO activity, which may be estimated via the Kyn/Trp ratio, is induced by a number of pro-inflammatory stimuli including endotoxin (LPS), bacterial DNA [18,19], pro-inflammatory T_H1-cytokines such as interferon-γ [10,20,21] and soluble cytokine receptors [10]. Increased IDO activity may thus be observed in states of chronic infection [22], malignant disease [23] and neuropsychiatric diseases [24].

Few data from animal models suggest that Trp and metabolites of the kynurenine pathway are excreted via the kidneys. However, Trp metabolism and IDO activation in chronic kidney disease (CKD) have not been widely studied, and the impact of haemodialysis on Trp and respective catabolites remains uncertain. This seems of interest as CKD is strongly attended by the presence of chronic inflammation, which has been convincingly demonstrated to be associated with adverse outcomes in CKD.

Here, we investigate the relationship between tryptophan, downstream Trp catabolites and IDO activity with measures of kidney function, clinical CKD stages and prognostically relevant indices of chronic inflammation, such as high-sensitivity C-reactive protein (hsCRP) and the soluble TNF-receptor-1 (sTNFR-I).

Subjects and methods

Study population

In a prospective study, 40 consecutive Caucasian patients [21 male, age 57 ± 14 years, serum creatinine 4.5 ± 2.7, serum urea 101.4 ± 42.0 mg/dl, estimated glomerular filtration rate (eGFR) 22.4 ± 13.6 ml/min/1.73 m^2, creatinine clearance 21.6 ± 14.3 ml/min, body weight 67.1 ± 2.8 kg and body height 165.5 ± 9.4 cm] with CKD according to National Kidney Foundation (NKF) stages 3–5 hospitalized in the Charité University Hospital, Berlin, and the University Hospital of Greifswald, Germany, were included in this analysis (Table 1). Seventeen patients were in stage 5 (end-stage renal disease, ESRD) and received chronic haemodialysis (three times per week, 4 h per session). Major underlying kidney disease was diabetic nephropathy ($n = 19$, 48%), hypertensive nephropathy ($n = 16$, 40%), glomerulonephritis ($n = 4$, 10%) and IgA-nephropathy ($n = 1$, 0.3%). Major concomitant diseases were coronary artery disease ($n = 32$, 80%), hyperlipoproteinaemia ($n = 26$, 65%) and congestive heart failure ($n = 14$, New York Heart association class 1–2, 35%). Forty healthy Caucasian volunteers (age 34 ± 9 years, 26 male, serum creatinine 1.1 ± 0.2, body weight 72 ± 3.9 kg) served as controls.

For inclusion into the analysis, participants were required to meet the following criteria: age >18 years and hospitalization related to any cause other than infection or acute inflammation. Patients were excluded when signs of systemic or localized infection (bacterial, fungal or viral) were present (dry cough, expectoration, positive chest X-ray, signs of urinary infection or diarrhoea). Patients with malignoma, and acute leukaemia, or patients on a medication known to interfere with Trp metabolism (e.g. selective serotonin reuptake inhibitors) were also excluded. The study was performed in accordance with the *Declaration of Helsinki.* Informed consent was achieved.

Assessment of kidney function and indices of inflammation

Kidney function was assessed using the following indices: serum creatinine (mg/dl), estimated creatinine clearance (Cockroft-Gault formula), serum urea (mg/dl) and estimated GFR [modification of diet in renal disease (MDRD) formula]. For assessment of chronic inflammation, the following indices were measured in a certified laboratory: hsCRP (assessed using immunoturbidimetry, mg/dl), white blood cell (WBC) count ($\times 10^9$/l), platelet count ($\times 10^9$/l) and sTNFR-I (pg/ml, upper and lower limit of detection 15.6–1000 pg/ml). For assessment of Trp metabolism, plasma ethylene-diaminetetraacetic acid samples were collected in the morning before breakfast (7:00–8:30 am) and in the early afternoon (1:00–2:30 pm), or directly before and 20 min after haemodialysis [for patients in kidney disease outcomes quality initiative (KDOQI) stage 5], respectively. All samples were peripheral venous blood samples and were stored at −80°C until assay.

Analysis of Trp metabolism

One hundred microlitre plasma was analysed after addition of 50 μl sulfosalicylic acid, 40 μl water and 10 μl standard solution [tryptophan-indole-d5 (d5-Trp) and 5-times deuterized kynurenic acid (d5-Kyna), Cambridge Isotope Laboratories, Andover, MA, USA]. The samples were mixed, stored at 4°C (30 min) and centrifuged (20 000*g*, 10 min). For recording, a Wallac MS2 tandem mass spectrometer (Perkin Elmer, Rodgau, Germany) equipped with an electrospray ion source was used. Ions were detected in a positive ion mode using multiple reaction monitoring. The first quadrupole selected the protonated ions at mass-to-charge ratio (*m*/*z*) 205, 210, 177, 209, 168, 190 and 195 for Trp, d5-Trp, 5-HT, Kyn, Quin, Kyna and d5-Kyna, respectively. Nitrogen served as collision gas. Fractioned ions *m*/*z* 188 for Trp, 193 for d5-Trp, 160 for 5-HT, 192 for Kyn, 78 for Quin, 144 for Kyna and 149 for d5-Kyna were detected in quadrupole Q3 (Q3) (flow solvent: 0.02% formic acid in 50% aqueous acetonitrile, flow rate 75 μl/min). For quantification, plasma samples were spiked with standards. Calibration curves were fitted by linear least-square regression and correlated with the concentration of d5-Trp and d5-Kyna.

Statistical analysis

Statistical analyses were performed using the MedCalc 9.0.1 software (MedCalc Software, Mariakerke, Belgium). All data were tested for normal distribution using the Kolmogorov–Smirnov test and were found to be normally distributed. The coefficient of determination is provided, when applicable. Univariate and multivariate regression analyses were applied to assess factors that independently predicted IDO activity. A

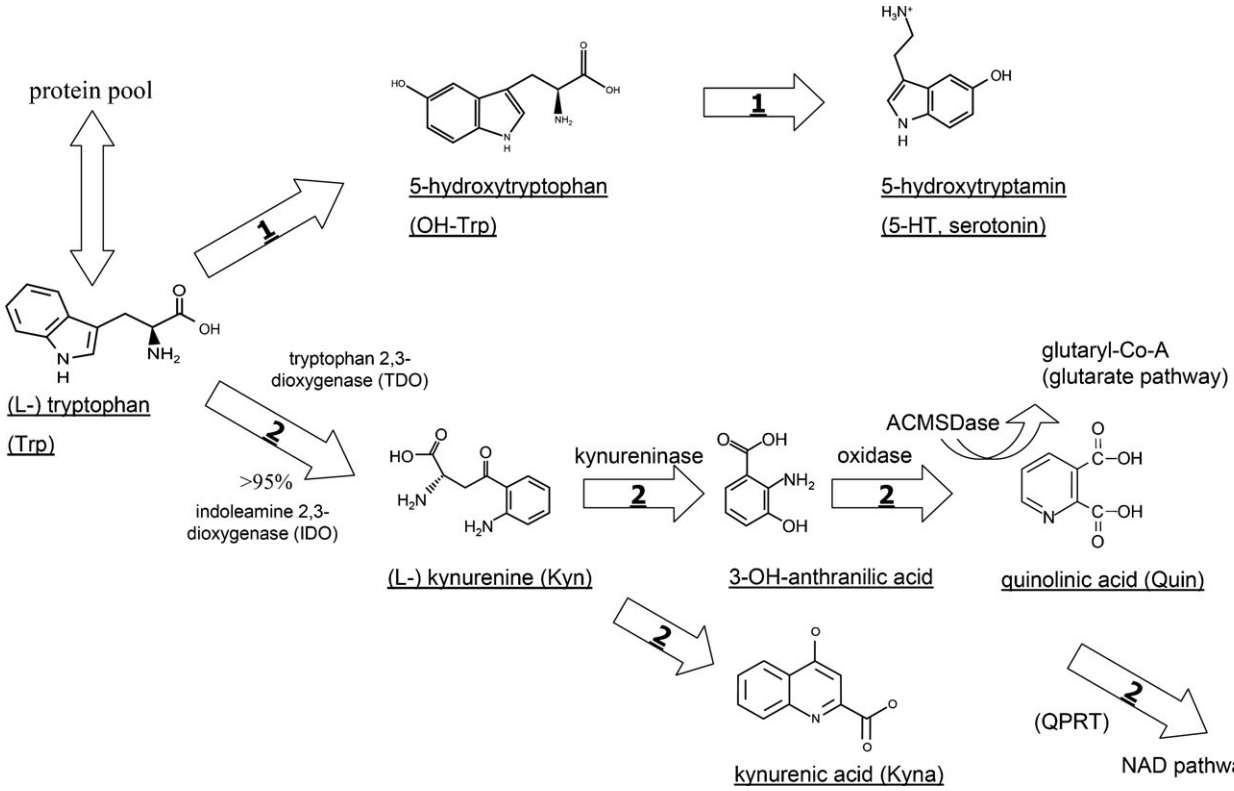

Fig. 1. Overview of Trp catabolism and the role of IDO. Two distinct pathways of Trp catabolism are indicated: 1 = 5-HT branch, 2 = kynurenine branch including both quinolinic branch and transamination branch. ACMSDase, amino-carboxymuconate-semialdehyde. QPRT, quinolinic acid phosphoribosyltransferase.

Table 1. Kidney function and indices of inflammation in CKD stages 3–5

	CKD stage 3 (30–59 ml/min/ 1.73 m^2) $n = 10$ (25%)	CKD stage 4 (15–29 ml/min/ 1.73 m^2) $n = 14$ (35%)	CKD stage 5 (<15 ml/min/ 1.73 m^2) $n = 17$ (42%)
Serum creatinine (mg/dl)	$2.0 \pm 0.7^{*, **}$	$3.7 \pm 1.3^{**}$	6.6 ± 1.5
Serum urea (mg/dl)	89.7 ± 37.0	102.6 ± 47.6	107.8 ± 40.9
Creatinine clearance (ml/min)	$41.9 \pm 12.5^{\#, \#\#\#}$	$20.7 \pm 5.2^{\#\#}$	10.3 ± 3.0
White blood cell count ($\times 10^9$/l)	8.5 ± 3.3	8.7 ± 6.4	7.9 ± 3.2
Platelet count ($\times 10^9$/l)	200.6 ± 93.8	240.6 ± 113.1	243.6 ± 142.0
High-sensitivity C-reactive protein (mg/dl)	$0.7 \pm 0.6^{\S}$	2.4 ± 2.1	3.2 ± 2.5
Soluble TNF receptor I (pg/ml)	$4365.6 \pm 4154.1^{\S, \S\S}$	$8594.8 \pm 5443.1^{\S\S\S}$	12617.2 ± 4935.0

$^{*}P = 0.001$ versus stage 4; $^{**}P < 0.0001$ versus stage 5; $^{\#}P \leq 0.01$ versus stage 4; $^{\#\#}P \leq 0.01$ versus stage 5; $^{\#\#\#}P < 0.001$ versus stage 5; $^{\S}P \leq 0.005$ versus stage 5; $^{\S\S}P = 0.04$ versus stage 4; $^{\S\S\S}P = 0.04$ versus stage 5.

value of $P < 0.05$ was considered to be significant. Results and relative changes are reported as means ± standard deviations (SD), if not indicated otherwise.

Results

Trp metabolism in controls

In controls ($n = 40$), the mean morning Trp level was 19.04 ± 3.7 μM. The following mean levels were measured in the morning of the respective day of assessment (Figure 2a–g): Kyn 2.6 ± 0.26 μM, Kyna 0.55 ± 0.23 μM, Quin 0.23 ± 0.09 μM and IDO activity 14.04 ± 2.4. In the 5-HT branch, OH-Trp levels were 0.07 ± 0.04 μM and 0.84 ± 0.19 μM (5-HT).

Trp and Trp catabolites over time

In order to assess potential changes in the levels of Trp, downstream Trp catabolites and IDO activity across the

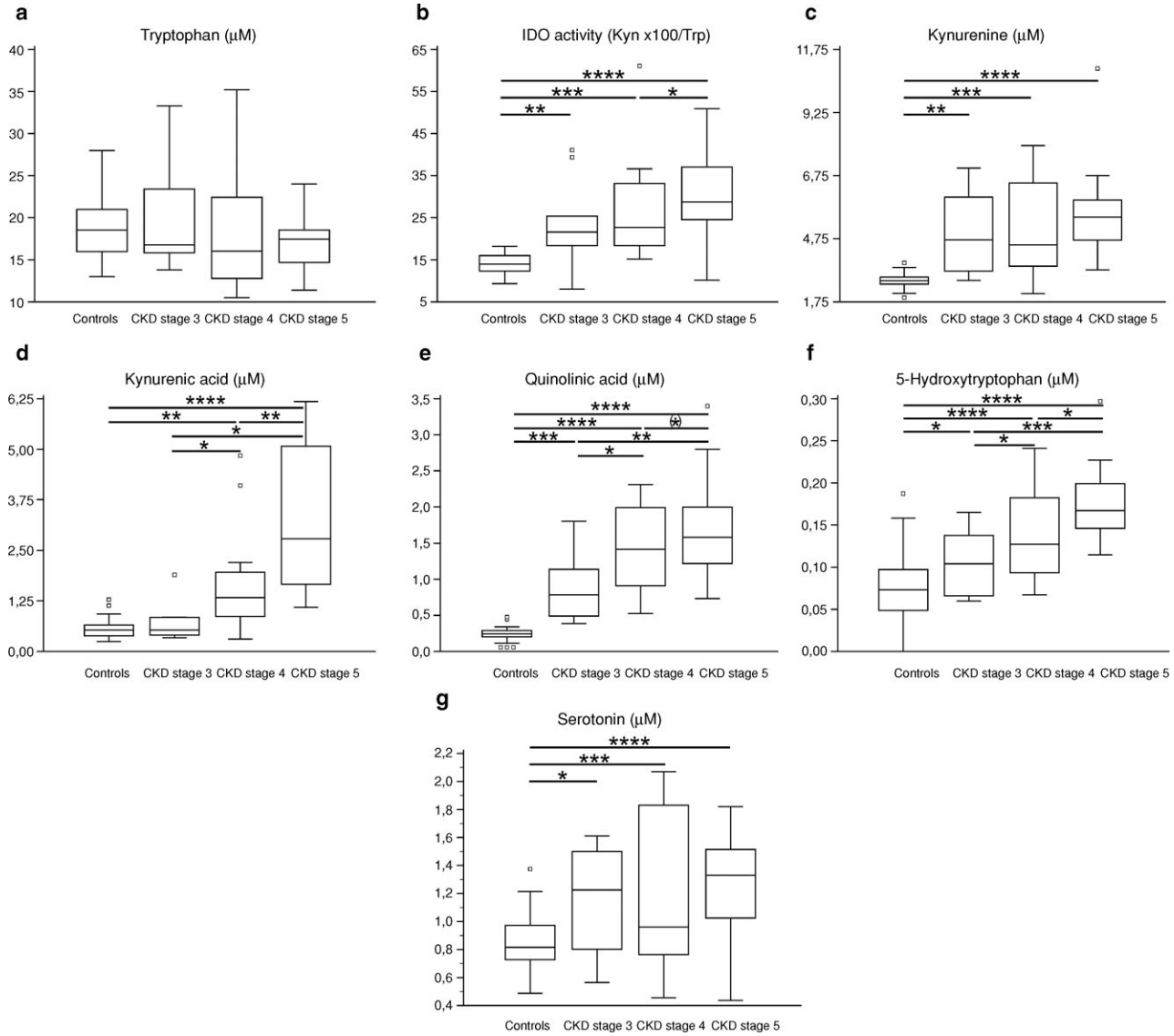

Fig. 2. Box and whisper plots demonstrating the course of tryptophan and respective metabolites through the clinical stages of CKD (a–g). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.005$; $^{****}P \leq 0.0001$.

day, morning and afternoon samples (or before and after haemodialysis for study patients receiving haemodialysis, i.e. CKD stage 5) were analysed. In all study cohorts, morning and afternoon samples were not found to differ significantly (all $P > 0.06$).

Trp metabolism, IDO activity and kidney function/clinical CKD stages

Trp and catabolites were checked for correlations with measures of kidney function (creatinine, urea, creatinine clearance, eGFR) and CKD stages.

(Trp): Trp levels were unchanged in CKD and did not correlate with measures of kidney function (*n.s.* versus all groups, Figure 2a).

(IDO): IDO activity increased with CKD severity (Figure 2b) and correlated with creatinine and creatinine clearance (both $P \leq 0.05$).

(Kyn): Kyn levels significantly increased in CKD stages 3–5 (Figure 2c) but did not directly correlate with kidney function (all $P \leq 0.05$).

(Kyna): Kyna levels increased significantly with respective CKD stages (Figure 2d) and correlated with serum creatinine ($P < 0.0001$, $r = 0.74$), creatinine clearance ($P < 0.0001$, $r = -0.65$) and eGFR ($P = 0.0046$, $r = -0.53$).

(Quin): Quin levels increased with respective CKD stages (Figure 2e) and correlated with serum creatinine, and creatinine clearance (both $P \leq 0.04$).

(OH-Trp): OH-Trp increased with CKD severity (Figure 2f) and correlated with creatinine ($P = 0.0001$,

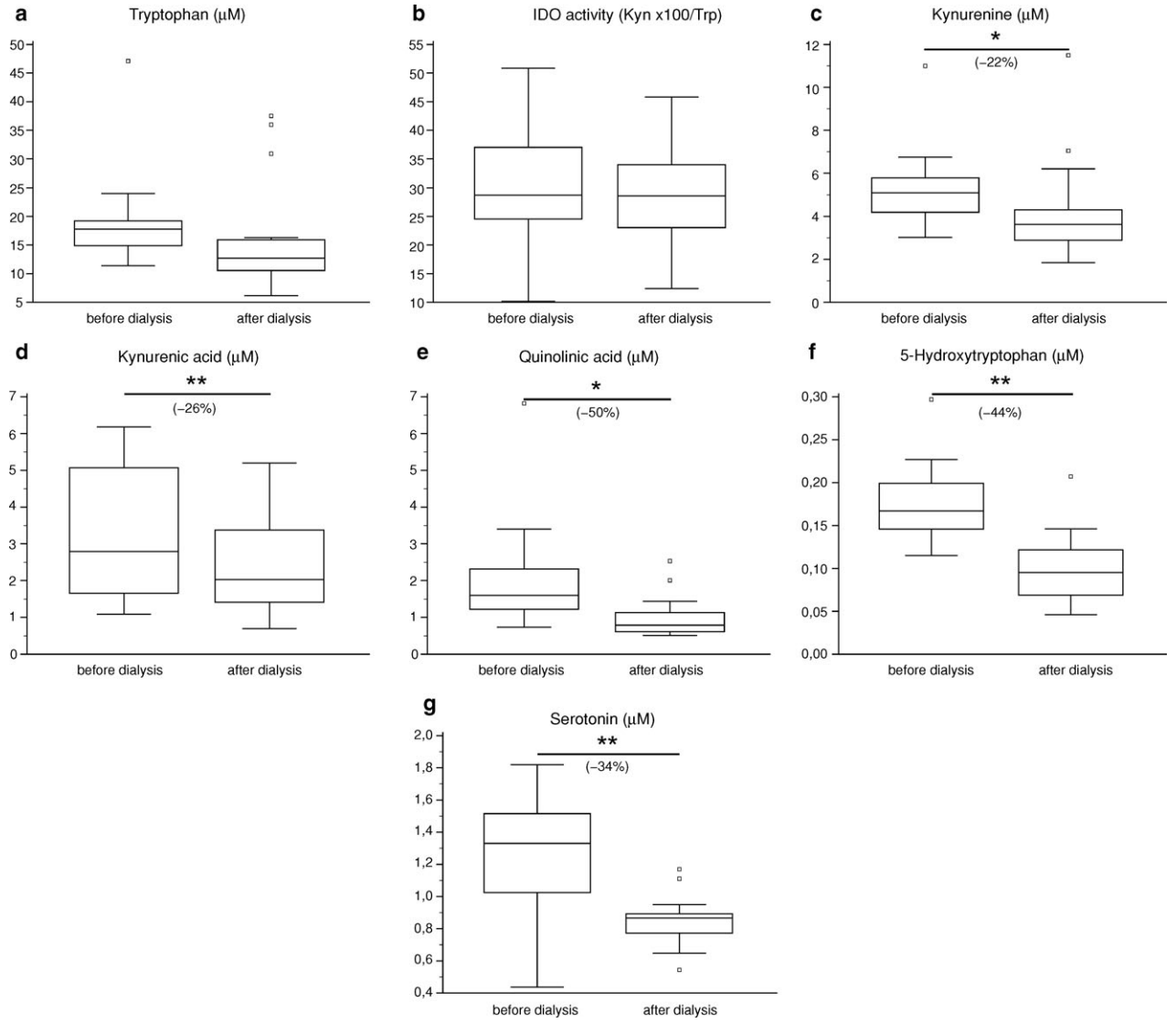

Fig. 3. Box and whisper plots indicating the levels of tryptophan and respective metabolites in patients before and after haemodialysis (a–g, $n = 17$). Respective changes (%) are indicated. $^{*}P \leq 0.002$; $^{**}P \leq 0.0001$.

$r = 0.57$), creatinine clearance ($P < 0.0001$, $r = -0.59$) and eGFR ($P = 0.006$, $r = -0.51$).

(5-HT): 5-HT levels increased in CKD (Figure 2g) but did not correlate with kidney function.

Markers of inflammation, Trp catabolism and kidney function

Markers of inflammation (WBC and platelet counts, hsCRP and sTNFR-I) were tested for correlations with Trp, respective catabolites and indices of kidney function. IDO, downstream catabolites and indices of kidney function correlated with hsCRP and sTNFR-I but not with WBC or platelet counts. Haemodialysis did not influence IDO activity ($P = 0.26$) and incompletely removed Kyn, Kyna, Quin, OH-Trp and 5-HT by 22, 26, 50, 44 and 34%, respectively (Figure 3).

HsCRP and sTNFR-I levels correlated with serum creatinine ($P = 0.0001$, $r = 0.58$ and $P = 0.04$, $r = 0.33$, respectively), creatinine clearance ($P = 0.0002$, $r = -0.56$ and $P = 0.02$, $r = -0.38$) and eGFR ($P = 0.04$, $r = -0.31$ and $P = 0.03$, $r = -0.27$). IDO activity, Kyn, Kyna, Quin and OH-Trp (but not Trp and 5-HT) correlated with hsCRP levels (all $P \leq 0.04$). IDO activity correlated with sTNFR-I levels ($P = 0.01$, $r = 0.4$). Moreover, sTNFR-I levels correlated with products downstream of IDO such as Kyn ($P = 0.04$, $r = 0.31$), Kyna ($P = 0.0002$, $r = 0.56$) and Quin ($P = 0.001$, $r = 0.50$). WBC counts did not correlate with respective parameters of Trp metabolism (all $P > 0.05$). Platelet counts correlated with Kyn and Kyna (both $P = 0.02$, $r = 0.27$), Quin ($P = 0.005$, $r = -0.31$)

and Trp ($P = 0.01$, $r = 0.28$), but not with IDO activity, 5-HT or OH-Trp.

Between-group comparison for indices of inflammation

WBC, platelet count, hsCRP and sTNFR-I levels were measured in order to assess the degree of chronic inflammation in the study population. WBC and platelet counts were rather unchanged, whereas hsCRP and sTNFR-I levels increased throughout CKD stages 3–5 (Table 1).

Multiple regression analysis

In multiple regression, IDO activity correlated with hsCRP and sTNFR-I ($P = 0.03$, $r = 0.42$ and $P = 0.01$, $r = 0.56$) independently of serum creatinine ($P = 0.27$, $r = 0.12$), age ($P = 0.50$, $r = -0.11$) and body weight ($P = 0.57$, $r = -0.16$). In this analysis, the multiple correlation coefficient was 0.59. The overall significance level for the analysis of variance was $P = 0.021$. IDO activity remained independent when eGFR instead of body weight entered the analysis ($P = 0.69$, $r = 0.03$).

Discussion

We found that Trp levels were relatively unchanged in CKD. Increasing CKD severity yielded elevated levels of Trp catabolites of the kynurenine pathway including both the quinolinic and transamination branch (Figure 1). When compared to controls, Kyn, Kyna and Quin levels increased in very severe kidney failure by about a factor 2, 6 and 8, respectively (Figure 2c–e). In addition, a significant correlation of Kyna and Quin with serum creatinine and creatinine clearance was noticed. IDO activity was found induced in CKD, correlated with CKD disease severity and with key inflammatory markers (hsCRP, sTNFR-I). Moreover, a correlation of catabolites downstream of IDO (Kyn, Kyna, Quin) with hsCRP and sTNFR-I was found. In multiple regression, IDO activity correlated with hsCRP and sTNFR-I independently of age, body weight and serum creatinine. This may indicate that induction of IDO in CKD may primarily be induced by inflammatory mechanisms.

Moreover, we found that low-flux haemodialysis did not influence IDO activity and only partially removed Kyn, Kyna, Quin, OH-Trp and 5-HT (Figure 3). Toxic Trp catabolites, which have been implicated in the pathogenesis of key uraemic syndromes [3,5–7,15,24–26,41], may thus only be incompletely removed under 4 h of routine low-flux haemodialysis. Such incomplete removal may be due to protein-binding characteristics, respective distribution volumes, washout effects and turnover of the respective molecules. Thus, although not investigated in the present analysis, even high-flux haemodialysis may not sufficiently remove respective mediators [27].

Chronic inflammation is a well-known feature in CKD. Mounting data points to an important cross-link of inflammation and cardiovascular disease as inflammation has been shown as an integral part of atherosclerosis [34]. Epidemiologically, this seems important as cardiovascular disease is the major cause of morbidity and mortality in CKD and end-stage renal disease (ESRD). Recent prospective epidemiological studies have linked elevated CRP with atherosclerosis in CKD. CRP has consistently been shown to predict cardiovascular and all-cause mortality, and independently predicts death in ESRD [28–33]. The strong association of elevated CRP with inflammation, atherosclerosis and cardiovascular mortality in CKD and ESRD is intriguing, as a single CRP assessment may predict future cardiovascular events [35, 36]. Moreover, sTNFR-I levels have been shown to be elevated in CKD, and a direct link to progression of CKD, vasculopathy and prognosis was established [37–40]. In the present analysis, we found that increased hsCRP and sTNFR-I levels, but not WBC or platelet counts, correlated with decreased kidney function. Indeed, this may indicate the presence of chronic inflammation and increased cardiovascular risk in the study cohort.

Although it was suggested that decreased glomerular filtration *per se* might lead to decreased excretion of Trp catabolites via the kidneys, data from animals models of renal insufficiency suggest the involvement of mechanisms other than just simple renal excretion failure as respective catabolites may also increase without a relevant decline in renal function [26]. However, IDO activity has previously been demonstrated to be induced by a number of immune activators including LPS, neopterin, IFN-γ, soluble cytokine receptors and others [10]. In the present analysis, we demonstrate that IDO activity correlates with CKD severity (Figure 2b). When compared to controls, an about 2-fold increase in IDO activity was observed from moderately reduced kidney function to very severe kidney failure (Figure 2b) and a significant correlation of IDO activity with hsCRP and sTNFR-I was noticed. In multiple regression, we found that IDO activity correlates with hsCRP and sTNFR-I independently of age, body weight and serum creatinine. This may indicate that although IDO activity is induced in CKD, induction of IDO may primarily be due to inflammatory stimuli. Next to inflammation, IDO activation in CKD may be influenced by factors other than inflammatory stimuli. This may include upregulation of hepatic tryptophan 2,3-dioxygenase, decreased Kyn metabolism, oxidative stress and increased Trp turnover.

About 1% of Trp catabolism occurs via the serotonin (5-HT) pathway (Figure 1). In this analysis, OH-Trp and 5-HT were noticed to increase in CKD (Figure 2f, g). The mechanism behind this may include a diminished 5-HT uptake by platelets in the uraemic environment and increased 5-HT production in intestinal enterochromaffin cells in response to inflammatory stimuli. However, the mechanism behind the increase in OH-Trp and 5-HT is beyond the scope of this analysis and deserve further analyses in subsequent investigations.

Some limitations of this analysis demand discussion. First, we did not access the dietary amino acid intake of our study population. Although Trp levels in CKD have also

been documented to differ from that of controls [27,41], we observed rather unchanged Trp levels in CKD (Figure 2a) and under haemodialysis (Figure 3a). This may reflect a relatively good nutritional condition of our study patients. However, although short-term dietary amino acid intake might be of minor impact on overall Trp and Trp catabolite levels, we are unable to rule out an effect of dietary intake. Nevertheless, morning and afternoon Trp levels were found unchanged in all CKD stages investigated. Secondly, we did not directly measure enzymatic IDO activity [42]. Although this might be performed in subsequent studies, estimated IDO activity has previously been shown to accurately assess respective enzymatic activities. Third, KDOQI stage 2 patients were not analysed. This was done in order to concentrate on patients with at least a moderately reduced kidney function. However, it may be interesting to assess Trp catabolism in the very early stages of CKD in subsequent analyses. Moreover, mean age was different between the CKD and control groups. Nevertheless, estimated IDO activity and respective Trp metabolites were found to be independent of age in both CKD patients and in controls.

Here, we demonstrate that Trp catabolites accumulate in CKD. These products, which have been shown to be crucially involved in the pathogenesis of key uraemic syndromes, are only partially removed under routine haemodialysis. IDO activity was found to be induced with increasing CKD disease severity but was not influenced by routine haemodialysis. In multiple regression, IDO activity was found to correlate with hsCRP and sTNFR-I independently of age, body weight and serum creatinine. Our results provide further evidence for the presence of chronic inflammation in CKD, and we propose a role for IDO in this context.

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Conflict of interest statement. None declared.

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Acetaminophen, aspirin and progression of advanced chronic kidney disease

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Abstract

Background. Although many studies have investigated the possible association between analgesic use (acetaminophen and aspirin) and the development of chronic kidney disease (CKD), the effect of analgesics on the progression of established CKD of any cause has not yet been investigated.

Methods. In this population-based Swedish cohort study, we investigated the decline over 5–7 years in estimated glomerular filtration rate (eGFR) among 801 patients with incident, advanced CKD (serum creatinine >3.4 mg/dL for men, >2.8 mg/dL for women for the first time) and with different analgesic exposures. Lifetime analgesic use and current regular use were ascertained through in-person interviews at inclusion while data on analgesic use during the follow-up was abstracted from the medical records at the end of the study period. A linear regression slope, based on their eGFR values during the follow-up, provided a summary of within-individual change. In the final multivariate analyses, a linear mixed effects model was implemented to assess the relation of analgesic use and change in eGFR over time.

Results. The progression rate for regular users of acetaminophen was slower than that for non-regular users (regular users progressed 0.93 mL/min/1.73 m^2 per year slower than non-regular users; 95% CI 0.03, 1.8). For regular users of aspirin, the progression rate was significantly slower than that for non-regular users (regular users progressed 0.80 mL/min/1.73 m^2 per year slower than non-regular users; 95% CI 0.1, 1.5). Different levels of lifetime cumulative dose of acetaminophen and aspirin did not significantly affect the progression rate.

Conclusion. We suggest that single substance acetaminophen and aspirin may be safe to use by patients