Plasma Amino Acid Profile and L-arginine Uptake in Red Blood Cells from Malnourished Uremic Patients

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Background: Patients with end-stage chronic renal failure (CRF) (uremia) have a high prevalence of inflammation, malnutrition, and oxidative stress. All of these features seem to be associated with the increased cardiovascular mortality observed in these patients. Nitric oxide (NO) is involved in the pathogenesis of CRF. The present study investigates the effects of nutritional status on L-arginine transport (NO precursor), plasma amino acid profile, and concentration of tumor necrosis factor (TNF)-α in uremic patients on hemodialysis (HD).

Methods: A total of 32 uremic patients on regular HD and 16 healthy controls were included in this study. Kinetic studies of L-arginine transport, mediated by cationic transport systems y^+ and y^+L into red blood cells, plasma concentrations of amino acids (measured by high-performance liquid chromatography), and plasma TNF-α level (evaluated by enzyme-linked immunosorbent assay), were analyzed in malnourished and well-nourished patients (isolated by body mass index).

Results: L-arginine influx by system y^+ in red blood cells (μmol/L cells^{-1}h^{-1}) was increased in both malnourished (377 $\pm$ 41) and well-nourished (461 $\pm$ 63) patients with CRF compared with controls (287 $\pm$ 28). Plasma levels of all cationic amino acids (L-arginine, L-ornithine, and L-lysine) were low in uremic patients compared with controls. Among the uremic population, the reduction in plasma cationic amino acids levels was greater in malnourished patients. L-cysteine and L-glutamate, precursors of glutathione, were dramatically increased in plasma from uremic patients, independently of nutritional status. In addition, TNF-α concentration in plasma was enhanced in malnourished uremic patients (3.4 $\pm$ 0.7 pg/mL) compared with controls (1.2 $\pm$ 0.1 pg/mL) and well-nourished patients (1.9 $\pm$ 0.1 pg/mL).

Conclusions: Our results suggest an increased catabolism of cationic amino acids, inflammatory markers, and oxidative stress in CRF, especially in malnourished patients. The reduced plasma concentration of plasma L-arginine is counterbalanced by enhanced rates of transport, resulting in an activation of NO synthesis in uremia.

NITRIC OXIDE (NO) is an inorganic gas associated with many physiologic functions, including the regulation of vascular function and homeostasis.[1,2] In mammalian cells, NO is synthesized from the semi-essential cationic amino acid L-arginine by a family of enzymes: nitric oxide synthase (NOS).[1–3] Inappropriate release of this mediator or impaired availability of its

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precursor L-arginine has been linked to the pathogenesis of various disease states.[4,5]

A disturbance in the L-arginine and NO pathway plays a crucial role in the pathogenesis of uremia, a syndrome present in patients with end-stage chronic renal failure (CRF).[4-8] Although inhibition of glomerular NOS is involved in the genesis of uremia, there are several studies in animal models and patients suggesting that systemic production of NO is increased in this syndrome.[4-9]

Cardiovascular disease remains the single most common cause of mortality in uremic patients.[10,11] Furthermore, although the prevalence of traditional cardiovascular risk factors is high in the population on hemodialysis (HD), the cardiovascular events remain disproportionate to these features. These epidemiologic findings have led to the search for additional motives for arteriosclerosis in uremia, such as oxidative stress and inflammation.[11-14]

Malnutrition is closely related to the increased oxidative stress and inflammation present in end-stage CRF.[13-17] Low body mass index (BMI) is related to the occurrence of cardiovascular disease in uremic patients on HD.[16] In this setting, patients on dialysis with higher BMI generally have a lower mortality rate, contrasted with findings in the general population.[13-16] However, a complete understanding of the mechanisms underlying the protective role of high BMI has not been reached.

Alterations in cell membrane transport are present in cells from uremic patients.[18] The first observation that cellular Na^+, K^+-ATPase activity was reduced in patients on HD was made by Welt et al.[19] in the 1960s, using red blood cells (RBCs) as a model. Subsequently, this uremic transport alteration has been shown to occur in a wide variety of cells.[19,20] Erythrocytes seem to possess NOS and are frequently used as surrogates for transport studies in different cells and tissues.[9,21]

L-arginine transport into RBCs is mediated by the cationic amino acid transport systems y^+ and y^+L.[22,23] System y^+L mediates high-affinity, Na^+-independent cationic and Na^+-dependent neutral amino acid transport.[22,23] Molecular biology studies revealed that y^+LAT and 4F2hc combine to induce y^+L system activity.[24] System y^+ is a lower-affinity, higher-capacity transport system specific to cationic amino acids.[22,23] It has been shown to be expressed by at least four cationic amino acid transporter proteins (CAT): CAT-1, CAT-2A, CAT-2B, and CAT-3.[25]

We previously reported that L-arginine transport into RBCs is increased, whereas circulating plasma concentration of L-arginine is reduced in patients with CRF.[4,5,9,26] However, these previous studies did not investigate the nutritional status of the uremic patients, and there are no reports of L-arginine transport in RBCs from malnourished uremic patients. In the present study we investigated L-arginine transport in RBCs and plasma amino acid profile and tumor necrosis factor (TNF)-α concentration in both well-nourished and malnourished patients with CRF on HD.

Methods

Subjects

Thirty-two patients with CRF on HD and 12 age-matched healthy volunteers participated in the study (Table 1). Patients were treated for at least 6 months with HD, three times per week. All patients were treated with erythropoietin (4000 U/wk). Blood samples were drawn by venipuncture before a 4-hour dialysis session. The exclusion criteria were heart failure, infection, dyslipidemia, and recent blood transfusion (at least 3 months before the study). The Pedro Ernesto Hospital Ethical Committee approved this work, and informed consent was obtained from each of the patients.

Anthropometric Measurement

Anthropometric measurements were performed using BMI, that is, the ratio of post-dialysis body weight (kilograms) divided by height squared (meters). BMI values less than 18.5 kg/m^2 were considered malnutrition.[27]

Measurement of L-[^{3}H]-arginine Influx in Red Blood Cells

Blood samples were centrifuged to separate the plasma (2000*g* for 5 minutes). The RBCs were then washed three times with saline (mM: NaCl 140, KCl 5, D-glucose 5, MOPS 10, pH 7.4) and centrifuged three times (2000*g* for 5 minutes). To eliminate effects caused by variations in intracellular substrate levels by *trans* stimulation and to

Table 1. Characteristics of Healthy Controls and Patients with Chronic Renal Failure on Hemodialysis

Data	Controls	Malnourished Patients with CRF	Well-nourished Patients with CRF
Number of patients	12	16	16
Age (y)	40 ± 9	46 ± 3	45 ± 4
Gender M/F	6/6	7/9	8/8
Dialysis session, min	—	240 ± 0	240 ± 0
Kt/V urea	—	1.2 ± 0.2	1.2 ± 0.1
Hypertension	—	9	8
EPO dose, units/week	—	4000 ± 0	4000 ± 0
Weight (kg)	64 ± 1	43 ± 2*	62 ± 2
Height (m)	1.67 ± 0.03	1.61 ± 0.02	1.68 ± 0.02
BMI (kg/m^2)	23 ± 2	16.5 ± 0.3*†	22 ± 0.5
Albumin (g/dL)	4.2 ± 0.7	3.6 ± 0.1	3.6 ± 0.1
Hematocrit (%)	44 ± 1	34 ± 1*	34 ± 1*
Creatinine (mg/dL)	1.05 ± 0.04	8.3 ± 0.4*	8.1 ± 0.6*
K^+ (mEq/L)	4.2 ± 0.15	5.7 ± 0.2*	5.8 ± 0.2*
TNF-alpha (pg/mL)	1.2 ± 0.1	3.4 ± 0.7*	1.9 ± 0.1

Abbreviations: CRF, chronic renal failure; EPO, erythropoietin; BMI, body mass index; TNF, tumor necrosis factor.
*$P < .01$ versus controls.
†$P < .01$ versus well-nourished patients.

achieve zero-*trans* conditions, the human RBCs in these experiments were incubated at 37°C in saline (mM: NaCl 140, KCl 5, D-glucose 5, MOPS 10, pH 7.4) for 3 hours, in the absence of any amino acid substrate. Influx of tritiated L-arginine was measured as described previously and resolved into the discrete transport components y^+ and y^+L by the selective inhibition of system y^+ with N-ethylmaleimide (200 μM, 10 minutes).[26] The hematocrit was evaluated by the Cell-Dyn 3700 SL laser hematology analyzer (Abbott Diagnostics, Santa Clara, CA).

Measurement of Plasma Amino Acid Concentrations by High-Performance Liquid Chromatography

As described previously,[26] individual amino acids were measured by reverse-phase high-performance liquid chromatography, using the fluorescence of the orthophthalaldehyde derivatives with an automated sample processing device (ASTED; Anachem, Luton, UK). The separation resolved all the known plasma amino acids over a 45-minute analysis period. Fluorescence was measured at 340 nm excitation and 440 emission wavelengths using a Jasco 4100 fluorimeter (Jasco, Thermo Separation Products, Stone, UK).

Measurement of Tumor Necrosis Factor-α Plasmatic Concentration

For a quantitative measurement of TNF-α in plasma, an enzyme-linked immunosorbent assay (Amersham Biosciences, Buckinghamshire, UK) was used.

Chemicals

All chemicals were purchased from Sigma, Poole, Dorset, United Kingdom.

Statistics

Data are expressed as the means ± standard error of the mean of measurements in the number of control subjects or patients with CRF. Statistical significance was determined at P less than .05 using one-way analysis of variance and post hoc Tukey tests (GraphPad Prism Program, GraphPad Software Inc, San Diego, Calif). Curves were fitted with Enzfitter (Elsevier, Cambridge, UK), a nonlinear least-squares fit to the Michaelis-Menten equation.

Results

Patient Profile and Biochemical and Nutritional Status

BMI was 16.5 ± 0.3 kg/m^2 in malnourished uremic patients, 22.02 ± 0.5 kg/m^2 in well-

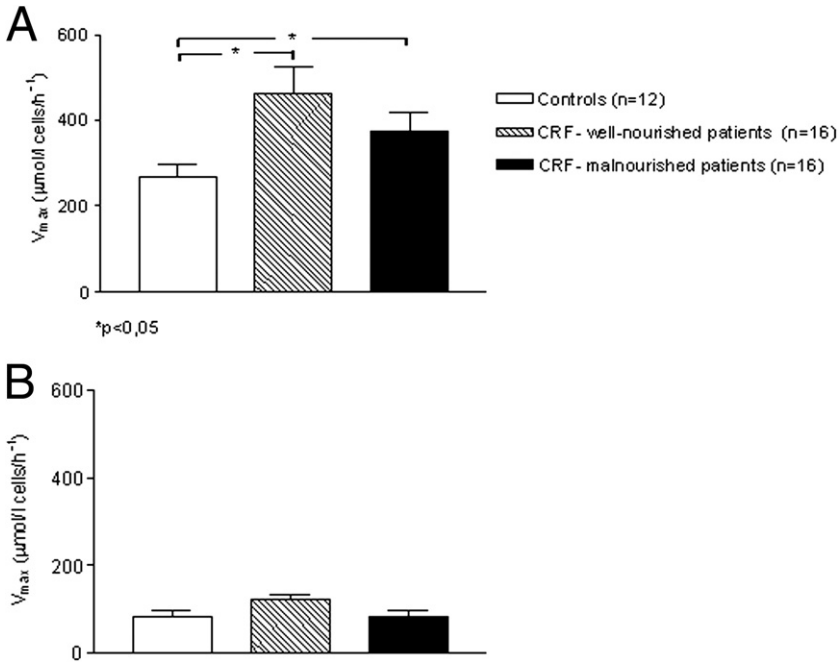

Figure 1. Effects of malnutrition on L-arginine transport in red blood cells from patients with chronic renal failure (CRF) on hemodialysis (HD). Kinetics of L-arginine transport by systems y^+ and y^+L were measured in red blood cells (RBCs) isolated from age-matched, healthy controls (n = 12) and patients with CRF on HD (n = 32). V_{max} values for L-arginine transport mediated by systems y^+ (A) and y^+L (B). Data denote the means ± standard error of the mean ($P < .05$).

nourished uremic patients, and 23 ± 2 kg/m^2 in controls. There was no difference in albumin concentration among the three groups. The hematocrit was reduced in the two groups of patients with CRF compared with controls (Table 1).

Tumor Necrosis Factor-α Plasma Concentration

TNF-α plasmatic levels were significantly increased in uremic malnourished patients compared with controls and well-nourished patients (Table 1).

L-arginine Influx Into Red Blood Cells

Total L-arginine influx into RBCs was resolved into the saturable transport components y^+ and y^+L by selective inhibition of the system y^+ with N-ethylmaleimide (200 μM, 10 minutes). L-arginine influx by system y^+ was activated in erythrocytes from all patients with CRF (Fig. 1A). The V_{max} values for L-arginine transport by system y^+L were the same for the three groups (Fig. 1B).

Plasma Amino Acid Profile in Well-nourished Versus Malnourished Uremic Patients

Plasmatic L-arginine concentration was significantly reduced in malnourished uremic patients and well-nourished uremic patients compared with controls. Similarly, plasma levels of the other cationic amino acids, L-lysine and L-ornithine, were decreased in both well-nourished and malnourished uremic patients. Plasma levels of all cationic amino acids were much lower in malnourished patients compared with well-nourished patients. The plasma levels of precursors for the synthesis of the antioxidant peptide glutathione, L-glutamate, and L-cysteine in all uremic patients were increased. The plasma concentrations of the amino acids are shown in Table 2.

Discussion

Our present results confirm previous findings of significant activation of L-arginine influx by system y^+ in erythrocytes from patients with CRF on HD.[26] The novel finding of the present study is that the observed activation of L-arginine transport is unaffected by nutritional status. The cationic amino acid transport system y^+ is known to be modulated over a wide range of mechanisms. As shown in Table 2, plasma concentration of L-arginine was low in both groups of uremic patients, because the kidneys are the most important source of L-arginine synthesis, contributing in a major way to the maintenance of plasma L-arginine concentration. It is possible that this finding may be involved in system y^+ activation in this disease. There is evidence that

Table 2. Amino Acid Concentrations in Plasma From Controls (n = 12) and Patients With Chronic Renal Failure on Hemodialysis (n = 23)

Amino acids (μmol/L)	Controls (n = 12)	Well-nourished Uremic Patients (n = 14)	Malnourished Uremic Patients (n = 9)
L-alanine	516 ± 40	242 ± 30*	212 ± 17*
L-arginine	125 ± 5	86 ± 9*	54 ± 14*#
L-aspartate	74 ± 8	34 ± 3*	34 ± 4*
L-citrulline	42 ± 4	83 ± 11*	70 ± 7*
L-cysteine	84 ± 6	500 ± 48*	557 ± 49*
L-glutamate	167 ± 10	306 ± 24*	263 ± 22*
L-glycine	250 ± 13	286 ± 19	205 ± 17
L-isoleucine	75 ± 4	113 ± 10	79 ± 4
L-leucine	145 ± 7	113 ± 10*	109 ± 10*
L-lysine	196 ± 10	76 ± 9*	58 ± 9*#
L-methionine	30 ± 3	27 ± 1	25 ± 2
L-ornithine	111 ± 6	58 ± 4*	42 ± 4*#
L-phenylalanine	68 ± 1	59 ± 6	51 ± 5
L-serine	144 ± 6	109 ± 6*	98 ± 10*
L-taurine	166 ± 5	60 ± 12*	53 ± 9*
L-threonine	126 ± 8	103 ± 11	88 ± 10*
L-tryptophan	58 ± 4	24 ± 14*	23 ± 4*
L-tyrosine	72 ± 3	40 ± 2*	38 ± 2*
L-valine	240 ± 13	173 ± 10*	172 ± 8*

*$P < .05$ versus controls.

low extracellular L-arginine up-regulates L-arginine transport by system y^+ in different types of cells.[22,28,29]

An additional potential mechanism of L-arginine transport enhancement may be proinflammatory mediators, alone or together with lipopolysaccharide.[22,30,31] However, whereas plasma concentration of TNF-α is significantly increased in malnourished uremic patients, it is unaltered in eutrophic patients. A systemic inflammatory response has been identified in uremic patients, characterized by release of cytokines such as TNF-α and interleukin-6.[11-14] This proinflammatory state is a result of CRF itself and dialysis. The association among malnutrition, inflammation, and atherosclerosis in this population has been well recognized and is known as the malnutrition-inflammation-atherosclerosis syndrome.[32] Further investigations are needed to determine the precise cytokine profile in these two groups of patients and to test its effects on L-arginine uptake by system y^+ in precursor cells of RBCs.

Anemia is a common complication in patients with CRF on HD (Table 1) and may be secondary to many factors such as inadequate erythropoietin production, iron deficiency, nutritional deficiencies, and increased RBC destruction.[33] The shortened survival time leads to a younger population of RBCs in uremic patients when compared with RBCs from control subjects.[5,20,26] As suggested before, there is an increased transport capacity for many transport systems in younger cells,[5,20,26] and it could be argued that the increased uptake of L-arginine by the y^+ transport system is a consequence of the younger population of RBCs present in uremic patients. However, many other amino acid transport systems are not affected by uremia,[20] and the age of RBCs in uremic patients is unlikely to be the whole explanation for the increased transport capacity of L-arginine observed in the experiments performed in this study.

In CRF, endothelium-dependent relaxation is impaired, whereas systemic production of NO seems to be increased.[4,34,35] Nevertheless, the fact that the majority of uremic patients are hypertensive indicates increased NO production in relation to blood pressure as a failing counter-regulatory mechanism. An important feature in CRF is an overproduction of reactive oxygen species, which can accelerate the inactivation of NO.[9,36,37] Glutathione (GSH) plays an important role in antioxidant defense, protecting proteins and lipids against free radical attack.[38] Our present findings show that two of three amino acids required for GSH synthesis, L-glutamate and L-cysteine, are increased in plasma from uremic patients, independently of nutritional status.

Recent studies provide evidence that L-cysteine is generally the limiting amino acid for GSH synthesis in humans and that it is immediately oxidized to L-cysteine in oxygenated extracellular solutions.[39–43] Because L-glutamate shares the same transport system with L-cysteine, high extracellular concentration of L-glutamate can competitively inhibit L-cysteine uptake and reduce GSH synthesis.[44] Furthermore, recent evidence suggests that the activity of the rate-limiting enzyme in GSH biosynthesis, γ-glutamylcysteine synthetase, is significantly decreased in uremic patients and patients on HD, probably because of inhibitory effects from uremic factors that are not removed by standard dialysis.[45] Our findings indicate a state of enhanced oxidative stress that could reduce the half-life of NO, thus explaining this paradox. We have made the hypothesis before that the enhancement in L-arginine transport in circulating cells reported by our group is widespread in vasculature. This serves to provide the necessary supply of substrate for maintaining increased systemic synthesis of NO in uremia in conditions of low plasma L-arginine[4–8,26] (Table 2). If this is true, a further decrease in the plasma levels of L-arginine is unable to further increase L-arginine uptake.

The y^+ transport system also mediates the transport of other cationic amino acids, such as L-lysine and L-ornithine.[22,23] As with plasma levels of L-arginine, plasma levels of L-lysine and L-ornithine were also reduced in patients on HD and were significantly lower in malnourished patients with CRF. The NOS and arginase enzymes are expressed simultaneously in a wide variety of cells; thus, the reduced plasma concentration of L-ornithine may result from the impairment of L-arginine metabolism by arginase, an enzyme that metabolizes L-arginine to urea and L-ornithine. In support of this hypothesis, a study suggests that increased NO synthesis in uremic endothelial cells may be the result of an inhibition of arginase, directing extra L-arginine to the NOS pathway.[34]

As in previous reports, our study shows that the neutral amino acid L-citrulline, the substrate for L-arginine synthesis and co-product of the L-arginine NO pathway, is increased independently of the nutritional status in uremic patients.[37] L-aspartate, another amino acid necessary to L-arginine production, together with L-citrulline by argininosuccinate synthetase, is dramatically reduced in uremic groups.

Conclusion

Transport of L-arginine is activated in erythrocytes from patients with CRF, independently of nutritional status, suggesting a limit to the adaptive increase in the activity of the cationic transport system y^+ in uremia. In malnourished uremic patients, conditions such as reduced plasma levels of L-arginine and increased TNF-α systemic concentrations are known to activate L-arginine transport. Nevertheless, the activation of L-arginine transport is not different in RBCs taken from well-nourished and malnourished patients. It is clear that further investigations are necessary to clarify the mechanisms involved in the association between the nutritional status and the L-arginine NO pathway.

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References

1. Moncada S, Palmer RMJ, Higgs A: Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109-142, 1991.

2. Ignarro LJ, Wei Lun: Visiting professorial lecture: nitric oxide in the regulation of vascular function: an historical overview. J Card Surg 17(4):301-306, 2002.

3. Gornik HL, Creager MA: Arginine and endothelial and vascular health. J Nutr 134:2880S-2887S, 2004.

4. Mendes-Ribeiro AC, Brunini TMC, Ellory JC, et al: Abnormalities in L-arginine transport and nitric oxide biosynthesis in chronic renal and heart failure. Cardiovasc Res 49:697-712, 2001.

5. Mendes-Ribeiro AC, Brunini TM: L-arginine transport in disease. Curr Med Chem Cardiovasc Hematol Agents 2(2):123-131, 2004.

6. Noris M, Benigni A, Boccardo P, et al: Enhanced nitric oxide synthesis in uraemia: implications for platelet dysfunction and dialysis hypotension. Kidney Int 44:445-450, 1993.

7. Aiello S, Noris M, Todeschini M, et al: Renal and systemic nitric oxide synthesis in rats with renal mass reduction. Kidney Int 52:171-181, 1997.

8. Sarkar SR, Kaitwatcharachai C, Levin NW: Nitric oxide and hemodialysis. Semin Dial 17(3):224-228, 2004.

9. Brunini TMC, Resende AC, Moss MB, et al: L-arginine availability as a pathological mechanism in essential hypertension, chronic renal and heart failure. Vasc Dis Prev 2:37-51, 2005.

10. Meguid El Nahas A, Bello AK: Chronic kidney disease: the global challenge. Lancet 365:331-340, 2005.

11. Tripepi G, Mallamaci F, Zoccali C: Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. J Am Soc Nephrol 16(1):S83-S88, 2005.

12. Himmelfarb J: Linking oxidative stress and inflammation in kidney disease: which is the chicken and which is the egg? Semin Dial 17(6):449-454, 2004.

13. Qureshi AR, Alvestrand A, Divino-Filho JC, et al: Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. J Am Soc Nephrol 13(Suppl 1):S28-S36, 2002.

14. Stenvinkel P: Malnutrition and chronic inflammation as risk factors for cardiovascular disease in chronic renal failure. Blood Purif 19:143-151, 2001.

15. Ritz E, Vallance P, Nowicki M: The effect of malnutrition on cardiovascular mortality in dialysis patients: is L-arginine the answer? Nephrol Dial Transplant 9:129-130, 1994.

16. Pupim LB, Caglar K, Hakim RM, et al: Uremic malnutrition is a predictor of death independent of inflammatory status. Kidney Int 66(5):2054-2060, 2004.

17. Young GA, Swanepoel CR, Croft MR, et al: Anthropometry and plasma valine, amino acids and proteins in the nutritional assessment of hemodialysis patients. Kidney Int 21: 492-499, 1982.

18. Swales JD: Blood pressure: from cells to populations. J R Coll Physicians 22:11-15, 1998.

19. Welt LG, Sachs JR, Mcmanus TJ: An ion transport defect from uremic patients. Trans Assoc Am Phys 77:169-181, 1964.

20. Hendry BM: Membrane transport in uraemia, in Raine AEG (ed): Advanced Renal Medicine, Oxford, Oxford University Press, 1992, pp 16-23.

21. Chen LY, Mehta JL: Evidence for the presence of L-arginine-nitric oxide pathway in human red blood cells: relevance in the effects of red blood cells on platelet function. J Cardiovasc Pharmacol 32(1):57-61, 1998.

22. Mann GE, Yudilech DL, Sobrevia L: Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. Physiol Rev 83:183-252, 2003.

23. Deves R, Boyd CA: Transporters for cationic amino acids in animal cells: discovery, structure, and function. Physiol Rev 78:487-545, 1998.

24. Torrents D, Estevez R, Pineda M, et al: Identification and characterization of a membrane protein (y^+LAT-1) that associates with 4F2hc to encode the amino acid transport activity y^+L. A candidate gene for lysinuric protein intolerance. J Biol Chem 273:32437-32445, 1998.

25. Closs EI, Simon A, Ve′kony N, Rotmann A: Plasma membrane transporters for arginine. J Nutr 134:2752S-2759S, 2004.

26. Mendes Ribeiro AC, Hanssen H, Kiessling K, et al: Transport of L-arginine and the nitric oxide inhibitor N^+-Monomethyl-L-arginine in human erythrocytes in chronic renal failure. Clin Sci 93:57-64, 1997.

27. World Health Organization: Physical Status: The Use and Interpretation of Anthropometry. Geneva, WHO, 1995.

28. Bogle RG, Baydoun AR, Pearson JD, et al: Regulation of L-arginine transport and nitric oxide release in superfused porcine aortic endothelial cells. J Physiol 490:229-241, 1996.

29. Hyatt SL, Aulak KS, Malandro M, et al: Adaptive modulation of the cationic amino acid transporter-1 (CAT-1) in Fao cells. J Biol Chem 272:19951-19957, 1997.

30. Durante W, Liao L, Iftikhar I, et al: Differential regulation of L-arginine transport and nitric oxide production by vascular smooth muscle and endothelium. Circ Res 78:1075-1082, 1996.

31. Shibazaki T, Fujiwara M, Sato H, et al: Relevance of the L-arginine transport activity to the nitric oxide synthesis in mouse peritoneal macrophages stimulated with bacterial lipopolysaccharide. Biochim Biophys Acta 1311:150-154, 1996.

32. Bamgbola FO, Kaskel FJ: Uremic malnutrition-inflammation syndrome in chronic renal disease: a pathobiologic entity. Ren Nutr 13(4):250-258, 2003.

33. Locatelli F, Del Vecchio L: Dialysis adequacy and response to erythropoietic agents: what is the evidence base? Nephrol Dial Transplant 18:29-35, 2003.

34. Thuraisingham RC, Roberts NB, Wilkes M, et al: Altered L-arginine metabolism results in increased nitric oxide release from uraemic endothelial cells. Clin Sci 103(1):31-41, 2002.

35. Hand MF, Haynes WG, Webb DJ: Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. Kidney Int 53(4):1068-1077, 1998.

36. Stenvinkel P, Holmberg I, Heimburger O, et al: A study of plasmalogen as an index of oxidative stress in patients with chronic renal failure. Evidence of increased oxidative stress in malnourished patients. Nephrol Dial Transplant 13:2594-2600, 1998.

37. Prabhakar SS: L-arginine-nitric oxide pathway in end-stage renal disease. Am J Kidney Dis 39(1):195-198, 2002.

38. Lu SC: Regulation of glutathione synthesis. Curr Top Cell Regul 36:95-116, 2000.

39. Lyons J, Rauh-Pfeiffer A, Yu Y, et al: Blood glutathione synthesis rates in healthy adults receiving a sulfur amino acid-free diet. Proc Natl Acad Sci U S A 97:5071-5076, 2000.

40. Jahoor F, Jackson A, Gazzard B, et al: Erythrocyte glutathione deficiency in symptom-free HIV infection is associated with decreased synthesis rate. Am J Physiol 276:E205-E211, 1999.

41. Chung TK, Funk MA, Baker DH: L-2-oxothiazolidine-4-carboxylate as a cysteine precursor—efficacy for growth and hepatic glutathione synthesis in chicks and rats. J Nutr 120:158-165, 1990.

42. Townsend DM, Tew KD, Tapiero H: The importance of glutathione in human diseases. Biomed Pharmacother 57:145-155, 2003.

43. Wu G, Fang YZ, Yang S, et al: Glutathione metabolism and its implications for health. J Nutr 134:489-492, 2004.

44. Tapiero H, Mathe G, Couvreur P, et al: Glutamine and glutamate. Biomed Pharmacother 56:446-457, 2002.

45. Alhamdani MS: Impairment of glutathione biosynthetic pathway in uraemia and dialysis. Nephrol Dial Transplant 20: 124-128, 2005.