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Urinary ortho-tyrosine excretion in diabetes mellitus and renal failure: Evidence for hydroxyl radical production

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Urinary ortho-tyrosine excretion in diabetes mellitus and renal failure: Evidence for hydroxyl radical production.

Background. Phenylalanine is converted to para- and ortho-tyrosine by hydroxyl free radical, or to para-tyrosine by the phenylalanine hydroxylase enzyme. The aim of this study was to measure para- and ortho-tyrosine in the urine and plasma of patients with chronic renal disease and/or diabetes, to obtain information on the renal handling of the different tyrosine isomers and, furthermore, to measure urinary levels of 8-epi-prostaglandin-$F_{2\alpha}$, a marker of lipid peroxidation.

Methods. In our cross-sectional study we measured para-, ortho-tyrosine, and phenylalanine levels, using high performance liquid chromatography and 8-epi-prostaglandin-$F_{2\alpha}$ with enzyme-linked immunosorbent assay (ELISA). We compared 4 groups: (*1*) controls (CONTR, $N = 14$), (*2*) patients with chronic kidney disease (CKD, $N = 12$), (*3*) patients with type 2 diabetes mellitus (DIAB, $N = 17$), (*4*) patients with chronic kidney disease and type 2 diabetes (DIAB-CKD, $N = 19$).

Results. We found a decreased plasma para-tyrosine level and decreased urinary para-tyrosine excretion in CKD patients, while the fractional excretion of para-tyrosine was similar in all 4 groups, approximately 1%. There was no difference in the plasma ortho-tyrosine levels between the groups. However, urinary ortho-tyrosine excretion was higher in all 3 groups of patients than in the CONTR group, and higher in DIAB and in DIAB-CKD patients than in CKD patients. The fractional excretion of ortho-tyrosine was significantly higher in DIAB and in DIAB-CKD patients than in the CONTR group. The fractional excretion of ortho-tyrosine exceeded 100% in the 2 diabetic groups. Urinary 8-epi-prostaglandin-$F_{2\alpha}$/creatinine ratio did not correlate with urinary ortho-tyrosine excretion.

Conclusion. The difference between para-tyrosine levels of the groups is probably due to renal impairment, while there is indirect evidence for an increased tubular secretion or production of ortho-tyrosine in the kidney in diabetic patients with or without CKD.

Key words: chronic renal disease, 8-epi-prostaglandin-$F_{2\alpha}$, ortho-tyrosine, para-tyrosine, type 2 diabetes mellitus.

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Oxidative stress has been implied for a long time as a key process in the development of complications of diabetes mellitus and chronic renal disease [1, 2]. The imbalance between free radicals and antioxidant systems gives rise to free radical-mediated damage. Metal-catalyzed oxidation reactions, like the Fenton reaction, where hydrogen peroxide is cleaved to hydroxyl free radical and hydroxyl anion, play an important role in the generation of oxidative stress [3]. In diabetes mellitus there is an increased oxidative stress (e.g., due to the high glucose concentrations). In chronic renal failure, there is a microinflammatory state, caused by uremic toxins, an enhanced formation and a decreased clearance of proinflammatory cytokines (e.g., TNF-α, IL-12), and an accumulation of advanced glycation end products [4].

Amino acid oxidation-products are stable and specific markers of free radical production [5]. Reactive oxygen species such as superoxide and hydroxyl free radicals damage protein-bound or free amino acids [6, 7]. Aromatic side chain amino acids are susceptible to oxidative stress, and their products (e.g., ortho-tyrosine, meta-tyrosine, and dityrosine) are stable [1].

The essential amino acid phenylalanine (Phe) is converted to the physiologic, semi-essential para-tyrosine (p-Tyr) by the phenylalanine hydroxylase enzyme. No other isoform of tyrosine is formed in this enzymatic reaction. In the presence of hydroxyl free radical Phe can be hydroxylated in para, meta, and ortho positions. Thus, para-, meta-, and ortho-tyrosine (p-, m-, o-Tyr) are all formed in this free radical-reaction (Fig. 1). Consequently, p-Tyr may be formed both physiologically and in the oxidative

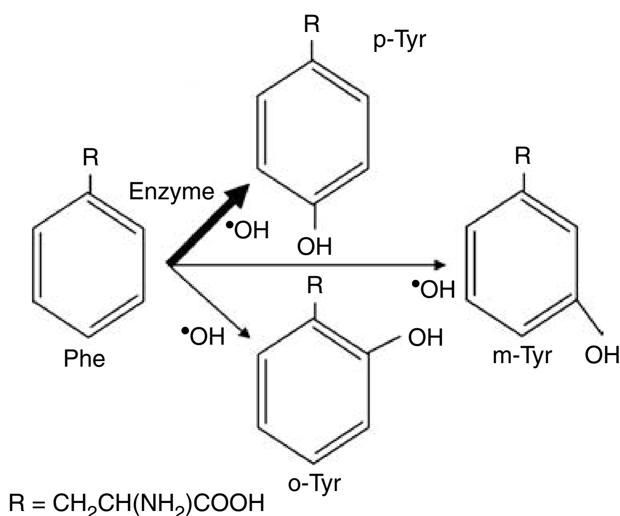

Fig. 1. Conversion reactions of phenylalanine (Phe) to para-meta- and ortho-tyrosine (p-, m-, o-Tyr). The wide (→) arrow represents the enzymatic reaction, while the narrow arrow (→) shows the reactions in the presence of hydroxyl free radical (˙OH). Abbreviations are: Phe, phenylalanine; p-Tyr, para-tyrosine; m-Tyr, meta-tyrosine; o-Tyr, ortho-tyrosine; ˙OH, hydroxyl free radical.

processes, while m- and o-Tyr are selective hydroxyl free radical markers [7, 8]. o- and m-Tyr have been used as markers of hydroxyl free radical formation, among others, in sera of euthyroid subjects after triiodothyronin administration [9], in cataractous lenses [10], and in the plasma after myocardial ischemia-reperfusion [11].

Accumulation of o- and m- Tyr has been widely described as correlating with an increase of malondialdehyde formation [12], an increase in NADPH oxidase activity, an increased level of oxidized fatty acids [13], a decrease in reduced glutathione levels [14], and others. Oxidized amino acid derivatives are superior to other oxidative stress markers (e.g., lipid peroxidation products, glycoxidation products) because they are reliable, specific, highly stable markers, and are not produced in sample preparation procedures. Moreover, o-Tyr can be detected well through its autofluorescence [6].

Amino acids are filtered in the glomeruli and reabsorbed from the primary filtrate in the renal tubules. The route of reabsorption depends on the nature and chemical structure of the amino acid. Both Phe and p-Tyr are reabsorbed via the transporters of the neutral amino acids. This process is of high efficacy, with only 1% of the filtered amino acids escaping with the urine [15]. Beside filtration, there are other sources of urinary amino acids, such as tubular secretion at the apical membrane and tubular brush border peptidases that split oligopeptides to amino acids. The fractional excretion (Fex) of p-Tyr and Phe varies at around 1% (mean Fex of p-Tyr, 1.18%; mean Fex of Phe, 0.9%) in humans [15].

The aims of this study were to measure p-, m-, o-Tyr, and Phe concentrations both in urine and in plasma samples of controls, of patients with type 2 diabetes mellitus or chronic kidney disease or a combination of both. We also investigated the correlation between the levels of urinary 8-epi-prostaglandin-$F_{2\alpha}$, a marker of lipid peroxidation, with the hydroxyl free radical marker, o-Tyr. We examined the renal handling of the physiologically produced and the hydroxyl radical-derived Tyr isomers.

METHODS

Patient groups

In a cross-sectional clinical study, 4 groups of patients were investigated: (*1*) a group of nondiabetic control subjects without chronic kidney disease (CONTR, $N = 14$); (*2*) a group of patients with stage III chronic kidney disease (CKD, $N = 12$); (*3*) type 2 diabetic patients (DIAB, $N = 17$); and (*4*) patients with type 2 diabetes and stage III. chronic kidney disease (DIAB-CKD, $N = 19$). Medians of measured and predicted clearance (Table 1) in the CKD and DIAB-CKD groups were in the range of stage III CKD (moderate decrease of GFR) according to the Definition and Classification of the Stages of Kidney Disease by the National Kidney Foundation (i.e., 30–59 mL/min). Diagnoses of the patients with chronic kidney disease were chronic pyelonephritis ($N = 4$), polycystic kidney disease ($N = 3$), nephrosclerosis ($N = 2$), IgA nephropathy ($N = 1$), minimal change disease ($N = 1$), and renal vasculitis ($N = 1$). The groups did not show any significant difference in regards to their age ($P = 0.426$) or gender ($P = 0.723$).

There was no significant difference between the CKD and the DIAB-CKD patients regarding the severity of their renal impairment [serum creatinine, $P = 0.795$; measured creatinine clearance, $P = 0.272$; predicted creatinine clearance (Cockroft-Gault), $P = 0.820$]. The DIAB and the DIAB-CKD groups did not differ in their fructosamine ($P = 0.797$) and hemoglobin A_{1c} levels ($P = 0.298$). There was no significant difference between the groups in the parameters of liver function (e.g., serum ALAT, lactic acid dehydrogenase, and alkaline phosphatase activities), serum total cholesterol, HDL and LDL cholesterol (data not shown). Important clinical characteristics of the 4 groups are shown in Table 1.

Twenty-four–hour collected urine and heparinized fasting plasma samples were obtained, and routine clinical parameters were also measured. As diet may influence plasma o-Tyr levels, fasting plasma samples were obtained. For determination of laboratory parameters, standard methods were used. The study was approved by the Ethical Committee of the Medical Faculty of the University of Pécs. All patients and controls gave informed consent.

Table 1. Clinical characteristics of the four groups of patients

	CONTR	CKD	DIAB	DIAB-CKD
Number of cases	14	12	17	19
Gender *male/female*	4/10	5/7	7/11	8/12
Age *years*	61 (54–65)	54 (51–65)	62 (57–69)	69 (59–73)
Blood urea nitrogen *mmol/L*	4.8 (4.1–6.0)	12.3[a] (10.2–15.8)	7.3[b] (6.5–9.5)	16.2[a,c] (13.1–23.9)
Serum creatinine *μmol/L*	79 (70–90)	190[a,c] (147–255)	81 (68–88)	196[a,c] (173–257)
Creatinine clearance				
Measured *mL/min*	109 (83–140)	31[a,c] (16–33)	109 (93–124)	38[a,c] (23–41)
Predicted (Cockroft) *mL/min*	80 (71–87)	35[a,c] (30–56)	109 (90–144)	38[a,c] (25–53)
Fructosamine *μmol/L*	–	–	267 (243–304)	259 (235–367)
Hemoglobin A_{1c}%	–	–	8.65 (8.22–9.59)	7.13 (6.23–9.01)

Abbreviations are: CONTR, group of healthy control subjects; CKD, group of patients with stage III chronic kidney disease; DIAB, group of diabetic patients; DIAB-CKD, group of patients with diabetes and stage III chronic kidney disease. Data are shown as median and (interquartile range). The Mann-Whitney *U* test was only used when the Kruskal-Wallis test for all groups was significant ($P < 0.05$).
[a] $P < 0.05$ vs. control subjects.
[b] $P < 0.05$ vs. CKD patients.
[c] $P < 0.05$ vs. type 2 diabetic patients.

Determination of non–protein-bound ortho- and para-tyrosine in urine and fasting plasma samples

A modification of the method described by Ishimitsu et al [16] was used for the analysis. Briefly, from the 24-hour collected urine or freshly obtained fasting heparinized plasma samples, aliquots of 250 μL were taken and handled on ice. One hundred and twenty-five microliters of 60% trichloroacetic acid was added to the samples, vortexed, and incubated 30 minutes on ice to precipitate protein content. To remove the precipitate, samples were then centrifuged at 15,000 rpm for 10 minutes in Eppendorf tubes. The supernatant was filtered through a 0.2 μm syringe filter (Millipore, Billerica, MA, USA), and 20 μL was injected into the manual injector of the high-performance liquid chromatography (HPLC) device.

The analysis was performed using a Shimadzu Class LC-10 AD_{VP} HPLC system (Shimadzu USA Manufacturing, Inc., Canby, OR, USA) equipped with a Shimadzu RF-10 A_{XL} fluorescent detector (Shimadzu USA Manufacturing, Inc.). The amino acids (p-, m-, o-Tyr, Phe) were measured upon their autofluorescence, the Tyr isoforms at 275 nm excitation and 305 nm emission wavelengths, while Phe at 258 nm excitation and 288 nm emission wavelengths. The analysis was performed using a Licrospher C-18 ODS column, in an isocratic run using an aqueous solution of 1% acetic acid and 1% sodium-acetate as the mobile phase. External standard calibration and measurement of areas under the curve were used to calculate the exact concentrations of the investigated amino acids. In some cases, standard peak-addition was also used to verify the elution time of the substances. p-, m-, Tyr, and Phe were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), while o-Tyr was from ICN Biochemicals, Inc. (Aurora, OH, USA).

Determination of urinary 8-epi-prostaglandin-$F_{2\alpha}$

Urinary 8-epi-prostaglandin-$F_{2\alpha}$ levels were determined by a competitive enzyme-linked immunosorbent assay (ELISA) assay kit purchased from Oxis Research (Oxis Health Products, Portland, OR, USA).

Statistical analyses

As most data were of non-normal distribution, we used the nonparametric Kruskall-Wallis test and Mann-Whitney *U* test and Spearman's rho correlation. Because of the non-normal distribution, median and interquartile ranges were used to characterize distribution of the data. To reduce false-positive results, multiple comparisons were carried out using Kruskall-Wallis and median-test, while the Mann-Whitney *U* test was only used for 2-group comparisons, where P values < 0.05 were regarded as statistically significant.

RESULTS

Evaluation of the method

Both o- and p-Tyr could be well detected in the urine samples (Fig. 2). Urinary Phe levels were below detection limit in the majority of cases, so urinary Phe levels were not measured. In the plasma samples, p-, o-Tyr, and Phe were present, all at well measurable concentrations. Lower theoretic limit of detection for o-Tyr has been calculated, proving to be 7 nmol/L. m-Tyr was either not detectable or coeluted with another substance

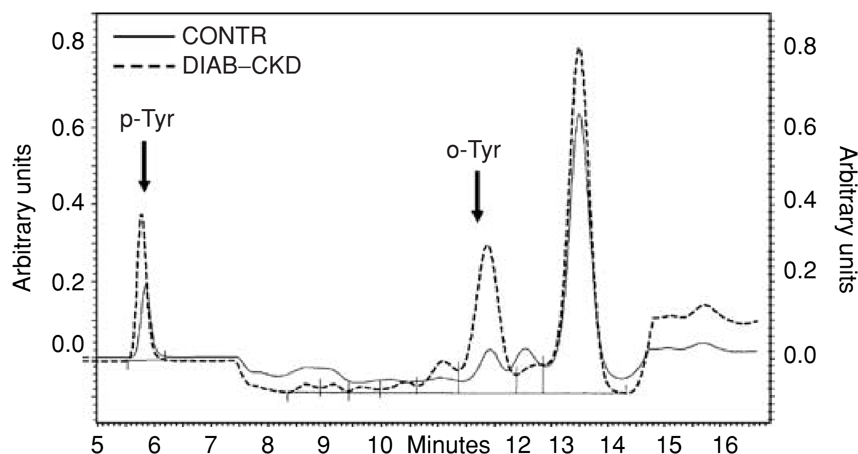

Fig. 2. Chromatogram of urine samples of a patient from the control group (—) and a patient with diabetes and chronic kidney disease (– – –). Abbreviations are: p-Tyr, para-tyrosine; o-Tyr, ortho-tyrosine.

Table 2. Plasma level and urinary excretion para- and ortho-tyrosine

	CONTR	CKD	DIAB	DIAB-CKD
Plasma p-Tyr *µmol/L*	55.96 (35.62–56.97)	28.45[a,c] (25.60–34.42)	46.11 (42.09–49.31)	32.46[c] (29.15–39.01)
Urine p-Tyr/creatinine *µmol/mmol*	4.27 (3.03–5.23)	1.80[a,c] (1.58–1.97)	5.31 (4.26–9.28)	1.95[c] (1.45–3.90)
Urinary p-Tyr excretion *µmol/day*	23.62 (16.79–81.57)	18.78[c] (5.10–40.98)	68.78 (43.84–113.63)	20.44[c] (9.11–29.74)
p-Tyr Fex %	0.67 (0.56–0.79)	1.36 (1.09–2.14)	0.98 (0.73–1.35)	1.06 (0.71–3.54)
Plasma o-Tyr *µmol/L*	0.022 (0.013–0.054)	0.050 (0.024–0.145)	0.023 (0.015–0.029)	0.054 (0.019–0.378)
Urine o-Tyr/creatinine *µmol/mmol*	0.034 (0.0001–0.035)	0.175[a] (0.056–0.481)	0.291[a] (0.103–0.330)	0.479[a,c] (0.367–0.701)
Urinary o-Tyr excretion *µmol/day*	0.24 (0.00–0.35)	1.22[a] (0.94–1.83)	3.41[a,b] (2.72–4.99)	4.03[ab] (2.58–6.51)
o-Tyr Fex%	7.86[d] (3.81–12.08)	27.28 (8.55–373.02)	125.29[a] (69.32–140.35)	111.89[a] (68.79–185.90)

Abbreviations are: CONTR, group of healthy control subjects; CKD, group of patients with stage III chronic kidney disease; DIAB, group of diabetic patients; DIAB-CKD, group of patients with diabetes and stage III chronic kidney disease; p-Tyr, para-tyrosine; o-Tyr, ortho-tyrosine; Fex, fractional excretion. Data are shown as median (interquartile range). The Mann-Whitney *U* test was only used when the Kruskal-Wallis test for all groups was significant ($P < 0.05$).

[a] $P < 0.05$ vs. control subjects.
[b] $P < 0.05$ vs. CKD patients.
[c] $P < 0.05$ vs. type 2 diabetic patients.
[d] $P < 0.05$ vs. p-Tyr Fex.

(proven by standard peak-addition); therefore, it was not determined.

To test the reproducibility of the method, interassay variations of 9 urine and plasma samples from 3 repeated measurements were calculated. Between the analyses, the samples were stored at −20°C, being frozen and thawed for each measurement. The tests were performed on 3 different days, using the same equipment, and each time the whole sample-handling procedure was carried out. The average interassay CVs in our study were 7.8% for urinary p-Tyr concentration and 7.7% for urinary o-Tyr concentration. Averages of CVs were 7.1% for plasma p-Tyr concentration and 6.3% for plasma o-Tyr concentration.

Median concentration of 8-epi-prostaglandin-$F_{2\alpha}$ in our study population was 1049 ng/mmol creatinine, which corresponds to the range described in the literature (10–1600 ng/mmol creatinine in an analysis of the Framingham study group) [17].

Plasma levels and urinary excretion of para-tyrosine

We found that the CKD group had a significantly lower plasma level of p-Tyr than the CONTR and the DIAB groups. Also, the DIAB-CKD group had a lower plasma p-Tyr level than the DIAB group (Table 2). We obtained similar results when correcting data to the plasma Phe levels (data not shown). Phe levels did not differ among the patient groups (median plasma Phe levels were 40.50 µmol/L for the CONTR group, 27.52 µmol/L for the CKD group, 31.08 µmol/L for the DIAB group, 30.03 µmol/L for the DIAB-CKD group, $P = 0.448$).

Urinary levels of p-Tyr were corrected for urinary concentrations of creatinine. Urinary concentrations of creatinine did not differ in the groups (data not shown). The CONTR group had a higher urinary p-Tyr/creatinine ratio than the CKD group. Also, the DIAB group had a higher p-Tyr/creatinine value than the CKD or the DIAB-CKD groups (Table 2). When daily p-Tyr excretion was

calculated, we found that the CKD and the DIAB-CKD groups had a lower p-Tyr excretion than the DIAB group (Table 2). Plasma levels of p-Tyr correlated with urinary levels of p-Tyr ($r = 0.468$, $P = 0.001$).

Renal clearance and fractional excretion of para-tyrosine

The CKD and the DIAB-CKD group had a lower p-Tyr clearance than the DIAB group [0.34 (0.12–0.64) and 0.46 (0.18–0.74) vs. 1.13 (0.58–1.64) mL/min, $P < 0.05$]. We found no difference when comparing the patient groups with the CONTR group [CONTR, 0.63 (0.26–0.99) mL/min]. To check the effect of the renal impairment on the handling of p-Tyr, fractional excretion (Fex) was calculated. There was no difference between the groups with regard to the Fex of p-Tyr (Table 2).

Plasma levels and urinary excretion of ortho-tyrosine

The CKD and the DIAB-CKD groups tended to have a higher plasma o-Tyr level than the CONTR group, but the observed difference was not significant ($P = 0.286$, Table 2). We obtained similar results when correcting data to the serum Phe levels (data not shown).

The urinary o-Tyr/creatinine ratio was higher in the 3 groups of patients than in the CONTR group, and higher in the DIAB-CKD group than in the DIAB group. The 3 groups of patients had significantly higher daily o-Tyr excretion than the CONTR group. The daily o-Tyr excretion of the 2 diabetic groups was also higher than in the CKD group (Table 2). The correlation between plasma levels of o-Tyr and urinary levels of o-Tyr was not significant ($r = 0.219$, $P = 0.130$).

Urinary 8-epi-prostaglandin-$F_{2\alpha}$/creatinine ratio did not correlate with plasma o-Tyr ($r = 0.04$), plasma o-Tyr/Phe ratio ($r = -0.06$), urinary o-Tyr/creatinine ratio ($r = 0.12$), or urinary o-Tyr excretion ($r = 0.16$, $P > 0.05$ for all).

Renal clearance and fractional excretion of ortho-tyrosine

There was no significant difference in the o-Tyr clearance among the groups [CONTR, 12.21 (2.13–21.26); CKD, 5.92 (2.64–59.68); DIAB, 122.34 (47.01–204.86); DIAB-CKD, 29.14 (8.94–76.22) mL/min, $P = 0.070$].

In the case of o-Tyr we found that the DIAB and the DIAB-CKD groups had a significantly higher Fex of o-Tyr than the CONTR group. The median Fex of o-Tyr exceeded 100% in both diabetic groups (Table 2). The Fex of o-Tyr was significantly higher than the Fex of p-Tyr in the control group (Table 2).

Urinary 8-epi-prostaglandin-$F_{2\alpha}$/creatinine ratio did not correlate with o-Tyr clearance or Fex of o-Tyr ($r = 0.01$ and $r = -0.167$, respectively; $P > 0.05$ for all).

DISCUSSION

In a cross-sectional study, we determined urinary and plasma p- and o-Tyr levels in diabetes and renal failure. We proved that our method was suitable for the measurement of both urinary and plasma non–protein-bound p- and o-Tyr with a good reproducibility. The plasma p-Tyr, o-Tyr, and Phe levels corresponded with the range described in the literature. Using this method, Ishimitsu et al found mean p-, o-Tyr, and Phe serum concentrations of 52 μmol/L, 17 nmol/L, and 58 μmol/L in healthy persons [16], while medians of our results for the CONTR group were 56 μmol/L (p-Tyr), 22 nmol/L (o-Tyr), and 40 μmol/L (Phe), respectively.

From data of an animal experiment [18] we know that serum levels of free o-Tyr reach highest concentrations at 15 minutes after oral, intramuscular or intraperitoneal Phe administration. Therefore, changes of free o-Tyr levels might be indicative of short-term free radical processes. However, the turnover of protein-bound o-Tyr is slower than that of free o-Tyr, and so we also tried to detect m- and o-Tyr in plasma protein hydrolysates. We found that m- and o-Tyr in the plasma protein hydrolysates did not resolve well from other peaks; therefore, they could not be measured using our method. According to the literature, the concentration of non–protein-bound free o-Tyr is 4.03 times higher than the concentration of protein-bound o-Tyr in the plasma [19]. A major aim of this study was to obtain information on the renal handling of p-Tyr and o-Tyr. To be able to calculate renal clearance and Fex of the substances, we needed to measure the non–protein-bound form of p-Tyr and o-Tyr.

It is known that in uremia there is an impaired amino acid metabolism [20, 21]. Through the amino acid analysis of uremic and azotemic sera, lower p-Tyr concentrations were found than in healthy controls (CKD vs. controls: 26 vs. 46 μmol/L [22], and 27 vs. 54 μmol/L [23]). Our corresponding data were 28 versus 56 μmol/L. The latest data of the literature imply that renal impairment causes a decreased renal phenylalanine-hydroxylase enzyme activity [24]. In our case, the p-Tyr Fex values were all below 100% (range for all groups 0.24–20.67%), indicating that p-Tyr is effectively retained by the kidney. A decreased synthesis of p-Tyr is indicated by the fact that patients with CKD had decreased plasma p-Tyr levels with a Fex similar to the CONTR group.

Free radical–derived damage has been suspected to have a biologically relevant role in the pathogenesis of complications of diabetes or chronic uremia [25]. Thus, identification of well detectable and stable markers of oxidative damage is of special interest. Among the oxidative stress markers, F_2-isoprostanes are specific to lipid peroxidation processes, and they are also generated during sample storage and handling [5]. On the contrary, o-Tyr

is specific for the hydroxyl free radical–derived damage of Phe. The fact that the 8-epi-prostaglandin-$F_{2\alpha}$ /creatinine ratio did not correlate with the parameters of o-Tyr excretion is probably due to the different origin of 8-epi-prostaglandin-$F_{2\alpha}$ and o-Tyr. Today, m-, o-Tyr, and dityrosine are accepted specific markers of hydroxyl free radical [26–28]. In our study, we observed an additive effect of diabetes and kidney failure on the excretion of the hydroxyl radical marker o-Tyr.

The abundance of plasma Phe (27.52 μmol/L Phe vs. 0.05 μmol/L o-Tyr in the CKD group) makes it unlikely that the amount of Phe would be rate-limiting in the o-Tyr formation. Plasma o-Tyr/Phe ratio and plasma o-Tyr concentration showed the same tendencies. This also supports that o-Tyr levels would rather reflect hydroxyl radical formation than the changes in Phe levels.

The lack of correlation between urinary excretion and plasma level of o-Tyr may indicate that the urinary excretion of o-Tyr is not determined by glomerular filtration alone, but by active renal transport processes, as well. Our data also confirm that the renal handling of o-Tyr is different among the patient groups. The median of fractional excretion of o-Tyr was above 100% in both diabetic groups. Such a Fex value of a substance could be a consequence of 2 processes (i.e., active tubular secretion of the substance or production of the substance in loco in the kidney). With our data, we cannot distinguish which process is superior. In diabetes, concentration of glucose in the urine may rise from 100 to 200 mmol/L (unpublished data). Glucose-derived oxidative stress on tubular cells may increase the production and, in this way, Fex of o-Tyr in the DIAB and the DIAB-CKD groups.

Our data also suggest that p-Tyr is more efficiently (approximately 10 times) retained by the kidney than o-Tyr, even in the control group (Table 2), which indicates a different renal handling of p- and o-Tyr even though the only difference between the 2 amino acids is the location of the hydroxyl group. This may be an adaptive process, as the physiologic Tyr isoform is retained, while the pathologic isoform is excreted.

CONCLUSION

We found that the physiologic p-Tyr is retained by both the healthy and the damaged kidney. Also, its production is decreased in renal impairment. According to our findings and data of the literature, measurement of urinary and plasma o-Tyr is a simple and valuable method for the indirect detection of hydroxyl free radical production. In patients with type 2 diabetes mellitus with or without renal failure, the renal excretion of o-Tyr is enhanced as a consequence of an increased renal tubular secretion or production.

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