

*Original Article***Plasma pteridine concentrations in patients with chronic renal failure**Keitaro Yokoyama<sup>1</sup>, Masamichi Tajima<sup>2</sup>, Hiraku Yoshida<sup>1</sup>, Masaaki Nakayama<sup>1</sup>, Goro Tokutome<sup>1</sup>, Hiroshi Sakagami<sup>2</sup> and Tatsuo Hosoya<sup>1</sup><sup>1</sup>The Division of Nephrology and Hypertension, Jikei University School of Medicine, Tokyo and <sup>2</sup>Department of Dental Pharmacology, Meikai University School of Dentistry, Sakado, Saitama, Japan**Abstract**

**Background.** Pteridine metabolism is impaired in the uraemic state. This may affect cardiovascular function and contribute to malnutrition. We wished to clarify further the impact of impaired pteridine metabolism.

**Methods.** Using the HPLC method, the plasma concentrations of endogenous pteridines were determined in 64 patients with chronic renal failure (33 on intermittent haemodialysis (HD) treatment vs 31 not yet on renal replacement therapy), and in 18 healthy controls. The patients were classified into three groups on the basis of creatinine clearance (Ccr): group (a), Ccr > 60 ml/min; group (b), Ccr = 10–60 ml/min; group (c), all patients receiving HD.

**Results.** Total neopterin (NP) and biopterin (BP) levels and the NP/BP ratio (a biomarker for macrophage activity) were significantly higher, whereas tetrahydrobiopterin (BH<sub>4</sub>)/dihydrobiopterin (BH<sub>2</sub>) ratio (a biomarker for nitric oxide synthase and phenylalanine hydroxylase activities) was significantly lower in group (c) (118.9 ± 11.7 ng/ml, 18.8 ± 1.2 ng/ml, 6.79 ± 0.53, and 0.26 ± 0.06) than in healthy subjects (5.17 ± 0.29 ng/ml, 2.83 ± 0.19 ng/ml, 1.92 ± 0.13, and 1.15 ± 0.11; *P* < 0.01). These significant differences were also observed between control and group (b) (12.4 ± 2.20 ng/ml, 4.48 ± 0.36 ng/ml, 2.81 ± 0.48, and 0.74 ± 0.08; *P* < 0.01). In groups (a) and (b), significant negative correlations were found between Ccr and the total NP level (*r* = -0.663, *P* < 0.01), the total BP level (*r* = -0.492, *P* < 0.01), the BH<sub>2</sub> level (*r* = -0.677, *P* < 0.01), and the NP/BP ratio (*r* = -0.493, *P* < 0.01). Conversely, significant positive correlations were found between Ccr and the BH<sub>4</sub>/BH<sub>2</sub> ratio (*r* = 0.602, *P* < 0.01).

**Conclusion.** The reduction of quinoid-type BH<sub>2</sub> to BH<sub>4</sub> is modified in patients with advanced chronic renal failure, before and after the initiation of regular

HD treatment. These metabolic alterations may play a role in the impaired macrophage, endothelial constitutive nitric oxide synthase, or phenylalanine hydroxylase (PH) activities observed in such patients.

**Keywords:** chronic renal failure; dihydrobiopterin; NADH; nitric oxide; superoxide; tetrahydrobiopterin

**Introduction**

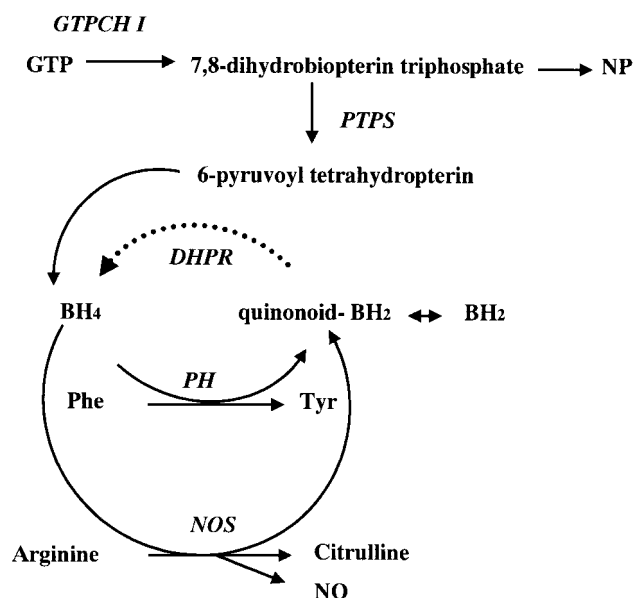
The major complications for patients on haemodialysis (HD) are cardiovascular diseases and malnutrition, which often determine their mortality [1–5]. Cardiovascular diseases are known to be related to the acceleration of inflammation that may cause the nutritional state to deteriorate in these patients. Therefore, many studies have focused on the relationship between inflammatory reactions and malnutrition in HD patients, although the exact mechanism has not been elucidated.

Tetrahydrobiopterin (BH<sub>4</sub>), a substance belonging to the pteridine group, is very important for the role it plays in the metabolism of some amino acids because it acts as a coenzyme, such as the ones for phenylalanine hydroxylase (PH), tyrosine hydroxylase, and other enzymes (Figure 1) [6]. Recently, in addition to the role for amino acid metabolism, it has been reported that BH<sub>4</sub> is related to the control of the endothelium-dependent vascular function by supporting nitric oxide (NO) production as a coenzyme of NO synthase (NOS).

In the absence of BH<sub>4</sub> activation, the plasma levels of phenylalanine can be elevated, and active oxygen might be generated in quantities greater than that released by NO because of the oxidase activity of NOS, which activates an inflammatory reaction [7–9]. In animals, it has been reported that the impairment of BH<sub>4</sub> causes a reduction in the activity of endothelium-derived NO [10]. This may suggest that BH<sub>4</sub> metabolism is adversely affected in a uraemic state, because it has been reported that NO production

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Correspondence and offprint requests to: Keitaro Yokoyama MD, The Division of Nephrology and Hypertension, Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8471, Japan. Email: keitaro@mrj.biglobe.ne.jp



**Fig. 1.** Pteridine metabolism. BH<sub>4</sub> is generated from GTP through the enzymes GTP cyclohydrolase I (GTPCH I) and PTPS. BH<sub>4</sub> acts as a coenzyme of PH during transformation of Phe to Tyr and is itself oxidized and transformed to BH<sub>2</sub> of the quinoid type. BH<sub>4</sub> is also produced by the reduction of quinonoid-type BH<sub>2</sub> by DHPR. This recycling reaction of BH<sub>4</sub> includes oxidation and reduction reactions of BH<sub>4</sub>: BH<sub>4</sub> is the reduced form, and BP and BH<sub>2</sub> are oxidized forms. When GTP is converted to 7,8-dihydrobiopterin triphosphate (BH<sub>2</sub>-3P) but bypasses PTPS, it becomes NP.

and phenylalanine metabolism are impaired, and the inflammation reaction takes place in such a state.

In this study, pteridine concentrations were determined in patients with chronic renal failure to prove the relationship of pteridine metabolism to the state of renal impairment and the possible role of pteridines in cardiovascular diseases and malnutrition in these patients.

## Subjects and methods

### Patients and control subjects

Our subjects included 33 patients receiving haemodialysis and 31 patients with less severe chronic renal failure who were not receiving haemodialysis. As an index of renal function, the creatinine clearance (Ccr) was determined in CRF patients without HD and compared with BH<sub>4</sub> levels. The patients were classified into three groups on the basis of Ccr (group (a) Ccr >60 ml/min, and group (b) Ccr=10–60 ml/min). All patients receiving haemodialysis were in group (c) (Table 1). The primary diagnoses were chronic glomerular nephritis and renal sclerosis in haemodialysis patients and chronic glomerular nephritis in non-haemodialysis patients. No patients had diabetic nephropathy. Diabetes mellitus is known to involve other factors that regulate endothelial constitutive nitric oxide synthase activity. Our subjects had received no previous treatment with nitrate, angiotensin-converting enzyme inhibitor, or angiotensin II antagonist. Eighteen healthy persons

**Table 1.** Patient profiles

	Healthy control	Group (a)	Group (b)	Group (c) (haemodialysis)
<i>n</i>	18	15	16	33
Age	55.2 ± 3.90	44.5 ± 3.74	58.5 ± 3.08	59.6 ± 1.74
Sex (M:F)	11:7	9:6	11:5	22:11
Ccr (ml/min)		78.1 ± 2.32 (>60.0)	36.5 ± 3.38 (10.0–60.0)	

Ccr, creatinine clearance.

(mean age 55.2 ± 3.90 years) were also recruited as control subjects. The study protocol was approved by the research and ethics committee of our institution.

### Blood samples

Blood samples were collected in the outpatient clinic from non-HD patients and before dialysis from HD patients in the morning, because glucocorticoids, which regulate the levels of BH<sub>4</sub>, are highest in the morning [11].

### Measurement of pteridines

Levels of neopterines (NPs) and biopterines (BPs) in plasma were measured with a modification of the high-performance liquid chromatography method of Fukushima and Nixon [12]. The concentration of reduced BP, i.e. BH<sub>4</sub>, was calculated from the total BP concentration (which equals concentrations of the reduced type plus the oxidized type) and the concentration of oxidized BP. Also, the ratio of the total NP to total BP (NP/BP) was calculated. Blood samples were collected immediately before dialysis or during outpatient consultation.

### Measurements of plasma levels of Phe and Tyr

The ratio of Tyr to Phe (Tyr/Phe) was determined by measuring levels of Phe and Tyr in the plasma with the high-performance liquid chromatography–ninhydrine luminescence method in both groups of patients.

### Statistical methods

Data are expressed as the means ± standard error of the mean (SEM). The two-sample *t*-test with Welch's correction and the Mann–Whitney test were used to analyse differences between groups. Pearson correlations was used to analyse data for correlations. All calculations were made using StatView v.4.5 for Macintosh (Abacus Concepts, Berkeley, CA, USA). Differences were considered to be significant when *P* < 0.05.

## Results

Serum levels of total NP and of total BP, and levels of BH<sub>2</sub> were significantly higher in group (c) than in groups (a), (b), or the control group. Furthermore, these same variables were significantly higher in

**Table 2.** The plasma levels of pteridines in patients with renal failure

	Healthy controls	Group (a)	Group (b)	Group (c) (haemodialysis)
Total NP (ng/ml)	5.17 ± 0.29	6.21 ± 0.49	12.4 ± 2.20 <sup>b,c</sup>	118.9 ± 11.7 <sup>b,c,d</sup>
Total BP (ng/ml)	2.83 ± 0.19	3.32 ± 0.19 <sup>a</sup>	4.48 ± 0.36 <sup>b,c</sup>	18.8 ± 1.2 <sup>b,c,d</sup>
BH <sub>2</sub> (ng/ml)	1.33 ± 0.07	1.53 ± 0.10	2.67 ± 0.25 <sup>b,c</sup>	15.2 ± 0.98 <sup>b,c,d</sup>
BH <sub>4</sub> (ng/ml)	1.49 ± 0.15	1.79 ± 0.15	1.81 ± 0.19	3.70 ± 0.85
Total NP/Total BP	1.92 ± 0.13	1.95 ± 0.20	2.81 ± 0.48	6.79 ± 0.53 <sup>b,c,d</sup>
BH <sub>4</sub> /BH <sub>2</sub>	1.15 ± 0.11	1.23 ± 0.12	0.74 ± 0.08 <sup>b,c</sup>	0.26 ± 0.06 <sup>b,c,d</sup>

NP, neopterin; BP, biopterin; BH<sub>2</sub>, dihydrobiopterin; BH<sub>4</sub>, tetrahydrobiopterin; <sup>a</sup>*P* < 0.05 vs control; <sup>b</sup>*P* < 0.01 vs control; <sup>c</sup>*P* < 0.01 vs Group (a); <sup>d</sup>*P* < 0.01 vs Group (b).

group (b) than in group (a) or the control group. In contrast, levels of BH<sub>4</sub> did not differ significantly between any of the groups. The total NP/total BP ratio was significantly higher in group (c) than in group (a), group (b), or the control group. The BH<sub>4</sub>/BH<sub>2</sub> ratio was significantly lower in group (c) than in group (b), group (a), or the control group, and was significantly lower in group (b) than in group (a) or the control group. None of these variables differed significantly between group (a) and the control group (Table 2). After dialysis in group (c), the levels of total BP and BH<sub>4</sub> had significantly decreased, but the BH<sub>4</sub>/BH<sub>2</sub> ratio was unchanged (Table 3). BH<sub>4</sub> levels after HD (1.1 ± 0.4 ng/ml) were lower than those in healthy subjects (1.49 ± 0.15 ng/ml). There were no significant differences between before HD and before the next HD.

In order to investigate the relations between our results and renal function, the levels of pteridine and Ccr were scattered in patients with renal failure not on dialysis (group (a) and group (b); *n* = 31). Significant negative correlations were found between Ccr and the total NP level (*r* = -0.663, *P* < 0.01), the total BP level (*r* = -0.492, *P* < 0.01), the BH<sub>2</sub> level (*r* = -0.677, *P* < 0.01), and the NP/BP ratio (*r* = -0.493, *P* < 0.01). Conversely, significant positive correlations were found between Ccr and the BH<sub>4</sub>/BH<sub>2</sub> ratio (*r* = 0.602, *P* < 0.01) (Figure 2).

Levels of Thr and the Tyr/Phe ratio were significantly lower in patients receiving HD (38.9 ± 3.23 ng/ml and 0.59 ± 0.02) than in control subjects (66.4 ± 6.03 ng/ml, *P* = 0.0002; 0.99 ± 0.08, *P* < 0.0001), but levels of Phe did not differ significantly (Table 4). Significant positive correlations were found between Ccr and the Tyr/Phe ratio (*P* = 0.62, *P* < 0.05). The Tyr/Phe ratio and the BH<sub>4</sub>/BH<sub>2</sub> ratio were positively correlated (*r* = 0.719, *P* < 0.0001; Figure 3).

## Discussion

In the present study, the actions of endogenous pteridines were investigated in patients with renal impairment and those on HD. When on HD, the total plasma levels of NP and BP and the NP/BP ratio increased, and the BH<sub>4</sub>/BH<sub>2</sub> ratio decreased significantly. In addition, Ccr was negatively correlated with the NP level and the NP/BP ratio was positively

**Table 3.** Changes in plasma levels of pteridines after haemodialysis and before the next haemodialysis

	Before/HD	After/HD	Before/next HD
Total NP (ng/ml)	118 ± 12.7	45.5 ± 5.3	101 ± 12.0
Total BP (ng/ml)	19.3 ± 2.2	6.6 ± 0.6	16.9 ± 2.0
BH <sub>2</sub> (ng/ml)	16.1 ± 1.9	5.5 ± 0.71	12.8 ± 1.9
BH <sub>4</sub> (ng/ml)	3.1 ± 1.7	1.1 ± 0.4	3.4 ± 1.8

HD, haemodialysis; NP, neopterin; BP, biopterin; BH<sub>2</sub>, dihydrobiopterin; BH<sub>4</sub>, tetrahydrobiopterin.

correlated with the BH<sub>4</sub>/BH<sub>2</sub> ratio. The results showed that the dialysate contents of NP, BP, BH<sub>2</sub>, and BH<sub>4</sub> were similar. These substances had a reduction rate of about 70%; however, the amount of generation until the next HD session was six times higher in NP than in BP, although their molecular weights were similar.

The BH<sub>4</sub>/BH<sub>2</sub> ratio in patients receiving HD decreased significantly. As the demand for BH<sub>4</sub> increases, BH<sub>4</sub> is required through a reduction of oxidized quinonoid BH<sub>2</sub>, in addition to its synthesis from guanosine triphosphate (GTP) [6]. However, the fact that the level of the reduced form of BH<sub>4</sub> is lower than those of the oxidized forms of BP and BH<sub>2</sub> in renal failure may suggest that there is a decreased reduction of dihydropteridine reductase (DHPR) during the conversion from quinonoid BH<sub>2</sub> to BH<sub>4</sub>.

To investigate this possibility, the DHPR activity in blood cells was determined after adding sufficient quantities of NADH. Because there was no difference in the DHPR activity between HD patients and healthy subjects, it was decided that such a change in the state of reduction might be involved in the pathophysiology of renal failure. Under oxidative stress, BH<sub>4</sub> acts as a radical scavenger similar to ascorbic acid by being oxidized to BH<sub>2</sub>. Miyata *et al.* [13] reported that the degree of ascorbic acid oxidation in dialysed patients was twice as high as it was in normal subjects.

In our study, there was no difference between those patients with a renal dysfunction and healthy subjects in their BH<sub>4</sub> level. However, it has been reported that BH<sub>2</sub> inhibits biological activity by competitive binding with endothelial constitutive NOS (eNOS) [14]. Therefore, in patients with renal failure, although BH<sub>4</sub> levels in the plasma did not change when the

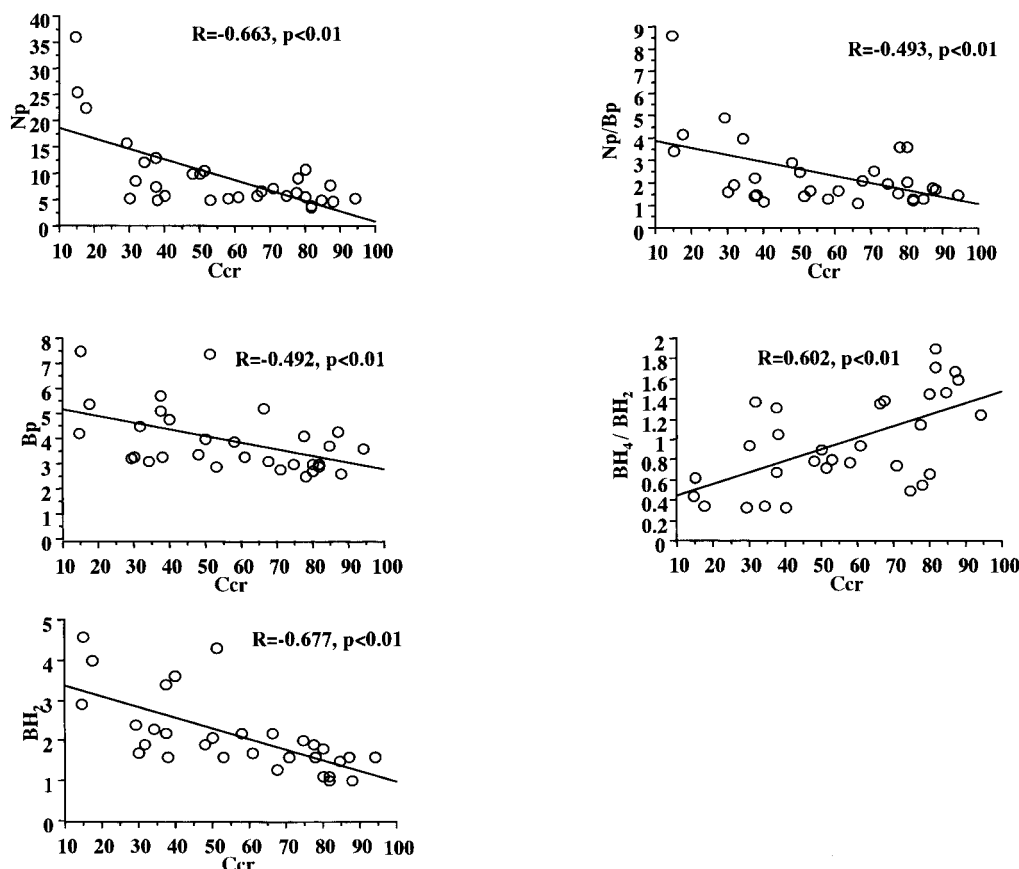


Fig. 2. The relationship between plasma levels of BH<sub>4</sub>s and Ccr. Upper left: The total NP level was negatively correlated with Ccr ( $r = -0.63$ ,  $P < 0.001$ ). Upper right: Similarly, the NP/BP ratio was negative correlated with Ccr ( $r = -0.53$ ,  $P < 0.001$ ). Lower left: BH<sub>2</sub> was negatively correlated with Ccr ( $r = -0.59$ ,  $P < 0.001$ ). Lower right: The BH<sub>4</sub>/BH<sub>2</sub> ratio was positively correlated with Ccr ( $r = 0.62$ ,  $P < 0.001$ ).

Table 4. Serum levels of Tyr and Phe in patients with renal failure

	Healthy control	Haemodialysis patients	P value
Tyr (ng/ml)	66.4 ± 6.03	38.9 ± 3.23	= 0.0002
Phe (ng/ml)	69.0 ± 7.56	65.2 ± 4.53	NS
Tyr/Phe	0.99 ± 0.08	0.59 ± 0.02	< 0.0001

renal function was compromised, BH<sub>4</sub> was relatively low because the elevation of BH<sub>2</sub> resulted in a decrease in NO production [15]. From their amino-acid profile, our results showed a BH<sub>4</sub> deficiency in patients with renal failure. In fact, the decreased Tyr/Phe ratio indicates that the conversion of Phe to Tyr by PH also decreased. The decreased Tyr/Phe ratio before HD suggests that the BH<sub>4</sub> deficiency also affects amino-acid metabolism. Moreover, the Tyr/Phe and BH<sub>4</sub>/BH<sub>2</sub> ratios were positively correlated ( $r = 0.719$ ,  $P < 0.0001$ ).

It has been suggested that in patients with renal failure there is a relative deficiency in NO production by eNOS. Guanidino compounds, such as asymmetric dimethyl-L-arginine, accumulate in the blood of patients with chronic renal failure, inhibit eNOS, and suppress endothelium-dependent vascular dilatation

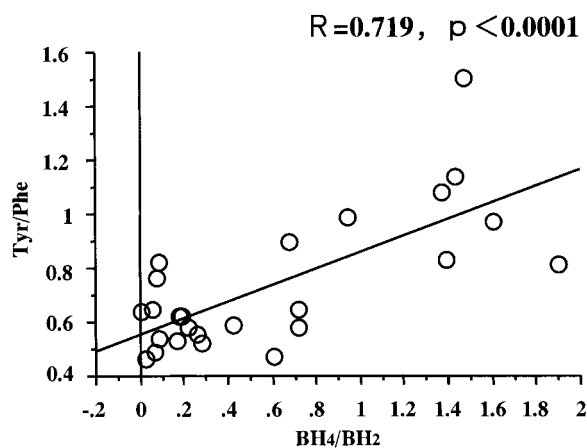


Fig. 3. The relationship between the BH<sub>4</sub>/BH<sub>2</sub> ratio and the Tyr/Phe ratio. The Tyr/Phe ratio and the BH<sub>4</sub>/BH<sub>2</sub> ratio, which reflects BH<sub>4</sub> activity, were positively correlated ( $r = 0.719$ ,  $P < 0.0001$ ).

[16,17]. Our results suggest that a BH<sub>4</sub> deficiency inhibits NOS. Kakoki *et al.* [10] reported that impairment of dimerization of eNOS by depleting BH<sub>4</sub> influences the decreased activity of endothelium-derived NO because BH<sub>4</sub> stabilizes eNOS in its dimeric form, which is an active form.

When BH<sub>4</sub> is deficient, NOS shows only oxidase activity: thus active oxygen (O<sub>2</sub><sup>-</sup>) is generated in greater quantities than NO [18]. Consequently, inflammation increases because the macrophages and monocytes are stimulated, which in turn stimulate inducible NOS (iNOS) synthesis. In patients with renal failure, although iNOS synthesis is increased when macrophages and monocytes are stimulated, the BH<sub>4</sub> level undergoes a relative reduction. Thus, the unbalanced iNOS and eNOS concentrations in those with renal failure may be closely related to the disorder of pteridine metabolism.

It was reported that the expression of iNOS, eNOS, and peroxynitrite-modified proteins in experimental anti-myeloperoxidase is associated with crescentic glomerulonephritis [19]. In our study the NP/BP ratio increased in patients with renal failure, which suggests that O<sub>2</sub><sup>-</sup> is generated as a consequence of a deficiency of BH<sub>4</sub>. Once macrophages have been activated, BP is not generated; but NP production increases after the phosphorylation of GTP because the 6-pyruvoyl tetrahydropterin synthase (PTPS) activity is low in macrophages and monocytes [20,21].

Our results suggest that the macrophages are activated in patients with renal failure. Therefore one may speculate that the NP level is a clinically significant index of both renal function and macrophage activity. Godai *et al.* [22] also reported that serum NP levels and serum creatinine concentration were positively correlated. In addition and of interest, it was reported that higher levels of serum NP serve as a marker for the progression of diabetic nephropathy [23]. It is important that the relationship between a deficiency in BH<sub>4</sub> and superoxide impairment with renal failure be clarified. Further study is needed here.

In summary, it has been shown that, in patients with chronic renal failure and on dialysis, the BH<sub>4</sub>/BH<sub>2</sub> ratio decreases and the Phe/Tyr ratio increases. This finding suggests that the reduction of quinonoid-type BH<sub>2</sub> is decreased, which plays a major role in causing impairment in NO production and phenylalanine metabolism. The disorder of pteridine metabolism in patients with renal failure may hold a key to the pathophysiology of cardiovascular diseases and malnutrition.

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## References

1. Arici M, Walls J. End-stage renal disease, atherosclerosis and cardiovascular mortality: is C-reactive protein the missing link? *Kidney Int* 2001; 59: 407–414
2. Levin A, Foley RN. Cardiovascular disease in chronic renal insufficiency. *Am J Kidney Dis* 2000; 36: S24–30
3. Stenvinkel P, Heimbürger O, Paulter F *et al.* Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 1999; 55: 1899–1911
4. Mitch WE, Maroni BJ. Nutritional considerations and the indications for dialysis. *Am J Kidney Dis* 1998; 31: 185–189
5. Bergstrom J. Nutrition and mortality in hemodialysis. *J Am Soc Nephrol* 1995; 6: 1329–1341
6. Werner ER, Werner-Felmayer G, Wachter H, Mayer B. Biosynthesis of nitric oxide: dependence on pterin metabolism. *Rev Physiol Biochem Pharmacol* 1996; 127: 97–123
7. Cosentino F, Katusic Z. Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. *Circulation* 1995; 91: 139–144
8. Cosentino F, Patton S, d'Uscio LV *et al.* Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. *J Clin Invest* 1998; 101: 1530–1537
9. Wever RMF, van Dam T, van Rijn HJ *et al.* Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem Biophys Res Commun* 1997; 237: 340–344
10. Kakoki M, Hirata Y, Hayakawa H *et al.* Effects of tetrahydrobiopterin on endothelial dysfunction in rats with ischemic acute renal failure. *J Am Soc Nephrol* 2000; 11: 301–309
11. Hattori Y, Akimoto K, Nakanishi N, Kasai K. Glucocorticoid regulation of nitric oxide and tetrahydrobiopterin in a rat model of endotoxic shock. *Biochem Biophys Res Commun* 1997; 240: 298–303
12. Fukushima T, Nixon JC. Analysis of reduced forms of biopterin in biological tissues and fluids. *Anal Biochem* 1980; 102: 176–188
13. Miyata T, Wada Y, Cai Z *et al.* Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 1997; 51: 1170–1181
14. Klatt P, Schmid M, Leopold E *et al.* The pterine binding site of brain nitric oxide synthase. *J Biol Chem* 1994; 269: 13861–13866
15. Klatt P, Schmid M, Brunner F, Mayer B. Inhibitors brain nitric oxide synthase. Binding kinetics, metabolism, and enzyme inactivation. *J Biol Chem* 1994; 269: 1674–1680
16. Vallance P, Leone A, Calver A *et al.* Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992; 339: 572–575
17. Vasquez-Vivar J, Kalyanaraman B, Martasek P *et al.* Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci USA* 1998; 95: 9220–9225
18. Huraux C, Makita T, Kurz S *et al.* Superoxide production, risk factors, and endothelium-dependent relaxations in human internal mammary arteries. *Circulation* 1999; 99: 53–59
19. Heeringa P, van Goor H, Moshage H *et al.* Expression of iNOS, eNOS, and peroxynitrite-modified proteins in experimental anti-myeloperoxidase associated crescentic glomerulonephritis. *Kidney Int* 1998; 53: 382–393
20. Fuchs D, Hausen A, Reibnegger G *et al.* Neopterin as a marker for activated cell mediated immunity: application in HIV infection. *Immunol Today* 1988; 9: 150–155
21. Werner E, Werner-Felmayer G, Fuchs D *et al.* Biochemistry and function of pterine synthesis in human and murine macrophages. *Pathobiology* 1991; 59: 276–279
22. Godai K, Uemasu J, Kawasaki H. Clinical significance of serum and urinary neopterin in patients with chronic renal disease. *Clin Nephrol* 1991; 36: 141–146
23. Weiss MF, Rodby RA, Justice AC, Hricik DE. Free pentosidine and neopterin as markers of progression rate in diabetic nephropathy. *Kidney Int* 1998; 54: 193–202

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