PART ONE

Peritoneal Kinetics and Anatomy
L-Arginine is the substrate for nitric oxide synthesis and may enter cells by the y+ and y+L transport systems. Peritoneal membrane characteristics may depend on vascular function and the L-arginine–nitric oxide pathway.

In a cross-sectional study, we evaluated erythrocyte L-arginine uptake in stable peritoneal dialysis (PD) patients with various categories of peritoneal transport function. We used 14C as a marker and N-ethyl-maleimide as an inhibitor of the y+ system to measure maximal uptake capacity ($V_{\text{max}}$ in $\mu$mol/L cell/h) and the half-saturation constant ($K_{\text{m}}$ in $\mu$mol/L) in erythrocytes.

The sample consisted of 41 patients (mean age: 50 ± 17 years; 5 with diabetes; 18 men). Mean dialysate-to-plasma creatinine (D/PCr) was 0.62 ± 0.14. Peritoneal membrane transport was classified as high, high-average, low-average, or low in 10, 11, 11, and 9 patients, respectively. Mean $y^{+}V_{\text{max}}$ was 208 ± 111 $\mu$mol/L cell/h, 494 ± 893 $\mu$mol/L cell/h, 222 ± 59 $\mu$mol/L cell/h, and 193 ± 63 $\mu$mol/L cell/h ($p = 0.404$, analysis of variance (ANOVA)) for the high, high-average, low-average, and low transporters respectively. Similarly, mean $y^{+}L V_{\text{max}}$ was 963 ± 1034 $\mu$mol/L cell/h, 843 ± 366 $\mu$mol/L cell/h, 639 ± 254 $\mu$mol/L cell/h, and 774 ± 378 $\mu$mol/L cell/h ($p = 0.647$, ANOVA). As with $V_{\text{max}}$, the $y^{+}L K_{\text{m}}$ and $y^{+} K_{\text{m}}$ values were not significantly different between the various peritoneal transport categories. A negative correlation was observed between $y^{+} V_{\text{max}}$ and Kt/V ($r = –0.393$, $p = 0.011$).

Erythrocyte uptake of L-arginine does not vary with peritoneal membrane transport characteristics, but maximal L-arginine uptake capacity is higher in patients with a lower Kt/V.

**Key words**
Membrane, amino acids, nitric oxide

**Introduction**
Nitric oxide, synthesized from the amino acid L-arginine, is a potent vasodilator; is involved in platelet aggregation; and is a key factor regulating blood flow, vascular tone, and permeability (1). Production of nitric oxide depends on the intracellular presence of its precursor, which enters erythrocytes through cationic amino-acid uptake systems, such as the y+ and y+L systems (2). Because ultrafiltration and peritoneal transport of solutes depend on the interaction between the peritoneal membrane and capillary surface areas, the L-arginine–nitric oxide pathway is likely to be involved in peritoneal permeability modulation during peritoneal dialysis (PD).

Patients treated with PD show large individual differences in solute and fluid exchange (3). Solute clearances depend on the effective peritoneal surface area—that is, the functional area of exchange between blood and dialysate—which is determined mainly by the perfused capillary surface area (4).

Changes in membrane transport systems have been described in erythrocytes from uremic patients, and researchers have postulated that the abnormalities are important contributors to the pathophysiology of the uremic syndrome (5–7). Increased maximal lysine uptake capacity via the y+ system has previously been shown (5).

In the present study, we separately evaluated two erythrocyte L-arginine uptake systems, y+ and y+L, in peritoneal dialysis (PD) patients with varying categories of peritoneal transport function.

**Patients and methods**
Our cross-sectional survey enrolled 41 adult PD patients, free of peritonitis for at least 1 month, at the Renal Unit, Hospital São Lucas. All patients were
clinically stable and free of inflammatory or infectious complications and of the use of nitric oxide donors. Informed consent was obtained from all participants, and the scientific and ethics committees approved the study protocol.

A standard peritoneal equilibration test (PET), following an overnight fasting period, evaluated peritoneal membrane transport as previously described (8,9). Quartiles of the 4-hour dialysate-to-plasma ratio of creatinine (D4/P Cr) were used to classify peritoneal membrane transport as low (0.39 – 0.50), low-average (0.52 – 0.60), high-average (0.62 – 0.71), or high (0.73 – 0.95) for each patient. This modified classification produced values quite similar to those proposed by Twardowski et al. (8) for low (0.34 – 0.50), low-average (0.51 – 0.64), high-average (0.65 – 0.80), or high transport (0.81 – 1.03) respectively.

A weekly urea clearance normalized to total body water volume (Kt/V) estimated adequacy of dialysis (10).

Creatinine, glucose, and urea concentrations were determined by automated kinetic methods (Advia 1650: Bayer Healthcare, Tarrytown, NY, U.S.A.). Dialysate creatinine concentrations were corrected for glucose interference using a locally calculated correction factor.

Erythrocyte L-arginine uptake assays were performed using the blood samples collected for the PET. Heparinized blood was centrifuged and washed three times with ice-cold saline solution (platelets and white-cell layer discarded) for separation of erythrocytes. All samples were kept on ice until uptake assays were performed. Erythrocytes were separated into two aliquots, and one aliquot was incubated with N-ethylmaleimide (NEM) before uptake measurements were made.

Total erythrocyte L-arginine uptake was determined by incubating cells for 3 minutes at 37°C and pH 7.4 in a water bath with progressive L-arginine concentrations (20, 40, 60, 80, 100, 120, 140, 180, 200, 300, and 500 μmol/L) and 14C as a marker. Uptake was interrupted by transferring the sample tubes to ice. Erythrocytes were then washed free of extracellular radioactivity, lysed using Triton (Rohm & Haas, Philadelphia, PA, U.S.A.) 0.1% volume:volume, and protein-precipitated using trichloroacetic acid 5% weight:volume to recover the intracellular content. A liquid-scintillation counter was then used to obtain a radioactivity count. Uptake was corrected to μmol/L cells/h. Maximal uptake capacity (V max in μmol/L cells/h) and half-saturation (K m in μmol/L) were derived from Michaelis–Menten kinetics, using the Enzfit computer software program for MS-DOS (Biosoft, Cambridge, U.K.). All l-arginine uptake assays were performed in duplicate. The NEM (0.8 mmol/L)–treated samples were used to measured NEM-insensitive fluxes, which correspond to the y+L system activity. The NEM-sensitive uptake was considered to be the uptake via the y+ system.

Results are expressed as percentages or as mean ± standard deviation (SD). Analyses of variance (ANOVA’s), with asymmetric variables rank-transformed before analysis, were used. The Pearson correlation coefficient was used to verify associations. The Statistical Package for Social Sciences software (SPSS version 11 for Windows: SPSS Inc., Chicago, IL, U.S.A.) was used in all statistical analyses.

Results
The sample included 41 patients, mean age 50 ± 17 years. Of the 41 patients, 23 (56%) were women, and 5 (12%) had diabetes. Mean time on dialysis was 25 months (range: 8.5 – 49.0 months). The main causes of end-stage renal disease were hypertensive nephropathy (n = 17), diabetic nephropathy (n = 5), polycystic kidney disease (n = 4), chronic glomerulonephritis (n = 3), systemic lupus erythematosus (n = 3), and other causes (n = 9).

Mean dialysate-to-plasma creatinine (D/P Cr) was 0.62 ± 0.14. The PET was used to classify patients as high (n = 10), high-average (n = 11), low-average (n = 11), or low (n = 9) transporters.

Table I shows Michaelis–Menten kinetics of L-arginine uptake via the y+ and y+L systems by PET category. No significant difference in V max or K m was detected between the various peritoneal membrane function categories for either L-arginine transporter (y+ or y+L).

Discussion
In the present study, we evaluated erythrocyte L-arginine transport in stable PD patients with varying PET categories. No significant difference in maximal uptake capacity or half-saturation constant was detected between the various peritoneal membrane function categories for either L-arginine transporter (y+ or y+L).
The negative correlation between maximal uptake capacity of the $y^+$ system and $K_t/V$ is interesting. Patients with a lower $K_t/V$ tend to be those receiving less dialysis, and they are therefore prone to being more uremic. Lower $K_t/V$ was associated with higher activity of the $y^+$ system. That finding accords with a previous report of an increased $y^+$ system $V_{\text{max}}$ in the erythrocytes of uremic patients (5). Increased $V_{\text{max}}$ may be the result of trans-stimulation, acceleration of uptake activity by L-arginine, or compatibility of other substrates for the L-arginine transport systems (2). Endogenous analogs of L-arginine—such as asymmetric dimethyl arginine—are increased in uremia (11), and they also have affinity for L-arginine uptake systems (2). The analogs are inhibitors of the nitric oxide synthases and would induce a reduction in nitric oxide production, despite increased L-arginine uptake. We did not measure plasma and intracellular L-arginine concentrations, nor levels of L-arginine analogs.

### Conclusions

Erythrocyte L-arginine uptake was similar across the various PET categories of peritoneal function in stable PD patients. A possible association between $y^+$ activity and dialysis adequacy is suggested.

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### References


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### TABLE  I Kinetics parameters of erythrocyte L-arginine uptake (n = 41)

<table>
<thead>
<tr>
<th>PET classification</th>
<th>$y^+ V_{\text{max}}$ (μmol/L cell/h)</th>
<th>$y^+ L V_{\text{max}}$ (μmol/L cell/h)</th>
<th>$y^+ K_m$ (μmol/L)</th>
<th>$y^+ L K_m$ (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (n=10)</td>
<td>963±1034</td>
<td>208±111</td>
<td>68±15</td>
<td>60±47</td>
</tr>
<tr>
<td>High average (n=11)</td>
<td>843±366</td>
<td>494±893</td>
<td>81±31</td>
<td>103±95</td>
</tr>
<tr>
<td>Low average (n=11)</td>
<td>639±254</td>
<td>222±59</td>
<td>75±23</td>
<td>91±44</td>
</tr>
<tr>
<td>Low (n=9)</td>
<td>774±378</td>
<td>193±63</td>
<td>99±31</td>
<td>56±30</td>
</tr>
</tbody>
</table>

a One-way analysis of variance.  
PET = peritoneal equilibration test; $V_{\text{max}}$ = maximal capacity of transport; $K_m$ = half-saturation constant.