ORIGINAL ARTICLE

Chronic kidney disease in adolescent and adult patients with phenylketonuria

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Abstract

Objectives A lifelong phenylalanine-restricted diet with supplementation of a phenylalanine-free amino acid formula is recommended in patients with phenylketonuria (PKU).

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The effect of a long-term PKU diet on renal function and blood pressure has not been investigated yet.

Design We analyzed renal function in 67 patients with PKU, aged 15–43 years, by measuring glomerular filtration rate (GFR) and effective renal plasma flow by isotope clearance (^{51}Cr-EDTA, ^{123}J-Hippuran), estimated GFR, blood retention parameters, urinary protein and electrolyte excretion. Renal ultrasound and 24 h ambulatory blood pressure monitoring were performed additionally. Patients were divided into three groups according to their: 1) current diet (CD), i.e., daily protein intake: I_{CD} <0.8 g/kg, II_{CD} 0.8–1.04 g/kg, III_{CD} >1.04 g/kg; 2) life-long diet time (LDT), i.e., cumulative years of life in which daily protein intake exceeded dietary recommendations: I_{LDT} <15 years, II_{LDT} 15–19 years, III_{LDT} >19 years.

Results GFR was decreased in 19 % of the patients. With increasing protein intake, GFR decreased significantly (I_{CD} 111 ml/min; II_{CD} 105 ml/min; III_{CD} 99 ml/min. I_{LDT} 112 ml/min; II_{LDT} 103 ml/min; III_{LDT} 99 ml/min). Proteinuria was detected in 31 %, microalbuminuria in 7 %, and hypercalciuria in 23 % of the patients. 23 % of the patients had arterial hypertension, and 41 % revealed a nocturnal non-dipping status.

Conclusions In patients with PKU on a lifelong diet we could detect impaired renal function in 19 %, proteinuria in 31 %, and arterial hypertension in 23 %. Thus, chronic kidney disease may develop in PKU patients, and routine renal function tests should be performed during long-term follow-up.

Abbreviations

AA	Amino acids
ABPM	Ambulatory blood pressure monitoring
BMI	Body mass index
BP	Blood pressure
CD	Current diet

CKD   Chronic kidney disease
eGFR   Estimated glomerular filtration rate
ERPF   Effective renal plasma flow
GFR   Glomerular filtration rate
LDT   Life-long diet time
PAH   Phenylalanine hydroxylase
Phe   Phenylalanine
PKU   Phenylketonuria
RDA   Recommended daily allowance

Introduction

Phenylketonuria (PKU, MIM 261600) is a rare autosomal recessive inborn error of metabolism caused by phenylalanine-4-hydroxylase (PAH, EC 1.14.16.1) deficiency (Scriver and Kaufman 2001). In untreated children, PKU results in severe neurological impairment with mental retardation, seizures, and behavioral disorders. Normal mental and motor activity skills can be achieved by early institution of a phenylalanine (Phe)-restricted diet consisting of a strong restriction of natural protein intake and substitution with a Phe-free L-amino acid (AA) formula (Scriver and Kaufman 2001). The Phe-restricted diet consists of foods with low protein content, e.g., vegetables, fruits and special low-protein food products. Most of the protein supply in PKU patients, e.g., 75–95 % of the whole protein intake, derives from the prescribed Phe-free AA formula (Mönch et al 1996). The allowed amount of the daily natural protein intake is dependent on the individual residual PAH enzyme activity corresponding to the severity of the disease. At the current state of knowledge, diet is recommended to be kept life-long.

To prevent a nutritional deficiency in essential AA, patients with PKU receive high amounts of the AA formula (Krauch et al 1996). Thus, protein intake in patients with PKU almost entirely consists of the AA formula, and often exceeds the current recommended daily allowance (RDA) for the general population, especially during the first years of life (D-A-CH Empfehlung 2000; Acosta et al 1998; Arnold et al 2002; Hoeksma et al 2005). AA formulas consist of synthetic mono AA, which have a lower biological efficacy than natural protein. The intake of the synthetic AA leads to peak plasma AA concentrations shortly after ingestion resulting in a high renal acid load (Mönch et al 1996; Manz et al 1977). This is in marked contrast to the stable plasma AA concentrations after the intake of intact natural protein (Gropper et al 1993).

Dietary protein is a well known modulator of kidney function (Brenner et al 1982; King and Levey 1993). The hyperfiltration theory suggests that protein consumption acutely results in an increase of renal plasma flow and glomerular filtration rate (GFR), leading to hyperfiltration and hypertension, thus resulting in chronic glomerular injury, fibrosis and mesangial cell proliferation (Bernstein et al 2007; Brenner et al 1996). In animal experiments, a high protein diet resulted in renal and glomerular enlargement, collagen deposition and tubulointerstitial infiltration, leading to cortical fibrosis and glomerulosclerosis (Jia et al 2010). In adult patients with chronic kidney disease (CKD), a low protein diet has been recommended to delay the progression of renal failure (Brenner et al 1982; Fouque and Aparicio 2007; Schena 2011).

Patients with PKU have to keep a life-long Phe-restricted diet, but it is unknown whether the relatively high total protein intake with the high proportion of synthetic AA may have an effect on renal function. We have therefore investigated the effect of the PKU diet on renal structure, function and blood pressure (BP) in adolescent and adult patients with PAH deficiency.

Study population and methods

Study population

Eighty patients with PKU treated in the metabolic unit of the Department of Pediatrics at the Charité Berlin were eligible for this cross-sectional study evaluating clinical and biochemical data, ultrasound studies, renal function, 24-h ambulatory blood pressure monitoring (ABPM), and dietary history. Exclusion criteria were: age <15 years, pregnancy or breastfeeding. Of a total of these 80 patients, 13 refused participation completely and 28 patients refused participation in some parts of the study. Therefore, of the 67 patients participating in the study, ultrasound was performed in 61 patients, ABPM in 44 patients and radioisotope clearance studies in 59 patients. Informed consent was obtained from all patients and/or their parents.

The median age of the patients was 24 years (15–43 years) with 38 females and 29 males. According to established criteria (Guldberg et al 1998; Güttler and Guldberg 1994), 60 % of the patients were classified as classic PKU (n=40), 37 % as mild PKU (n=25) and 3 % as hyperphenylalaninemia (n=2). Fifty-eight patients were diagnosed by newborn screening, nine patients were diagnosed by selective screening at a median age of 42 months (3–171 months). In total, we collected data of cumulative 1600 patient-years.

Chart review showed that dietary treatment had been started in all patients immediately after diagnosis of PKU. At the time of examination, 82 % of the patients (n=55) kept a Phe-restricted diet with supplementation of a Phe-free AA formula, 3 % (n=2) kept a diet low in natural protein without additional AA formula, and 15 % (n=10) had

stopped adhering to the diet. Protein intake was calculated regularly by three-day-dietary-protocols. At the time of examination AA formula was applied thrice a day (AA formulas and hydrolysates supplemented in the patients are shown in Supplementary Table 1).

Methods

Clinical examination including body mass index (BMI) was performed in every patient. According to the World Health Organization "overweight" was defined as a BMI equal to or more than 25, and "obesity" as a BMI equal to or more than 30. ABPM was performed by using Model 90207(−32), Spacelabs Medical (USA). Arterial hypertension was defined according to established criteria as a daytime average above systolic 135 mmHg and/or systolic 85 mmHg (Parati and Pickering 2009; O'Brien et al 2005; Wühl et al 2002). A decrease of systolic BP during nocturnal time by ≥10 % of the diurnal BP was defined as a "dipper", a decrease of <10 % as a "non-dipper" (Kastarinen et al 2010). In three patients without nocturnal sleeping phase during ABPM, "dipping"-/"non-dipping"-status was not discriminated. Renal ultrasound was performed with a 50/60 Hz transducer (Sonoline Antares, Siemens, Germany).

Laboratory analysis was performed in the fasting state in all patients and included the determination of serum creatinine, urea, uric acid, cystatin C, blood gases, electrolytes, total protein, albumin, vitamin D status and parathormone. Excretion of creatinine, protein, albumin, alpha-1-microglobuline, immunglobulin G, glucose, and electrolytes was analyzed in a 24-h urine collection. AA in plasma and urine were measured by cation exchange chromatography (Biotronic/Eppendorf). Plasma Phe was analyzed regularly in all study patients, according to the German recommendations for the treatment in PKU (Burgard et al 1999).

Renal function was determined by radio-isotope clearance: 80 kBq ^{51}Cr-EDTA/kg and 15 kBq ^{123}J-Hippuran/kg were simultaneously applied intravenously, and measured 30 min after application in a 2.0 ml plasma sample. ^{51}Cr-EDTA clearance was used to evaluate GFR, ^{123}J-Hippuran clearance to evaluate effective renal plasma flow (ERPF), and the ratio of ^{51}Cr-EDTA/^{123}J-Hippuran clearances to evaluate the filtration fraction (Hüseman et al 1999). Upper and lower cut off levels of GFR and ERPF were defined as ±2 SD. Additional informed consent for performing radio-isotope clearance was obtained from all patients and/or their parents.

The estimated GFR (eGFR) was calculated using the formula of Schwartz for adolescent patients ≤16 years (n= 6) and the "modification of diet in renal disease" (MDRD) formula for adult patients (n=61) (Kooman 2009; Schwartz et al 2009; Staples et al 2010). Urinary creatinine clearance

was not evaluated, since inaccuracy of sampling might have resulted in a falsely low estimation of GFR.

PAH genotype was determined in 60 patients, as previously described (Hennermann et al 2000). According to their genotype patients were classified into two different groups. Group 1: patients with two null mutations, corresponding to a complete loss of residual PAH activity; group 2: patients with at least one putative milder mutation, corresponding to a certain residual PAH activity. In three patients only one mutation was identified; they were not included in any group.

Statistics

For statistic analyses PASW Statistics, Version 18.0, was used. Differences between patients groups were analyzed by non-parametric tests, the Mann-Whitney-test, and the Jonckheere-Terpstra test. Significance of correlations of variables was tested with the Spearman-Rho-correlation coefficient. Multiple forward stepwise regression analysis was performed to analyze the significance of effects of several variables on the outcome variable, GFR measured by isotope clearance.

Results

Protein intake

Median current total protein intake in all 67 PKU patients was 0.96±0.23 g/kg/day. Current total protein intake was 1.01±0.23 g/kg/day in patients on a Phe-restricted diet with AA formula (n=55), 0.57±0.01 g/kg/day in patients on a low protein diet without AA formula (n=2), and 0.75± 0.00 g/kg/day in patients off PKU diet (n=10).

According to their dietary protein intake, patients were divided into three groups: according to the amount of their *current diet* (CD), and according to their *life-long diet time* (LDT). CD was determined by the amount of total daily protein intake and daily AA intake (Table 1). LDT was determined by the number of cumulative years of life in which the daily protein intake has exceeded German RDA of 1985 (DGE Empfehlung 1985) (Table 2).

Clinical examination

Clinical examinations revealed no significant abnormalities in any patient. Median BMI was 23.9±4.8 kg/m^2 and was increased in 25/67 patients (37.3 %). Sixteen patients (23.9 %) were overweight, nine patients were obese (13.4 %). There was an inverse correlation between BMI and actual total and synthetic protein intake (p=0.000; r=−0.470, r=−0.421, respectively). None of the patients was suffering from any other

Table 1 Classification of PKU patients according to their actual protein intake (CD)

Current total protein intake and current synthetic protein intake are indicated in mean with minimum and maximum.

Group	Number of patients	Current total protein intake (g/kg/day)	Current AA intake (g synthetic protein intake/kg/day)
I-CD	21	0.75 (0.56−0.79)	0 (0−0.57)
II-CD	22	0.90 (0.80−1.04)	0.70 (0.58−0.79)
III-CD	22	1.14 (1.05−1.67)	0.94 (0.80−1.45)

inborn error of metabolism or from insulin dependent or non-insulin dependent diabetes.

Renal ultrasound

Renal ultrasound revealed bilateral nephrocalcinosis in 1/61 patient, associated with increased calcium excretion. Increased renal echogenity was found in 4/61 patients (6.6 %), associated with arterial hypertension and increased calcium excretion in 3/4. None of these patients received calcium supplementation. One patient showed unilateral polycystic changes, one patient a renal position abnormality. The size of both kidneys was within normal range with a mean size of 10.7 ± 0.75 cm (left), and 10.4 ± 0.78 cm (right), respectively, and correlated significantly with serum creatinine ($p = 0.041$).

Hypertension

Mean 24-h systolic BP was 127.0 ± 14.1 mmHg (105–182 mmHg), diastolic BP 77.6 ± 9.6 mmHg (58–118 mmHg), and MAD 93.4 ± 10.4 mmHg (73–138 mmHg). Arterial hypertension was diagnosed in 22.7 % of the patients (10/44). 41 % of the patients (17/41) revealed a nocturnal non-dipping status. Neither arterial pressure nor dipping status were correlated with GFR, eGFR, protein intake (CD, LDT), or proteinuria. There was a significant correlation of all BP parameters with BMI ($p = 0.000$; $r = 0.510$).

GFR

GFR measured by ^{51}Cr-EDTA was 107 ± 15.5 ml/min/1.73 m^2 (range 64–149; norm 95–161). GFR was decreased in 18.6 %

Table 2 Classification of PKU patients according to their life-long protein intake (LDT)

Group	Number of patients	Number of years exceeding protein intake
I-LDT	19	12 (4−14)
II-LDT	17	17 (15−19)
III-LDT	19	22 (20−34)

Indicated are the number of years in which the total protein intake in PKU patients exceeded the German RDA from 1985 (DGE Empfehlung 1985)

Values are indicated in mean with minimum and maximum

of the patients ($n = 11$) and normal in 81.4 % of the patients ($n = 48$). ERPF measured by ^{123}J-Hippuran was 707 ± 159 ml/min/1.73 m^2 (range 426–1197; norm 515–916), and revealed an increase corresponding to hyperfiltration in four patients (6.8 %), a decrease in five patients (8.5 %), and normal values in 50 patients (84.7 %). ERPF correlated significantly with GFR ($p = 0.000$; $r = 0.512$). Filtration fraction was increased in five patients (8.5 %) and normal in 54 patients (91.5 %). GFR decreased significantly with increasing protein intake, both was correlated with CD and LDT (Figs. 1, 2, and 3). GFR was associated with BMI ($p = 0.025$; $r = 0.295$); in contrast, there was no correlation of GFR with the patient's age. Neither ERPF nor filtration fraction were correlated with CD or LDT. Data of patients with diminished GFR are included in Supplementary Table 2.

The eGFR correlated significantly with ^{51}Cr-EDTA (Fig. 4). eGFR was 108 ± 18.1 ml/min/1.73 m^2 (range 74–157; norm 90–160). eGFR was decreased in 10.4 % of the patients ($n = 7$) and normal in 89.6 % of the patients ($n = 60$). The eGFR did not correlate with CD, LDT or BMI.

By multiple forward stepwise regression analysis, only proteinuria and the current total protein intake were independent predictors of the GFR estimated by isotope clearance ($p < 0.005$; $r^2 = 0.201$).
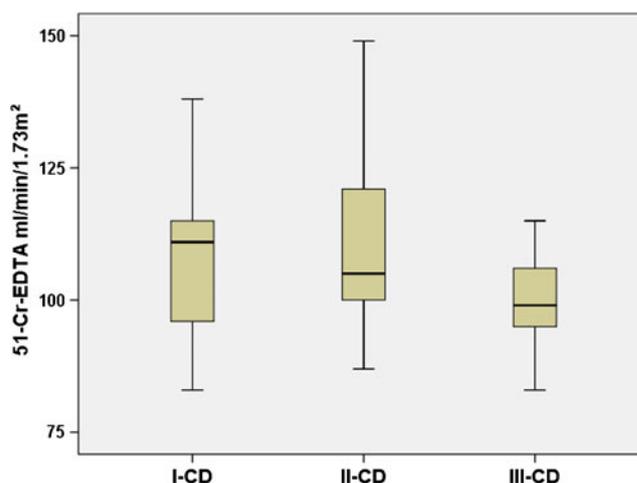

Fig. 1 ^{51}Cr-EDTA related to current total protein intake ^{51}Cr-EDTA was measured in 59 patients. Median ^{51}Cr-EDTA was 111 ml/min in the group with the lowest total protein intake (I-CD), 105 ml/min in the group with the medium total protein intake (II-CD), and 99 ml/min in the group with the highest total protein intake (III-CD). The correlation of ^{51}Cr-EDTA with current total protein intake was significant ($p = 0.008$; $r = 0.341$)
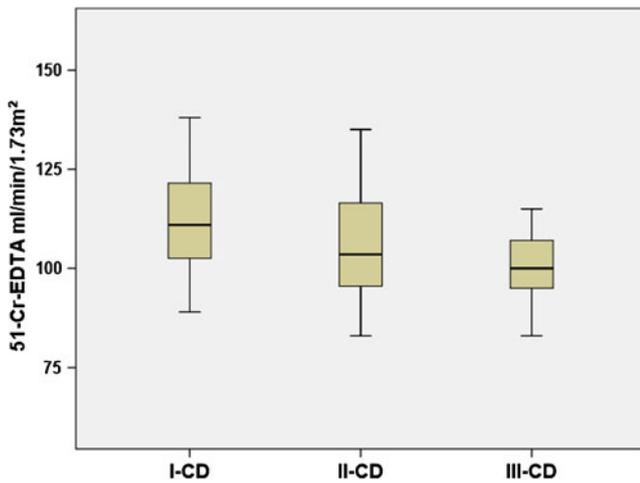
Fig. 2 ^{51}Cr-EDTA related to current AA intake (synthetic protein intake) ^{51}Cr-EDTA was measured in 59 patients. Median ^{51}Cr-EDTA was 111 ml/min in the group with the lowest AA intake (I-CD), 105 ml/min in the group with the medium AA intake (II-CD), and 100 ml/min in the group with the highest AA intake (III-CD). The correlation of ^{51}Cr-EDTA with current AA intake was significant ($p=0.011$; $r=-0.331$)

Creatinine and cystatin C serum concentrations were within normal ranges in all patients. There was a significant correlation between serum creatinine and ^{51}Cr-EDTA ($p=0.000$; $r=0.519$). Both, creatinine and cystatin C, increased non-significantly with increasing protein consumption.

Proteinuria and Phe excretion

Mean serum levels of protein and albumin were within normal ranges. Proteinuria, defined as a protein excretion
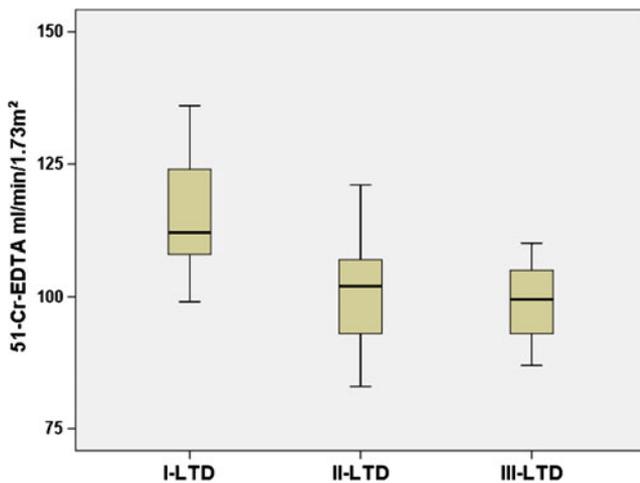

Fig. 3 ^{51}Cr-EDTA related to life-long-diet ^{51}Cr-EDTA was measured in 59 patients. Median ^{51}Cr-EDTA was 112 ml/min in the group with the lowest life-long protein intake (I-LDT), 103 ml/min in the group with the medium life-long protein intake (II-LDT), and 99 ml/min in the group with the highest life-long protein intake (III-LDT). The correlation of ^{51}Cr-EDTA with life-long-diet was significant ($p=0.003$; $r=-0.410$)
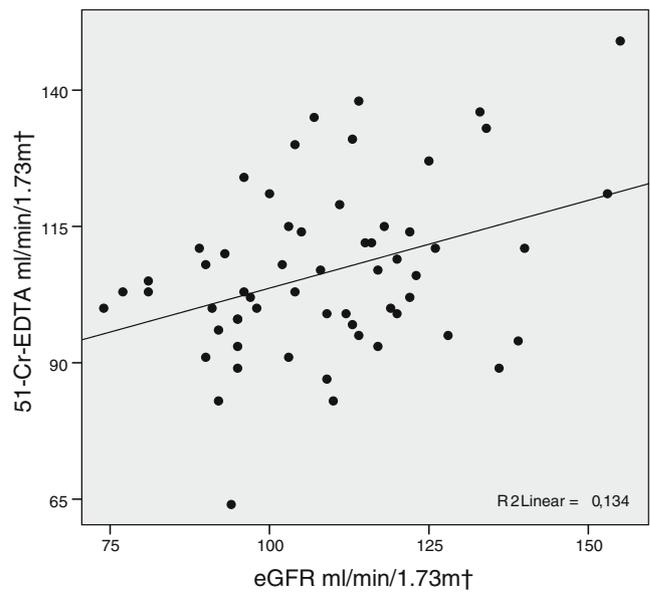

Fig. 4 ^{51}Cr-EDTA related to eGFR ^{51}Cr-EDTA was measured in 59 patients, eGFR in 67 patients. The correlation of ^{51}Cr-EDTA and eGFR was significant ($p=0.029$; $r=0.285$)

of >150 mg/24 h (Guy et al 2009), was found in 19/62 patients (30.6 %). Microalbuminuria, defined as an albumin excretion of 30–300 mg/g creatinine (Blecker et al 2011), was detected in 4/62 patients (6.5 %). Proteinuria was not correlated to BMI. The excretion of alpha-1-microglobulin was increased in one patient (18.3 mg/g creatinine), the excretion of immunglobulin G in two patients (maximum 25.1 mg/g creatinine).

Urinary AA analysis revealed no hyperaminoaciduria in any patient. Urinary Phe concentrations were increased to 0.713±0.375 mmol/g creatinine (range 0.087–2.429; norm <0.19) and correlated significantly with actual and life-long Phe plasma concentrations ($p=0.000$, $p=0.001$; $r=0.727$, $r=0.488$, respectively). Urinary Phe excretion correlated significantly with GFR ($p=0.011$; $r=0.332$), proteinuria ($p=0.007$; $r=0.338$), and systolic BP ($p=0.040$; $r=0.311$). Actual Phe plasma levels were 796±317 μmol/L (range 169–2,143; recommendations for PKU patients >15 years 40–1,200) and exceeded actual German recommendations in only 4/67 patients. However, actual or life-long plasma Phe concentrations did not correlate with GFR, eGFR, proteinuria, or BP.

Calcium, phosphate, hypercalciuria and vitamin D

Serum calcium was slightly increased in 3/61 patients (4.9 %), and serum phosphate was decreased in 3/59 patients (5.1 %). Calcium excretion was 0.17±0.09 g/g creatinine (range 0.04–0.54; norm <0.2) and was increased in 22.7 % of the patients (15/66). Renal tubular reabsorption

of phosphate (TmP/GFR) was within the normal range (0.96±0.27 mmol/L; norm 0.6–1.7 mmol/L). In only two patients, both, calcium and phosphate excretion were increased. The serum calcium phosphate product was normal in all patients. An increase in calcium excretion was significantly associated with CD, mainly with actual synthetic AA intake (Fig. 5), but there was no correlation to LDT. Phosphate reabsorption was not correlated to CD or LDT. Venous blood gas analysis revealed no abnormalities in any patient.

Mean parathormone concentrations were 2.89± 1.11 pmol/L (norm 1.6–6.9 pmol/L) and revealed no signs of hyperparathyreoidism. The 25-hydroxy-vitamin D(3) serum levels were within the normal range (24.3±8.8 ng/ml; norm 9.2–45.2 ng/ml). Mean 1,25-dihydroxy-vitamin D(3) serum levels were 50.4±17.7 ng/L (norm 17–53 ng/L) and were increased in 24/64 patients (median: 69.6 ng/L, range: 56–113 ng/L), 21 of them on synthetic AA substitution (which contained 0.13–0.2 μg vitamin D3/gram protein). However, there was no correlation between nutritional supply with D3 and either 25-hydroxy-vitamin D(3) or 1,25-dihydroxy-vitamin D(3) levels. Vitamin D deficiency was not diagnosed in any of the patients. There was no correlation between vitamin D3 intake and hypercalciuria.

PAH genotype

34/57 patients carried two null mutations (genotype group 1), 23/57 patients carried at least one putative milder *PAH* mutation (genotype group 2). No significant differences
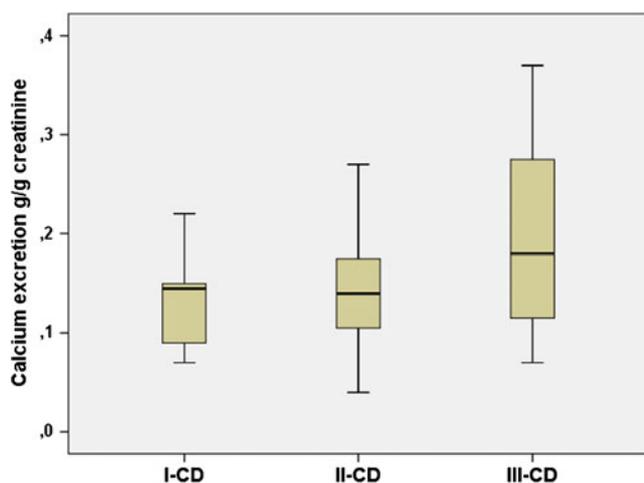

Fig. 5 Calcium excretion related to current synthetic protein intake calcium excretion was measured in 66 patients. Median urinary calcium concentration was 0.15 g/g creatinine in the group with the lowest AA intake (I-CD), 0.12 g/g creatinine in the group with the medium AA intake (II-CD), and 0.21 g/g in the group with the highest AA intake (III-CD). The correlation of calcium excretion and current synthetic protein intake (AA intake) was significant (p=0.008)

between both groups were found for CD, LTD, GFR, eGFR, and proteinuria (Table 3).

Discussion

Dietary protein is a well known modulator of kidney function (Brenner et al 1982; King and Levey 1993). The effect of the PKU diet, which is characterized by a relative high protein content with a high proportion of synthetic AA, on renal function has not been previously examined. We show that adolescent and adult PKU patients may develop CKD (impaired renal function, proteinuria) and arterial hypertension. In contrast, CKD in the general population is mainly prevalent at older ages (Zhang and Rothenbacher 2008).

The RDA for the daily protein intake has changed within the last decades, and a lower nutritional protein intake is currently recommended (D-A-CH Empfehlung 2000; DGE Empfehlung 1985; DGE Empfehlung 1991). Although median total protein intake in PKU patients exceeded these RDA, the amount of total protein intake was still within the range of a typical Western diet and, thus, may not account for the development of CKD in our patients (Fouque and Aparicio 2007; Halbesma et al 2009). However, we could demonstrate that GFR decreased significantly with increasing total protein and increasing AA intake, both related to CD as well to LDT. Overall, we found hyperfiltration in only 7 % of the patients, but a decrease of GFR in 19 % of the patients. Although Phe might interfere with renal clearance of hippurate due to the competition for renal anion transporters (Enomoto and Niwa 2007), there were no correlations with plasma or urine Phe values and ERPF measured by [123]J-Hippuran.

Importantly, protein composition in PKU diet differs from that of healthy population and mainly derives from the synthetic AA formula. Ingestion of AA formula results in plasma peak AA concentrations (Mönch et al 1996; Gropper et al 1993), and high plasma AA concentrations have been shown to be nephrotoxic in animals, resulting in a significant decrease of GFR, an increase in albuminuria and histological changes consistent with tubular damage (Zager et al 1983). A similar pathomechanism may account for the renal damage in PKU patients.

An increase in renal protein excretion was detected in more than 30 % of the PKU patients, and microalbuminuria in 7 % of the patients. Multivariant linear regression analysis revealed a significant association of GFR measured by ^{51}Cr-EDTA with both, current total protein intake and proteinuria, indicating that protein intake and proteinuria are involved independently in the pathomechanism of renal injury in phenylketonuric patients.

Table 3 Protein intake and renal function in PKU patients related to their genotype

Genotype group	Number of patients	Frequency of *PAH* mutations		Total protein intake (g/kg/day)	Natural protein intake (g/kg/day)	Synthetic protein intake (g/kg/day)	LDT	GFR (ml/min/1.73 m^2)	eGFR (ml/min/1.73 m^2)	Urinary protein excretion (mg/24 h)
Genotype group 1	34	p.R408W	50 %	0.98±0.25	0.27±0.18	0.72±0.35	18.0±5.1	105.4±17.5	110.0±18.8	149±299
		IVS12-1G >A	16 %							
		IVS10-11G >A	15 %							
		p.R243X	4 %							
		p.G272X	3 %							
		p.F299C	3 %							
		others	15 %							
Genotype group 2	23	p.R408W	17 %	0.96±0.24	0.30±0.18	0.66±0.34	15.4±5.6	104.7±12.4	113.0±17.4	62±74
		p.R261Q	13 %							
		p.Y414C	9 %							
		p.R413P	4 %							
		IVS10-11G >A	4 %							
		p.G272X	4 %							
		p.P281L	4 %							
		p.A395P	4 %							
		others	39 %							

Genotype group 1: patients with two null mutations, corresponding to a complete loss of residual PAH activity

Genotype group 2: patients with at least one putative milder mutation, corresponding to a certain residual PAH activity

Data are given in mean ± SD. Differences between genotype group 1 and genotype group 2 are not significant

Arterial hypertension was found in one fourth of the PKU patients, reflecting an important secondary health problem in PKU patients. Furthermore, 40 % of the patients revealed a nocturnal non-dipping status, which is known to be associated with CKD (Kastarinen et al 2010). Arterial BP was significantly associated with BMI, but not with actual or lifelong protein intake, GFR or proteinuria. More than one third of the examined patients revealed an increase in BMI. It has been reported before that obesity is a common problem in PKU patients (MacDonald et al 2011). Though, it is our experience and that of others that caloric intake in PKU patients is within normal limits (Acosta 1996). An increase in BMI in PKU patients may be due to inadequate energy expenditure (Acosta et al 2003; White et al 1982). Furthermore, our data reveal that BMI is inversely correlated with the actual total and synthetic protein intake. Obesity is a known risk factor for glomerulosclerosis (Kambham et al 2001), which is reflected by our data, revealing an association of BMI and GFR. Though, BMI was not associated proteinuria in our study. Hence, obesity is a further risk factor for CKD in PKU patients.

Calcium excretion significantly increased with increasing protein intake, mainly with increasing synthetic AA intake, whereas the time of the lifelong diet (LDT) seemed not to influence calcium excretion. A high protein intake is associated with an increase in calcium excretion (Itoh et al 1998). An increase in calcium excretion (found in 23 % of patients) could imply an additional long-term risk for PKU patients on the current diet. However nephrocalcinosis was rarely observed and the presence of hypercalciuria was not associated with diminished GFR, proteinuria or hypertension. 1,25-dihydroxy-vitamin D(3) serum concentrations were increased in 38 % of the PKU patients, but there was no correlation between the nutritional vitamin D3 supply with either 25-hydroxy-vitamin D(3) or 1,25-dihydroxy-vitamin D(3) levels. However, 1,25-dihydroxy-vitamin D(3) levels are tightly regulated on the level of synthesis, independent of nutritional supply (Anderson et al 2004). Furthermore, an increase in vitamin D(3) serum concentrations was not associated with hypercalciuria. Although actual recommendations indicate a higher vitamin D intake in adolescents and adults (German Nutrition Society 2012), none of our patients had vitamin D deficiency.

Several putative pathomechanism could explain the association of (synthetic) protein intake and a decrease in renal function. First, Phe excretion was significantly correlated with GFR and proteinuria. Although a nephrotoxic effect of Phe has not been described yet, renal damage by high urinary Phe concentrations may contribute to the development of CKD in PKU. Second, increased oxidative stress has been demonstrated in PKU patients and attributed to an increased free radical generation, a deprivation of micronutrients or an increase of phenylalanine and its metabolites (Sirtori et al 2005; Sitta et al 2009; Ribas et al 2011). Therefore, oxidative stress could be involved in the pathophysiology of the tissue damage and chronic renal injury found in PKU. Third, it has been postulated that human kidney PAH may play a significant role in phenylalanine homeostasis. PAH has been shown to be expressed in human kidneys (Lichter-Konecki et al 1999), and impaired phenylalanine hydroxylation has been observed in renal failure (Zhao et al 2012). Thus, renal PAH expression could influence renal function in PKU patients, although in our study an association with the *PAH* genotype could not be found. In contrast, hyperfiltration, a well-known mediator of renal injury associated with a high protein intake (Bernstein et al 2007; Brenner et al 1996), was not observed in our patient's cohort.

CKD in patients with PKU may benefit from an early start of treatment. Although the effects of a low-protein diet on the progression of CKD has been the subject of controversy, a protein restriction of 0.6–0.8 g/kg/day is recommended in adult patients with end-stage CKD (Fouque and Aparicio 2007). In contrast, a protein-restricted diet seems to have no significant impact on the progression of CKD in children (Wingen et al 1997; Chaturvedi and Jones 2007). Thus, at the current state of knowledge, recommendations for protein intake in PKU patients with CKD are difficult to establish. However, given the potential nephrotoxicity of AA, we recommended substitution of the AA formula in at least three daily doses (Mönch et al 1996). In addition to dietary protein restriction, ACE inhibitors or angiotensin-receptor blockers were shown to have a beneficial effect on progression of CKD (Kriz 2004). Therapy with these drugs could be beneficial in view of the high incidence of arterial hypertension in patients with PKU; however, efficacy of such treatment remains to be established in this patient population.

Conclusions

In conclusion, we found a high prevalence of proteinuria, decreased GFR, hypercalciuria, and arterial hypertension in adolescent and adult PKU patients. An increased intake of protein with a high content of synthetic AA due to lifelong prescription of a PKU diet and the presence of proteinuria were independently associated with the decrease in GFR. Although GFR was within the normal range in the whole group of patients, there was a continuous and graded relationship of both, CD and LTD, with decreasing GFR. The intake of high amounts of synthetic AA, increased oxidative stress, and local damage through high Phe excretion

may contribute to renal damage in PKU. Arterial hypertension was mainly explained by increased BMI and unrelated to protein intake or proteinuria. Altogether, PKU patients constitute a high risk group for the development of CKD.

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