Evidence In Vivo Showing Increase of Baseline Nitric Oxide Generation and Impairment of Endothelium-Dependent Vasodilation in Normotensive Patients on Chronic Hemodialysis

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Abstract. Cardiovascular mortality is excessive in hemodialyzed patients. Observations in atherosclerosis suggest that endothelial dysfunction and impaired nitric oxide (NO) may be involved. However, the relation of endothelial NO to its vascular effects has not been studied conclusively in uremia. Therefore, to study these questions an invasive technique was used in normotensive patients who were on hemodialysis (HD; \( n = 11 \)) and in matched control subjects (\( n = 11 \)). Pharmacologic agents were infused into the brachial artery to test the chain of events from NO generation to smooth muscle cell relaxation, measuring forearm blood flow by venous occlusion plethysmography. Glyceroltrinitrate (GTN 1:2.2 nmol/min; GTN 2:4.4; GTN 3:8.8), infused to establish the reaction of the vessel wall to defined doses of NO, caused a reduced response in HD patients (control subjects: 183 ± 20 [SEM], 246 ± 26, and 338 ± 29%; HD patients: 161 ± 7, 206 ± 12, and 262 ± 24%; baseline = 100% for each group, \( P = 0.032 \) by ANOVA). All subsequent data were corrected for this decreased response to defined doses of NO in HD patients. L-arginine (10 mg/min), given to exclude substrate deficiency of NO synthase (NOS), caused no significant changes (control subjects: 108 ± 4%; HD patients: 103 ± 4%; \( P = \text{NS} \)). Acetylcholine (ACH 1:55 nmol/min; ACH 2:110; ACH 3:220), infused to stimulate endothelial NOS, had a significantly reduced effect in HD patients (control subjects: 246 ± 32, 340 ± 40, and 465 ± 52%; HD patients: 251 ± 55, 244 ± 36, and 318 ± 50%; \( P = 0.002 \)). N-monomethyl-L-arginine (LMA 1:1 \( \mu \text{mol/min}; \text{LMA 2:2; LMA 3:4} \)), given to block baseline NO generation, showed an enhanced response in HD patients (control subjects: 90 ± 2, 83 ± 2, and 74 ± 4%; HD patients: 84 ± 3, 73 ± 3, and 64 ± 4%; \( P = 0.037 \)). Vascular response to three doses of norepinephrine (60, 120, and 240 pmol/min) was comparable in both groups, which indicated similar endothelium-independent vasoconstriction. In summary, in normotensive HD patients, (1) vasodilation to defined doses of exogenous NO was reduced, (2) there was no evidence of substrate deficiency of NOS, and (3) stimulation of NOS was impaired; however, (4) baseline NO generation was increased. It is concluded that in HD patients, the NO system has a reduced capacity to regulate vascular tone and this impairment is most significant under conditions of NOS stimulation.

Considerable progress has been made in the treatment of end-stage renal disease over the past 20 yr. Nonetheless, the dialysis population continues to carry an excess mortality from fatal cardiovascular events (1–3). One possible explanation derives from the association of uremia and accelerated atherosclerosis (4–7). The latter may be related to predialysis factors such as preceding arterial hypertension, glucose intolerance, secondary hyperparathyroidism, dyslipidemia, and others. However, dialysis itself may further contribute to atherosclerosis by oxidative stress, cytokine stimulation, and other events inherent to hemodialysis (HD).

Endothelial dysfunction has been shown recently to be a major initiating factor in atherosclerosis (reviewed in reference (8)). Endothelial dysfunction is associated primarily with decreased nitric oxide (NO). However, endothelial dysfunction of uremia has received little attention. There are only a few studies of NO in uremia, and they have reached contradictory conclusions. NO is difficult to measure, and some of the discrepancies may be technique related.

To clarify the role of NO in vivo, we used the forearm perfusion technique to study endothelial function in hemodialyzed patients. This invasive method permits dissociation of endothelial mechanisms from those of the smooth muscle cell layer. This enabled us to use a more complex set of functional tests than has been used by other investigators of uremia. We generated dose–response patterns for acetylcholine (ACH), a direct stimulus of endothelial NO, and for N-monomethyl-L-
arginine (LMA), a blocker of NO synthases (NOS). We also infused l-arginine (l-ARG), the substrate of NOS. Because these tests are functional tests, depending on the ability of arterial resistance vessels to respond to NO, we took specific care to establish a dose–response curve for NO itself. To do so, we infused glyceroltrinitrate (GTN). To evaluate further the principal constrictory properties of uremic resistance vessels, we also tested the vascular response to norepinephrine (NE).

In this study, we focused on normotensive patients on HD. We matched the control subjects for age, gender, height, weight, BP, and serum cholesterol.

**Materials and Methods**

**Patients**

The study was approved by the ethics committee of our institution. All participants gave informed written consent. The studies were carried out in 11 patients on chronic HD and 11 matched healthy control subjects (Table 1). All participants were nonsmokers. They did not have hypercholesterolemia, congestive heart failure, diabetes mellitus, or liver cirrhosis. None of the patients or control subjects was receiving antihypertensive medication or nitrate therapy. All subjects were asked to refrain from large meals and beverages that contained alcohol or caffeine during the 6 h before the study.

**Forearm Blood Flow Studies**

Studies were performed in a quiet, temperature-controlled room (23 to 25°C) between 9 a.m. and 4 p.m. with the subjects resting supine.

| Table 1. General characteristics of HD patients and healthy control subjects* |
|------------------|------------------|------------------|------------------|
| Characteristic   | Control Subjects (mean ± SEM) | HD Patients (mean ± SEM) | P Value |
| Gender (F/M)     | 5/6               | 4/7               | 0.87             |
| Age (yr)         | 38 ± 4           | 39 ± 5           | 0.87             |
| Height (cm)      | 169 ± 3          | 168 ± 3          | 0.82             |
| Weight (kg)      | 65.9 ± 3.8       | 64.9 ± 3.4       | 0.84             |
| Mean arterial pressure (mmHg) | 90 ± 3       | 91 ± 4          | 0.88             |
| Baseline FBF (ml/100 ml) | 2.6 ± 0.2 | 2.5 ± 0.2       | 0.85             |
| Total cholesterol (mmol/L) | 5.1 ± 0.1 | 5.2 ± 0.2       | 0.83             |
| Hemoglobin (mmol/L) | 9.0 ± 0.3       | 7.3 ± 0.2       | <0.0001          |
| Hematocrit       | 0.42 ± 0.01      | 0.36 ± 0.01      | 0.001            |
| Time on HD (mo)  | 81 ± 4           | 81 ± 4           | 0.001            |
| Primary renal disease | 5 chronic GN  | 3 ADPKD-I       | 3 chronic PN (urinary tract malformations) |

* HD, hemodialysis; FBF, forearm blood flow; GN, glomerulonephritis; ADPKD-I, autosomal dominant polycystic kidney disease, type I; PN, pyelonephritis.

HD patients were studied during their interdialytic day. All infusions were administered in the brachial artery. In control subjects, we used the brachial artery of the nondominant arm, whereas in HD patients, the arm opposite the one carrying the arteriovenous fistula was cannulated. We inserted a 20-gauge silicon catheter (Vygon; Ecouen, France) under local anesthesia (1% lignocaine) using the Seldinger technique. A pressure transducer (Baxter; Unterschleißheim, Germany) was connected to the arterial catheter via a three-way stopcock for continuous monitoring of arterial BP. Forearm blood flow (FBF) was measured by strain gauge plethysmography (Periquant 815; Gutmann Medizinelektronik, EURASBURG, Germany) as published (9), with modifications. The modifications were required because HD patients have only one arm for flow measurements (the other arm usually has the dialysis fistula and is therefore unavailable). Accordingly, flow measurements were obtained in the cannulated arm only in all participants. The upper arm cuff for venous congestion was kept inflated at 40 mmHg for 10 s during each 15-s cycle of FBF measurement. The circumferential increase of the forearm in response to the cuff–induced venous congestion was measured by a calibrated strain gauge that was placed around the widest part of the forearm. Arterial inflow during venous outflow obstruction is proportional to the increase in circumference (10). Arterial inflow is expressed as milliliters per 100 ml of forearm volume. To provide for a rapid venous outflow after cessation of venous congestion, the forearm was placed 10 cm above the level of the right atrium. During all recording periods, the hand was excluded from the circulation by a wrist cuff inflated to a suprasystolic pressure of 200 mmHg.

Determinations of baseline FBF were started 20 min after insertion of the arterial catheter. As shown in Figure 1, at least two baseline determinations of FBF were obtained before the beginning of the experimental protocol. Each determination took 2.5 min. We made 10 individual measurements in succession during each 2.5-min period of FBF determination. Determinations of FBF were repeated during all subsequent periods of drug infusion. Each drug dose was given for 4 min at a constant rate of 1 ml/min. We made 16 measurements of FBF during each dosing period. Between the infusions of different agents, a control period of 20 min was allowed, and baseline FBF was determined thereafter by six individual measurements before the infusion of any new agent (Figure 1).

**Study Protocol and Agents**

After the end of the baseline period, participants received an infusion of l-ARG at one dose (57 μmol/min = 10 μg/min). In the second part of the protocol, ACH was given in increasing doses of 55, 110, and 220 nmol/min. In the third part of the protocol, subjects received LMA in three doses (1, 2, and 4 μmol/min). The study protocol was finished by observations of the effects of three doses (2.2, 4.4, and 8.8 nmol/min) of GTN. In a different session, six HD patients and six matched control subjects received three doses of NE (60, 120, and 240 pmol/min).

Agents were obtained from the following: l-ARG and LMA from Clinalfa (Laufelfingen, Switzerland), ACH as Acetylcholinum ophthalnicum dispersa from Ciba Vision (Germering, Germany), GTN as Perlagant from Schwarz-Pharma (Monheim, Germany), and NE as Arterenol from Hoechst Marion Roussel (Frankfurt, Germany). All drug solutions were prepared fresh before each study using isotonic saline to obtain the required concentrations.

**Statistical Analyses**

For each determination of FBF, the mean of the last 10 individual FBF measurements was calculated (in several control periods, the
mean of six individual measurements was calculated) (Figure 1). Observations are reported in percentage of the FBF baseline value (FBF observed/baseline FBF \times 100) \pm SEM. For the baseline FBF, we used the one determined just before the period of observation. As shown in the studies using GTN, the response of FBF to defined doses of NO was different between HD patients and control subjects; we therefore report the results of observations involving L-ARG, ACH, and LMA corrected for this difference. For these corrections, we used the equation 100 + [(FBF response to test agent (in percentage of baseline) − 100)/[FBF response to GTN 3 (in percentage of baseline) − 100] \times 100.

Comparisons between HD and control groups were done by two-way ANOVA for repeated measurements. The data for baseline FBF as well as those for observations under L-ARG were evaluated by \( t \) test. \( P < 0.05 \) was considered statistically significant. Statistical calculations were performed by means of the computer software Statistical Package for the Social Sciences, version 8.0.1.

Results

General characteristics of study patients and control subjects were comparable (Table 1). All participants tolerated the studies well. There were no side effects or complications. The concentrations of parathyroid hormone in plasma (292 ± 62 ng/ml [SEM]) and the mean dose of erythropoietin (4182 ± 700 U/wk) in the HD patient group were neither excessive nor unusual for our unit. BP and heart rate remained unchanged in all participants throughout the entire study period.

Baseline FBF values in the second half of the protocol before LMA and GTN in HD patients and control subjects differed slightly from initial baseline FBF (before L-ARG). However, this variation was similar in HD patients and control subjects (baseline before LMA: 142 ± 11% [SEM] of initial baseline in control subjects versus 131 ± 11% in HD patients, \( P = 0.1 \); baseline before GTN: 91 ± 7% in control subjects versus 77 ± 4% in HD patients, \( P = 0.44 \)). In this section, the presentation of our observations is given in a sequence other than that of the actual experiments to facilitate interpretation of results. Table 2 lists all observed raw

### Table 2. FBF in response to infusions of L-arginine (L-ARG), acetylcholine (ACH), N-monomethyl-L-arginine (LMA), glyceroltrinitrate (GTN), and norepinephrine (NE) including the foregoing baseline FBF values

<table>
<thead>
<tr>
<th>Variable</th>
<th>FBF Control ((n = 11)) (ml/100 ml × min)</th>
<th>FBF HD patients ((n = 11)) (ml/100 ml × min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>L-ARG</td>
<td>3.0 ± 0.3</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>ACH 1</td>
<td>10.2 ± 1.4</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>ACH 2</td>
<td>15.4 ± 2.3</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>ACH 3</td>
<td>21.9 ± 3.2</td>
<td>9.8 ± 1.5</td>
</tr>
<tr>
<td>Baseline</td>
<td>3.6 ± 0.4</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>LMA 1</td>
<td>2.9 ± 0.4</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>LMA 2</td>
<td>2.4 ± 0.4</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>LMA 3</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.3 ± 0.3</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>GTN 1</td>
<td>4.2 ± 0.5</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>GTN 2</td>
<td>5.6 ± 0.7</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>GTN 3</td>
<td>7.5 ± 0.7</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.2 ± 0.3(^b)</td>
<td>2.4 ± 0.4(^b)</td>
</tr>
<tr>
<td>NE 1</td>
<td>1.8 ± 0.3(^b)</td>
<td>2.1 ± 0.4(^b)</td>
</tr>
<tr>
<td>NE 2</td>
<td>1.5 ± 0.2(^b)</td>
<td>1.8 ± 0.3(^b)</td>
</tr>
<tr>
<td>NE 3</td>
<td>1.3 ± 0.2(^b)</td>
<td>1.3 ± 0.3(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Data represent mean ± SEM.  
\(^b\) \( n = 6 \).
data. Table 3 basically reports the same data as Table 2 in percentage of baseline. GTN infusion (Figure 2) resulted in a significant and dose-dependent increase in FBF. Control subjects: 183 ± 20% (GTN 1); 246 ± 26% (GTN 2); and 338 ± 29% (GTN 3); HD patients: 161 ± 7% (GTN 1); 206 ± 12% (GTN 2); and 262 ± 24% (GTN 3). The HD group had a significantly smaller response to GTN than did control subjects ($P = 0.032$).

Because the effects of L-arginine, ACH, and LMA depended on the ability of arterial resistance vessels to respond to NO and because HD patients showed a significantly smaller response to defined doses of NO than control subjects did, we decided to report all results of those observations after correction (Figures 3 to 5) for a standard NO-mediated dilation (GTN 3).

L-arginine infusion (Figure 3) resulted in an increase in FBF to 108 ± 4% in control subjects and 103 ± 4% in HD patients. There was no difference between both groups ($P = 0.36$).

ACH infusion (Figure 4) was followed by a dose-dependent rise in FBF. Control subjects: 246 ± 32% (ACH 1), 340 ± 40% (ACH 2), and 465 ± 52% (ACH 3); HD patients: 251 ± 55% (ACH 1), 244 ± 36% (ACH 2), and 318 ± 50% (ACH 3). Comparison of both groups revealed significantly less ACH-induced vasodilation in the HD group than in the control group ($P = 0.002$).

NE induced a significant and dose-dependent decrease of FBF in both groups. Control subjects: 83 ± 5% (NE 1), 67 ± 4% (NE 2), and 56 ± 5% (NE 3); HD patients: 85 ± 3% (NE 1), 71 ± 5% (NE 2), and 52 ± 5% (NE 3). These responses statistically were not different ($P = 0.15$), thus indicating similar endothelium-independent vasoconstriction in both groups.

LMA reduced FBF in a dose-dependent manner (Table 2). We reasoned that the impairment of response to defined doses of NO (GTN experiment) most likely would prevail in the LMA observations as well. We therefore corrected the FBF response to LMA accordingly (i.e., reporting the FBF data as fraction of the standard NO-induced vasodilation [GTN 3]). The results ($90 ± 2%$ [LMA 1], $83 ± 2%$ [LMA 2], and $74 ± 4%$ [LMA 3] in control subjects versus $84 ± 3%$ [LMA 1], $73 ± 3%$ [LMA 2], and $64 ± 4%$ [LMA 3] in HD patients) (Figure 5) demonstrated a significant difference of the calculated data. This probably shows an increased constrictor response to LMA in the HD group ($P = 0.037$).

**Discussion**

These studies are an attempt to describe the functional state of the NO system in patients on chronic HD, using carefully matched control subjects. Because it is presently impossible to measure NO directly *in vivo*, we used a battery of related functional tests. All of them are based on FBF and its regulation by NO. We used the standard forearm perfusion technique (9) with modifications. In an HD patient, only one arm is available for measurement of FBF; the arm carrying the arteriovenous fistula is considered unavailable. Standard procedures, however, recommend simultaneous measurements in both arms. The latter are used to exclude systemic changes during the protocol (11). To account for this limitation in our patients, we obtained control measurements for baseline FBF between all infusions of agents in our protocol. All agents were given in doses that are known to have local effects only. Furthermore, we avoided noise, pain, or fright in our patients as best as possible. In this way, we were able to demonstrate stability of baseline values and a lack of change of BP and heart rate. This is compatible with a lack of systemic effects throughout the protocol. To account for the small numerical changes of the baseline FBF that did occur between the infusions of agents, we reported all results corrected for baseline control obtained immediately preceding the respective experimental step.

Four different aspects of the NO system were studied: (1) We infused L-arginine to assess the role(s) of potential L-arginine deficiency and of possible endogenous NOS inhibitors and to obtain an indication of the effect of L-arginine on NO formation. (2) We infused ACH to stimulate endothelial NOS (eNOS, NOS III). (3) LMA was given to block the activity of all isoforms of NOS. This was used to explore the contribution of endogenous NO to basal FBF. (4) We infused GTN to determine the underlying capacity of resistance vessels to respond to defined doses of (exogenous) NO. (5) In an additional experiment, we tested vascular responsiveness to the endothelium-independent vasoconstrictor NE for comparison with the results obtained under LMA infusion.

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**Table 3.** FBF response to one dose of L-arginine (L-ARG), three doses of acetylcholine (ACH), and three doses of $N$-monomethyl-L-arginine (LMA)$^a$

<table>
<thead>
<tr>
<th>Agent/Dose</th>
<th>FBF (% of baseline) Control</th>
<th>FBF (% of baseline) HD Patients</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-ARG</td>
<td>116 ± 7%</td>
<td>107 ± 6%</td>
<td>NS (by $t$ test)</td>
</tr>
<tr>
<td>ACH 1</td>
<td>418 ± 60%</td>
<td>296 ± 42%</td>
<td>0.003 (by ANOVA)</td>
</tr>
<tr>
<td>ACH 2</td>
<td>597 ± 75%</td>
<td>315 ± 46%</td>
<td></td>
</tr>
<tr>
<td>ACH 3</td>
<td>865 ± 100%</td>
<td>433 ± 76%</td>
<td></td>
</tr>
<tr>
<td>LMA 1</td>
<td>78 ± 4%</td>
<td>77 ± 4%</td>
<td>NS (by ANOVA)</td>
</tr>
<tr>
<td>LMA 2</td>
<td>63 ± 4%</td>
<td>63 ± 4%</td>
<td></td>
</tr>
<tr>
<td>LMA 3</td>
<td>49 ± 4%</td>
<td>51 ± 3%</td>
<td></td>
</tr>
</tbody>
</table>

$^a$These data were not corrected for impaired NO response in HD. Values represent mean ± SEM.
Few published studies have attempted to delineate the role of the NO system in uremic patients in vivo. Most of them used noninvasive techniques. Several authors measured vasodilation in arterial conduit vessels in response to postischemic flow and to sublingual GTN (12–14) by high-resolution ultrasound. Flow-dependent vasodilation in conduit vessels is known to be dependent on endothelium-derived NO (15). In another study, the vasomotion of dorsal hand veins in response to local infusion of ACH, GTN, and L-ARG was tested (16). For several reasons, it is difficult to compare our results with the studies mentioned previously. First, NO effects were studied in different types of vessels. Second, the age of the participants as well as the duration and state of uremia in the individual study groups differed. Third, none of the techniques mentioned is able to provide information on the contribution of NO to the basal vascular tone. Compared with these methods, the technique that we used may offer a technically more direct and a pharmacologically better defined approach to arterial vascular NO function. In a recently published abstract (17), the authors also documented use of our technique in patients with renal insufficiency; however, the patients were not undergoing dialysis. In comparison with these studies, we offer the following comments concerning our results.

Response to GTN

We considered the dilatory properties of NO in the muscular vessel wall per se. As shown in Figure 2, the response to GTN was reduced consistently and significantly in the HD patients. Recently, Adams et al. (18) performed a large, ultrasound-based study of endothelium-dependent and -independent vasodilation in 800 volunteers. They demonstrated that the vasodilator response to exogenous NO is impaired in subjects who are at risk for atherosclerosis (cigarette smoking, diabetes mellitus, hypercholesterolemia) independently from endothelium-dependent vasodilation. Joannides et al. (14) and Hand et al. (17) in their studies found the response to GTN to be decreased in uremic subjects. Conversely, Kari et al. (12), van Guldener et al. (13), and Hand et al. (16) found in their patients a response to GTN that was not significantly different from that in control subjects. We are unable to explain these different findings. They may be related in part to methodologic aspects and different study populations as mentioned previously. It is conceivable that the preuremic state and/or the young age of subjects in the study of Kari et al. is associated with lesser influences on the vasodilatory effects to exogenous NO than those to be found in fully established uremia of adults.

There are several conceivable explanations for the diminished response to exogenous NO in HD patients. Oxidative stress—believed to be present in HD patients (19,20)—may have contributed to inactivation of NO (21,22). This possibility
might be tested using antioxidants. Furthermore, there may be a defect in the vascular smooth muscle cell signaling pathway downstream from NO. For instance, an impairment of the activity of guanylate cyclase could result in a reduced vasodilation in response to exogenous and endogenous NO. Finally, fibrosis of the arterial wall may cause increased stiffness and reduced distensibility of the vessels in HD patients. Such observations have been reported by Mourad et al. (7). However, this observation was made in conduit vessels, and it is unclear to what extent their findings might apply to resistance vessels (that have been studied by us) as well. Recent, albeit preliminary, studies in our laboratory have tested the effects of adenosine (75, 150, and 300 μg/min) instead of GTN as a vasodilator. Under these conditions, resistance vessels of HD patients dilated comparably with control subjects. These preliminary findings therefore may indicate that a defect in the cellular signaling cascade of NO or increased breakdown of NO may be involved in the deficient response to GTN in HD patients.

Figure 4. FBF in response to three doses of N-monomethyl-L-arginine (1, 2, and 4 mmol/min) after correction for standard nitric oxide-mediated vasodilation (GTN 3). The corrected FBF response was significantly increased in patients on HD (P < 0.05, control subjects versus HD patients by ANOVA), whereas the uncorrected FBF response was similar in both groups.

Figure 5. FBF in response to three doses of ACH (55, 110, and 220 nmol/min) after correction for standard nitric oxide-mediated vasodilation (GTN 3). The corrected and the uncorrected FBF responses were significantly reduced in patients on HD (P < 0.005, control subjects versus HD patients by ANOVA).
patients. Our finding of a decreased vascular response to exogenous NO may have implications for nitrate therapy in uremic patients.

**Basal NO Tone**

After correction for standard NO-induced vasodilation (GTN 3), LMA induced greater vasoconstriction in HD patients than in control subjects (Figure 5). Studies using intrabrachial infusions of LMA in uremic patients have not been reported in the literature. Given that the principal constrictory properties are comparable in both groups (response to NE), our result suggests an increased baseline vascular NO release in uremic patients. A note of caution may be appropriate. On the basis of Figure 2 (dose–response curve for GTN), we made the assumption that both curves would continue steadily in the range of very low NO and even at FBF levels below 100% of baseline. Although this assumption seems justified from the results in Figure 2, it has not been tested directly (infusion of NO scavengers). Studies of NO production in uremia are controversial. Most laboratories performed measurements of stable NO metabolites—as markers of NO—in uremic patients. They were usually found to be elevated (23–26), but contrary results have been reported, too. A recent study determined whole-body NO production by radionuclide-based measurement of arginine to citrullin conversion and reported a reduced release of NO in uremic patients compared with matched control subjects (27). However, the participants of this study were markedly older and at least some of them had hypertension and atherosclerosis. Thus, conditions other than uremia may have contributed to the reduced NO production in these patients. In addition, these individuals were not undergoing renal replacement therapy. It has been suggested that the frequent blood-membrane contact in HD patients might be associated with NOS activation and hence increased NO production (28). Thus, the results of this study (27) may not be representative of the population reported here. Furthermore, endothelial cells in culture produced more NO after exposure to uremic than to normal serum (29). In addition, Aiello et al. (30) found an increased baseline NO production in the systemic circulation of rats with renal insufficiency compared with control subjects. This was associated with an increased vascular expression of eNOS and inducible NOS. It is unclear whether this finding also applies to uremia in humans. Taken together, uremia in normotensive HD patients seems not to be a state of NO deficiency under baseline conditions but rather a state of NO excess. Uremia has been described as a state of chronic endothelial injury, as shown by a typical pattern of endothelial markers in the sera of HD patients (31). It is conceivable that an elevated baseline NO production in the presence of oxidative stress would result in increased peroxynitrite formation, thus leading to maintained endothelial damage. Future studies should be directed at the isoform(s) of NOS in uremia and its stimuli. In addition, it might be useful to test whether uremic toxins or factors related to the mode of the dialysis treatment are involved.

In our study, we did not measure L-ARG analogues—such as dimethyl-L-arginine—directly. It has been proposed that L-ARG analogues accumulate in renal failure, causing competitive inhibition of NOS (32). In the present studies, however, the effects of LMA in uremic patients were not reduced, as would be expected in the presence of significant levels of L-ARG analogues. Our observations therefore did not show a major role of these compounds in normotensive patients on HD. A similar result was reached by Anderstam et al. (33). However, these observations do not exclude a potential role of L-ARG analogues in the setting of hypertensive uremia.

**Intrabrachial Infusion of L-ARG**

These infusions, delivered at a dose of 10 mg/min, led to a small increase in FBF in control subjects. In HD patients, FBF tended to increase as well, but this did not become significant. The mechanism(s) by which L-ARG induces vasodilation has not been clarified conclusively. One suggestion is that L-ARG stimulates NO via its effect to increase the release of insulin (34), but other mechanisms also may be involved (35–37). Imaizumi et al. (38) described a threshold dose for L-ARG infusions of 20 mg/min in healthy volunteers. We cannot explain the differences in threshold dose; however, it is obvious that the dose that we used was a relatively small one. It has been suggested in the literature that uremia is a state of potential L-ARG deficiency (39). We did not measure L-ARG in the plasma of our participants. However, the response to L-ARG in HD patients (Figure 3) was not greater than that of control subjects, as we would have expected in L-ARG deficiency. In fact, the response seemed to be less in HD patients than in control subjects. Therefore, our observations fail to support the possibility of L-ARG deficiency in uremic patients on HD, at least under baseline conditions.

**Stimulation of eNOS by ACH**

The FBF response to intrabrachial infusion of ACH was considerably decreased in the HD group compared with control subjects (Figure 4). These observations are basically in accordance with previous reports in the literature (12–14,16). The techniques used and the vascular tributaries examined by these studies were different from our own approach. Nonetheless, the summarized data suggest reduced ACH-triggered endothelium-dependent vasodilation in uremia. ACH stimulates the endothelial release of NO, vasodilating and/or constricting prostaglandins, and endothelium-derived hyperpolarizing factor(s) (8). On the basis of our study, we cannot ascribe the reduced responsiveness of uremic resistance vessels to ACH to the absence or presence of one of these factors alone. Additional human studies are necessary to delineate the potential role of vasoconstrictor prostaglandins and of LMA-resistant vasodilation.

With respect to the NO system, it would be useful to test whether L-ARG can improve the blunted vascular response to ACH, which has been shown in human dorsal hand veins (16) but could not be demonstrated in experimental renal failure (40). Similarly, a reduced availability of critical cofactors for NO synthesis, such as tetrahydrobiopterin, might be involved. Furthermore, proinflammatory cytokines (e.g., tumor necrosis factor α), which usually are present in HD patients (41,42),
may cause endothelial dysfunction (43). Alternative explanations include changes in the expression of eNOS and/or inducible NOS (44). Additional studies would be useful to delineate the role of these potentially important factors in uremia.

References


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