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Indices of Activity of the Nitric Oxide System in Hemodialysis Patients

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Abstract

Arginine deficiency and/or increased levels of circulating nitric oxide (NO) synthesis (NOS) inhibitors can cause reduced NOS, which may contribute to hypertension in patients with end-stage renal disease (ESRD). To test these hypotheses, NO oxidation products ($\text{NO}_2 + \text{NO}_3 = \text{NO}_x$) and cyclic guanosine monophosphate (cGMP), the vasodilatory second messenger of NO, were measured in the blood, urine, and dialysate effluent of hemodialysis (HD) patients and compared with the blood and urine of healthy subjects. The subjects ate a controlled low-nitrate diet ($\sim 330 \mu\text{mol/d}$) for 48 hours before and during blood, dialysis effluent, and 24-hour urine collection. NO_x output was significantly reduced in HD patients versus controls (552 ± 51 v $824 \pm 96 \mu\text{mol/24 h}$; $P < 0.001$), whereas cGMP output was not low versus controls. Plasma arginine level was normal and plasma levels of citrulline and the endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA), were markedly elevated in patients with ESRD versus controls. Systolic blood pressure was greater in HD patients compared with controls despite concurrent antihypertensive therapy in most patients with ESRD. These studies suggest NO production is low in patients with ESRD undergoing HD, possibly because of the increased ratio of plasma ADMA to arginine.

Index Words

End-stage renal disease; hypertension; arginine; cyclic guanosine monophosphate (cGMP); endogenous nitric oxide synthases (NOS) inhibitors

Nitric oxide (NO) is a potent vasodilator made from L-arginine by the enzymatic action of several widely distributed nitric oxide synthases (NOSs). The constitutively expressed, vascular endothelial and brain-type NOSs have major roles in the physiological control of vascular tone and kidney function, and many of these actions are mediated by cyclic guanosine monophosphate (cGMP).^{1,2} NO deficiency can be produced experimentally by the administration of substituted L-arginine analogues that function as competitive inhibitors of NOS. Chronic experimental NOS inhibition in animals produces hypertension, and NOS may be defective in some individuals with primary and secondary hypertension.³

Hypertension is particularly prevalent in hemodialysis (HD) patients with end-stage renal disease (ESRD),⁴⁻⁷ and there are valid reasons to suspect NO deficiency might occur and contribute to the hypertension in this population. We previously reported that patients with ESRD undergoing peritoneal dialysis (PD) produce less NO, indicated by the removal rate of the stable inert oxidation products of NO ($\text{NO}_2 + \text{NO}_3$ [NO_x]) in peritoneal dialysate plus urine.

⁸ In addition, the plasma levels of naturally occurring compounds that inhibit the L-arginine–NO pathway accumulate in patients with ESRD. For example, plasma levels of asymmetric dimethylarginine (ADMA) increase in HD patients, possibly to concentrations that inhibit NOS, although this remains controversial.^{9,10} This, together with plasma arginine levels in the low-normal range,^{9,11} means the ratio of plasma levels of endogenous NOS inhibitors to L-arginine is high in patients with ESRD undergoing HD, which would predispose to reduced NOS.

We conducted the present study to assess the status of the L-arginine–NO system in patients with ESRD undergoing HD. In this study, we measured NO_x and cGMP (NO second messenger) removal rates through the kidney or HD, as well as plasma levels in HD patients both before and immediately after dialysis and in healthy controls under conditions of low dietary NO_x intake. Other indices of the L-arginine–NO system were also measured; plasma levels of arginine, citrulline, and the endogenous NOS inhibitors, ADMA, symmetric dimethylarginine (SDMA), and n-monomethyl-L-arginine (L-NMA).

Methods

Seventeen HD patients were randomly selected and asked to participate on the study for the measurement of 24-hour NO_x production. HD patients were recruited from two morning midweek dialysis shifts. Of the 17 HD patients, 8 had been diagnosed with diabetic nephropathy, 4 with glomerulonephritis, 2 with hypertensive nephrosclerosis, and 1 each with renovascular disease, Alport's syndrome, renal atheroembolism, and obstructive uropathy as the cause of ESRD. Of these, 15 of 17 HD patients were receiving antihypertensive medication. Thirteen normotensive, healthy subjects were recruited from the spouses of patients with ESRD and the West Virginia University Health Science Center (Morgantown, WV) faculty, students, and staff to serve as the control group. Criteria for controls were as follows: normotensive, healthy individuals with no known illnesses and taking no cardiovascular medications.

All participants on the study were provided with complete meals of low nitrate content for the 48 hours before blood sampling. Meals were nutritionally complete (~2,000 calories/24 h) and contained low NO_x content (330 μmol/24 h), low sodium content (60 mEq/24 h), and potassium and phosphorus restrictions appropriate for dialysis-dependent patients. Subjects were instructed to eat only what food was provided during this period and to note any uneaten food. We asked all participants to refrain from exercise during the 48-hour study period. Control subjects collected all their urine over the second 24-hour period of the low-NO_x diet. Blood pressure (BP) was measured (two to three estimates at 5-minute intervals while subjects were quietly seated), and a blood sample was obtained at the end of the 48-hour period, coincident with the end of the 24-hour urine collection. In HD patients, the low-NO_x diet was begun at the end of an HD treatment. During the subsequent dialysis (2 days later), all dialysate ultrafiltrate was collected; this was considered to represent the excretion of substances that had accumulated over the previous 48-hour period because none of the HD patients produced any urine. In all individuals, the exact times of urine/dialysate collection were recorded. In HD patients, BP was measured and blood samples were obtained before and after the HD treatment on day 2 of the low-NO_x diet. BP and heart rate were also measured throughout the entire period of HD, which is routine dialysis protocol.

Sample Collection and Preparation

Urine collections were placed in sterile, dry plastic containers with 2% boric acid as antibacterial. The dialysate and effluent from HD patients were collected in a 55-gal sterile plastic vat with a tap at the bottom. The volumes of all urine and dialysate samples were measured, and then samples were aliquoted and stored frozen until later analysis. Blood samples were collected into chilled tubes using ethylenediaminetetraacetic acid as

anticoagulant, centrifuged at 4°C, and plasma was aliquoted, frozen, and stored at -20°C until later analysis. The plasma to be used for cGMP analysis was stored with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine [IBMX] (30 µL of 10 mmol/L of IBMX to 500 µL of plasma) to prevent the breakdown of cGMP in vitro.

The following analyses were conducted: NO_x concentrations of plasma, urine, peritoneal dialysate, and hemodialysate were measured using the Griess assay after conversion of NO₃ to NO₂ with the NO₃-reductase enzyme, as described previously by us.¹² In preliminary studies, we obtained the complete recovery of 50 µmol/L of NO₃ added to urine and 5 and 10 µmol/L of NO₃ added to HD fluid (both n = 2). Plasma and urine cGMP were measured by the Cayman Chemicals enzyme-linked immunosorbent assay kit (Ann Arbor, MI). Plasma arginine and citrulline levels were measured by reverse-phase high-performance liquid chromatography with precolumn derivatization and fluorescent detection using a modification of the AccQ Tag system for amino acid analysis (Waters, Milford, MA) as follows: with α-aminobutyric acid as an internal standard, plasma samples were ultrafiltered using centrifugation filters with a 10,000-molecular weight cutoff (Nanosep; Pall Filtron Corp, North-borough, MA) to remove proteins. Filtered samples were then derivatized at 55°C with 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate.¹³ Dual pumps (Waters 510) were used to deliver the binary mobile phase (140 mmol/L of sodium acetate, with 17 mmol/L of triethylamine (TEA) titrated to pH 5.05, and 60% acetonitrile) at 1 mL/min using the AccQ Tag gradient table to a C18 column (Nova-Pak, 4µm, 3.9 × 150 mm; Waters) that was maintained at 60°C. Ten-microliter samples were injected by autosampler (Waters 717 plus) with the eluting products measured by a fluorescence detector (Waters 474) fit with a 5-µL flow cell, at excitation and emission wave lengths of 250 and 395 nm (gain, 100), respectively. Heating the column at 60°C allowed the resolution of citrulline and threonine, which coelute at lower temperatures.

For measurement of the endogenous NOS inhibitors, ADMA, SDMA, and L-NMA, the reverse-phase high-performance liquid chromatography with AccQ Tag method of Anderstam et al¹⁰ was used with minor modifications. This method was established by us after the NO_x production studies were completed, and therefore measurements were made on separate groups, all n = 8. All controls were a subset of the NO_x production series, and the HD group contained four original and four new patients. Four patients had diabetic nephropathy; two patients, glomerulonephritis; and one patient each, renovascular disease and Alport's syndrome. The assay was as follows: blood samples were kept on ice for exactly 90 minutes before centrifugation, precipitation, filtration, and storage at -70°C, as described.¹⁰ This arbitrary time period was chosen to standardize the handling of all samples whether they were acquired in our laboratory or at remote sites and transported to us. Samples were prepared as described,¹⁰ but instead of spiking individual samples, we constructed standard curves (Fig 1). Standards contained equal concentrations of L-NMA, ADMA, and SDMA in the concentration range of 0.625 to 10.0 µmol/L, made up in AccQ fluor borate buffer in a stock of deproteinized control plasma. Samples and standards (30 µL) were injected onto a Waters AccQ Tag 3.9- × 150-mm, 4-µm, silica-based Nova-Pak C18 reversed-phase column at 37°C using a Waters column heater and control module. Fluorescence intensity was measured at 250 nm (excitation) and 395 nm emission (gain, 100) using a Waters 474 scanning fluorescence detector. A flow rate of 1 mL/min was attained using a Waters 600s controller and 616 pump system, using the identical gradient elution table and buffers described by Anderstam et al,¹⁰ with the exception that we used double the concentration of triethylamine in buffer A, which improved our peak resolution (Fig 2). Recoveries of spiked synthetic ADMA, SDMA, and L-NMA added to control plasma were approximately 100%. Concentrations of arginine, citrulline, and methylarginines were calculated using Millennium chromatography manager (version 2.10) software (Waters Corp, Milford, MA) for integrations and calculations based on the established standard curves for each run.

Plasma and urine sodium and potassium levels were measured by flame photometer, creatinine concentrations were by colorimetric assay of the Janovski complex (Sigma kit no. 555-A; St. Louis, MO).

All data were reported as mean $\pm$ SE, and statistics were by paired and unpaired *t*-test and one-way analysis of variance. Statistical significance was considered *P* less than 0.05.

Results

The 24-hour output of NO_x was less in HD patients versus controls (*P* < 0.001; Table 1), based on the rate of removal of NO_x from the body by either HD or, in the case of controls, renal excretion. There was no obvious impact of sex on NO_x output in either controls (women, 740 $\pm$ 103 μ mol/L/24 h; *n* = 8; men, 960 $\pm$ 188 μ mol/L/24 h; *n* = 5; not significant) or HD patients (women, 526 $\pm$ 51 μ mol/L/24 h; *n* = 10; men, 424 $\pm$ 88 μ mol/L/24 h; *n* = 7; not significant). Because dietary NO_x intake was similar (Table 1), the difference between output and intake, which provides a qualitative estimate of total NO production, was less in HD patients versus controls. The groups were well matched; in HD patients (*n* = 17; 7 men, 10 women), the average age was 63 $\pm$ 3 years, body weight was 72 $\pm$ 5 kg, and height was 167 $\pm$ 2 cm. There were no differences compared with demographics of controls (*n* = 13; 5 men, 8 women), in whom age was 56 $\pm$ 4 years, body weight was 73 $\pm$ 2 kg, and height was 169 $\pm$ 2 cm. In contrast to the reduction in total NO production seen in HD patients, the 24-hour output of cGMP (a major second messenger of NO) was not reduced in HD patients (Table 1).

Systolic BP was high immediately in pre-HD patients versus controls (Table 2) despite that 15 of 17 HD patients were receiving one or more antihypertensive medications. Of the 17 HD patients studied, 12 had pre- and postdialysis measurements taken and, as shown in Table 3, systolic and diastolic BPs tended to decline during dialysis but were not significantly different pre-versus post-HD. Weight loss because of fluid removal averaged 2.56 $\pm$ 0.52 kg per treatment in HD patients (*n* = 17), and adequate HD was confirmed by urea reduction ratio (67% $\pm$ 2%). The 24-hour creatinine clearance in the control group was 129 $\pm$ 8 mL/min and 24-hour urine volume was 1,620 $\pm$ 158 mL. In HD patients, 24-hour urine volume was 210 $\pm$ 61 mL and the total fluid removed by hemodialysis was 2,565 $\pm$ 507 mL.

Despite reduced production of NO_x (indicated by low 24-hour NO_x outputs; Table 1), plasma concentrations of NO_x were greater in pre-HD patients (*P* < 0.001) versus controls, reflecting inadequate plasma clearance (Table 2). Plasma NO_x levels decreased significantly and were no longer different from control values immediately post-HD (Table 3), reflecting the very rapid dialytic clearance of the small NO₂ and NO₃ molecules (molecular weight, 46 and 62, respectively). The plasma cGMP values were also greater in pre-HD patients (*P* < 0.001) versus controls and decreased to control values post-HD. Blood urea nitrogen levels were also reduced post-HD but remained slightly elevated versus normal values. Plasma arginine values were not significantly less in HD patients versus controls (Table 2) and did not change during HD (Table 3), whereas pre-HD plasma citrulline concentrations were high versus controls (*P* < 0.001; Table 2). Although plasma citrulline concentration decreased during HD, values remained elevated versus controls (Tables 2 and 3; *P* < 0.005).

The plasma ADMA and SDMA values were obtained in separate groups. There were eight controls (5 men, 3 women) aged 47 $\pm$ 5 years, with body weight of 76 $\pm$ 6 kg and height of 171 $\pm$ 2 cm. The eight HD patients (2 men, 6 women) were not significantly different with respect to age (63 $\pm$ 4 years), body weight (65 $\pm$ 6 kg), and height (165 $\pm$ 3 cm). Plasma levels of the NOS inhibitor, ADMA, were elevated approximately 10 times in pre-HD plasma versus controls (Table 2). SDMA levels were also greater, but L-NMA was undetectable in most plasma samples irrespective of group and is therefore not reported. Figure 2 shows

representative chromatograms from normal plasma (upper panel) in which very small peaks of ADMA and SDMA are evident, whereas in the middle panel, pre-HD plasma contains large ADMA and SDMA peaks. Plasma creatinine and potassium values were high in HD patients versus controls, as expected (Table 2), whereas plasma sodium levels were similar in both groups.

Discussion

Hypertension is a serious problem in ESRD, and systolic hypertension has been singled out for its adverse impact on survival in dialysis-dependent patients.⁷ Irrespective of the cause of ESRD, survival is better in normotensive than hypertensive patients with ESRD. Renal failure–associated hypertension occurs in patients of all volume states and commonly accompanies advancing renal failure before dialysis dependence, when total body water is near normal. A number of patients with ESRD have difficult-to-control hypertension without clinical evidence of volume overload.^{4–7} The contention that refractory hypertension in ESRD is not solely caused by fluid accumulation is supported by clinical experience with patients undergoing PD, who are less often fluid overloaded but commonly require antihypertensive therapy. In the present study, 15 of 17 HD patients in the NO_x production study were receiving antihypertensive medications despite which the pre-HD systolic BP was elevated versus controls.

The present study was designed to test the hypothesis that impaired NO production may occur in ESRD, using NO_x production as our index. NO deficiency has been suggested to occur in renal failure,^{9,11} but as recently noted, direct evidence of NO production in anephric subjects is lacking.¹⁴ We recently suggested NO production is low in patients with ESRD undergoing PD,⁸ and in the present study, a marked reduction in the rate of production of the stable NO oxidation product, NO_x, was found in patients with ESRD undergoing HD, indicating total NO production is also reduced in this group. These measurements were made during controlled low dietary NO_x intake, a uniquely important aspect of this study because uncontrolled dietary NO_x intake is variable and cannot be discriminated from NO_x formed endogenously (because of NO production). In healthy individuals, even when NO_x intake is low and known, NO_x output cannot be used as a quantitative measure of NO production because NO_x leaves the body through several routes. Only approximately 60% to 70% of ingested NO_x (and presumably a similar percentage of endogenously formed NO_x) is removed from the body through the kidneys. Because only approximately 1% to 3% of administered NO_x is lost through the feces, bacterial degradation of NO_x in the gut is believed to account for most of the balance.¹⁵ In the present study, we assume the proportion of NO_x cleared from the body by HD, as a surrogate of functioning kidneys, is equivalent to that removed in the urine in controls.

These observations indicate total NO production is low in patients with ESRD undergoing HD despite the suggestion that the HD process itself can stimulate NO production (through inducible NOS) as part of an inflammatory response.¹⁶ The extent to which this occurs in our study is uncertain because our HD patients were dialyzed with biocompatible membranes, which have low inducible NOS–activating potential.^{16–18} However, some intradialytic NO production may occur even with biocompatible dialyzers, and if that occurred in the present study, the amount of physiologically regulated NO produced in HD patients would be even less than suggested by the reduced NO_x output.

Despite evidence from the NO_x output data that NO production is reduced in HD patients, plasma NO_x levels measured after strict dietary nitrate restriction and after an overnight fast were much greater in pre-HD patients compared with controls. This does not contradict the NO_x output data because NO_x in the body of the patients with ESRD (whether exogenous or endogenous) has accumulated in the plasma for 44 hours since the previous HD. It is very

important to recognize that plasma NO_x values must be interpreted in the context of renal function and volume status.^{15,19} An increase in plasma NO_x may simply reflect loss of renal clearance and cannot automatically be considered to indicate increased NO production. In the present study, plasma NO_x decreased to control values immediately post-HD, which suggests HD adequately replaces the kidney as far as NO_x clearance is concerned.

Soluble guanylyl cyclase is activated by NO to produce the second messenger cGMP,² but in the present study, cGMP output is not different in HD patients versus controls, whereas NO_x output is reduced. Thus, in the HD population, NO_x and cGMP outputs do not change in parallel, with cGMP output greater than predicted based on the NO_x profile. Elevated cGMP output in patients undergoing HD may occur for a number of reasons, including activation of atrial natriuretic peptide, stimulated by the interdialytic volume overload experienced by many HD patients. Increases in plasma atrial natriuretic peptide concentration have been reported in HD-dependent patients with ESRD.²⁰ Greater than predicted outputs of cGMP (and to a lesser extent, NO_x) may also result from HD-induced disruption of blood cells which, if lysed, will extrude their contents into the extracellular fluid. Finally, because cGMP removal from the body is normally by a combination of excretion and metabolism, the use of cGMP output may not provide a quantitative index of NO production in any given state because the contribution caused by metabolism may vary. It has been shown that tissue cGMP, rather than the concentration of cGMP in body fluids, correlates with NOS activity in the chronically NOS-inhibited rat.²¹ Thus, although the patients with ESRD undergoing HD do not show parallel declines in cGMP output, this fragile and hemodynamically complicated population shows low NO_x output.

What causes the relative NO deficiency in ESRD? Arginine used for NOS throughout the body is both derived from the diet and made endogenously, mainly by the kidney.^{11,22} A lack of functional renal mass would compromise endogenous arginine generation and could lead to arginine deficiency and impaired NOS. Citrulline is the substrate for intrarenal arginine generation,²² and this accumulates in anephric patients,¹¹ as also shown by us. Plasma arginine levels have been variously reported as normal, low-normal, or subnormal in patients with ESRD^{9,11} and were at the low end of normal in HD patients in our study. Thus, arginine deficiency is not evident in this population, although plasma arginine levels may not reflect intracellular arginine availability. We recently reported plasma from patients with ESRD inhibits arginine transport into cultured endothelial cells²³; thus, there may be cellular arginine depletion in ESRD. In support of this possibility, orotic acid (a marker for arginine deficiency) levels increase in renal failure.¹¹

Irrespective of whether NOS substrate deficiency occurs in ESRD (which remains an open question), there is evidence that circulating levels of endogenous NOS inhibitors increase in renal failure. Vallance et al⁹ first reported the presence of endogenous NOS inhibitors and their accumulation in patients with renal failure. Of these NOS inhibitors, whereas SDMA is ineffective, ADMA acts as a potent nonselective NOS inhibitor, blocking NO production through inducible NOS (iNOS) and constitutive NOS.⁹ Administered ADMA leads to vasoconstriction in healthy humans and guinea pigs.⁹ If plasma ADMA levels increase and arginine levels are unchanged or decrease, NOS should be inhibited, as suggested by Vallance et al.⁹ Not all investigators agree, however, that plasma ADMA levels increase sufficiently in ESRD to have an impact on NOS activity.^{10,24,25} In the present study, however, we observed marked increases in plasma ADMA levels in uremia to values that, although less than originally reported,⁹ are sufficient to exert some NOS inhibitory effect. Therefore, although this area remains controversial, the present study supports the possibility that an increase in the ADMA-arginine ratio contributes to NO deficiency in patients with ESRD. The endogenous NOS inhibitors are cleared by HD, as reported previously,⁹ although the plasma concentration of ADMA remains elevated versus controls, even immediately post-HD. This, together with the

relative stability of plasma arginine concentration observed by us during HD, should favor increased NOS activity at the end of dialysis and thus provide a mechanism for BP reduction. In a recent study, Madore et al²⁶ used exhaled NO concentrations as an index of NO production pre- and post-HD. Expired NO concentration was greater in HD patients versus controls and decreased during HD, findings at variance with the present observations. As noted by us previously, it is difficult to assess which pool of endogenous NO is being sampled by measurements of NO content in expired air⁹; thus, these findings are difficult to interpret. We consider NO_x removal rate through the kidneys or their surrogate (when measured on a low NO_x intake) should provide a picture of overall body NO production. Whether this reflects the production of NO that controls vascular tone, however, remains to be seen.

In conclusion, this clinical study, conducted under conditions of dietary NO_x control, provides evidence that total NO generation is less in hypertensive patients with ESRD undergoing HD versus normotensive controls. If this reflects hemodynamically active NO, then decreased NOS may contribute to the hypertension of ESRD, although other alterations in the NO system (eg, decreased guanyl cyclase responsiveness to NO and more rapid oxidation of NO to NO_x, thus shortening its active life) may also contribute. Conversely, total cGMP removal rate is not reduced in HD patients (in contrast to our earlier findings in patients with ESRD undergoing PD⁸), probably secondary to hemodynamic complications of HD. Thus, NO_x and cGMP values do not correlate in the anephric population undergoing HD. Increased plasma concentrations of the endogenous NOS inhibitor, ADMA, occur in patients with ESRD undergoing HD, which may contribute to NO deficiency in this population.

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Fig 1. Standard curves for lNMA, ADMA, and SDMA, which are linear in the concentration range of 0.625 to 10.0 μmol/L

Fig 2. Representative chromatograms for (top) control plasma, (middle) HD patient plasma, and (bottom) control plasma spiked with 2.5 $\mu\text{mol/L}$ of L-NMMA, ADMA, and SDMA. All peaks are within the linear range of the standard curves shown in Fig 1

Table 1

Summary of Nitrate + Nitrite (= NO_x) Intake and Output and cGMP Output in Control Subjects and ESRD Patients Undergoing Hemodialysis

	Control	HD Patients
NO _x intake (μmol/L/24 h)	314 ± 10	293 ± 15
NO _x output (μmol/L/24 h)	824 ± 96	552 ± 51 [*]
Δ NO _x (output – intake; μmol/L/24 h)	525 ± 108	191 ± 51 [*]
cGMP output (nmol/L/24 h)	147 ± 48	216 ± 37

^{*} $P < 0.05$ v control.

Table 2
Blood Pressure and Plasma Concentrations in Controls and HD Patients Measured Immediately Pre-HD

	Control	HD Patients
BP (mm Hg)	119 ± 5/78 ± 3	147 ± 5/73 ± 3 [*]
Plasma NO _x (μmol/mL)	28 ± 3	63 ± 8 [*]
Plasma cGMP (pmol/mL)	5 ± 1	25 ± 3 [*]
Plasma arginine (μmol/L)	84 ± 11	77 ± 7
Plasma ADMA (μmol/L) [†]	0.40 ± 0.08	4.14 ± 0.78 [*]
Plasma SDMA (μmol/L) [†]	0.12 ± 0.02	1.41 ± 0.19 [*]
Plasma citrulline (μmol/L)	26 ± 1	104 ± 10 [*]
Plasma cr (mg/dL)	0.7 ± 0.1	8.3 ± 0.4 [*]
Plasma Na ⁺ (mEq/L)	137 ± 1	136 ± 1
Plasma K ⁺ (mEq/L)	4.6 ± 0.1	5.1 ± 0.2 [*]

Abbreviations: HD, hemodialysis; BP, blood pressure; NO_x, nitric oxide oxidation products; cGMP, cyclic guanosine monophosphate; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; cr, creatinine.

^{*} $P < 0.05$ v controls.

[†] These data were obtained from a separate group of HD patients, described in the text.

Table 3
BP and Plasma Concentrations in HD Patients (n = 11) Immediately Before and After a 3.5- to 4-Hour Dialysis Treatment

	Pre-HD	Post-HD
BP (mm Hg)	148 ± 7/75 ± 5	130 ± 9/70 ± 5
BUN (mg/100 mL)	62 ± 2	22 ± 3 [*]
Plasma NO _x (μmol/mL)	61 ± 6	21 ± 2 [*]
Plasma cGMP (pmol/mL)	27 ± 4	8 ± 2 [*]
Plasma arginine (μmol/L)	81 ± 9	66 ± 11
Plasma ADMA (μmol/L) [†]	4.14 ± 0.78	1.45 ± 0.42 [*]
Plasma SDMA (μmol/L) [†]	1.41 ± 0.19	0.62 ± 0.10 [*]
Plasma citrulline (μmol/L)	112 ± 14	44 ± 3 [*]

Abbreviations: BP, blood pressure; BUN, blood urea nitrogen; NO_x, nitric oxide oxidation products; cGMP, cyclic guanosine monophosphate; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; HD, hemodialysis.

^{*} p = 0.05 pre-HD v post-HD.

[†] These data were obtained from a separate group of HD patients, described in the text.