Hemodialysis Impairs Endothelial Function via Oxidative Stress
Effects of Vitamin E–Coated Dialyzer

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**Background**—Patients who undergo hemodialysis experience accelerated atherosclerosis and premature death. Recent evidence suggests that endothelial dysfunction proceeds to and exacerbates atherosclerosis. It remains unknown whether hemodialysis per se causes endothelial dysfunction.

**Methods and Results**—We evaluated endothelial function estimated by flow-mediated vasodilation during reactive hyperemia using high-resolution ultrasound Doppler echocardiography before and after a single session in patients on maintenance hemodialysis. Several studies have shown that the imbalance between pro-oxidant and antioxidant activities in hemodialyzed patients results in high oxidative stress, which causes lipid peroxidation and endothelial injury. Accordingly, we investigated the effects of antioxidative modification during hemodialysis on endothelial function using a vitamin E–coated cellulose membrane dialyzer. Nonspecific endothelium-independent vasodilation was measured after administration of a sublingual glyceryl trinitrate spray (0.3 mg). A single session of hemodialysis by noncoated dialyzer impaired flow-mediated vasodilation (*P*<0.05) associated with increased plasma levels of oxidized LDL (*P*<0.05), an index of oxidative stress. Hemodialysis by vitamin E–coated membrane prevented dialysis-induced endothelial dysfunction and increases in oxidized LDL. Plasma levels of oxidized LDL were inversely correlated with the magnitudes of flow-mediated vasodilation (*r* = −0.53, *P* < 0.001). Hemodialysis by noncoated or vitamin E–coated membrane did not affect glyceryl trinitrate–induced endothelium-independent vasodilation.

**Conclusions**—Our findings indicate that hemodialysis per se impairs endothelial function, possibly by increasing oxidative stress. (*Circulation. 2000;101:1002-1006.)*

**Key Words:** antioxidants ■ atherosclerosis ■ vasodilation ■ kidney ■ lipoproteins

Patients with end-stage renal diseases who undergo hemodialysis experience accelerated atherosclerosis, and the high prevalence of atherosclerotic cardiovascular death accounts for a great majority of mortality in this population.1–4 Morphological and mechanical alterations of conduit arteries have been characterized by atherosclerosis in hemodialyzed patients.5–9 Endothelium-derived NO plays a crucial role not only in control of vascular tone of conduit arteries but also in antiatherosclerosis by several mechanisms, such as inhibition of platelet aggregation/adhesion or of smooth muscle cell proliferation.10–12 Recent reports demonstrated that NO–dependent flow-mediated vasodilation is blunted in patients undergoing hemodialysis,13,14 suggesting that the impaired bioactivity of endothelium-derived NO in the conduit artery may contribute to the progression of atherosclerosis in hemodialyzed patients.

Oxidative stress plays a pivotal role in the pathogenesis of vascular injury and in the progression of atherosclerosis by several mechanisms, some of which are associated with the inhibition of NO synthase activity and the inactivation of NO by reactive oxygen species.15 Previous studies have shown the imbalance in pro-oxidant and antioxidant activities in hemodialyzed patients,16 resulting in high oxidative stress demonstrated as enhanced lipid peroxidation.17,18 Hemodialysis per se has been suggested to induce oxidative stress, with reactive oxygen species being generated on the surface of dialysis membranes by activation of polymorphonuclear leukocytes.19–23 Indeed, it has been well documented that even a single session of hemodialysis significantly increases lipid peroxides and decreases antioxidants.24–27 Thus, it is plausible that hemodialysis induces oxidative stress and impairs the bioactivity of NO, resulting in accelerated atherosclerosis. To attenuate the activation of polymorphonuclear leukocytes on the surface of dialysis membranes, a vitamin E–coated multilayer hemodialysis filter was recently produced.28,29 With this dialyzer, it has been shown that the
consumption of blood antioxidants, oxidative demineralization of lipids, and activation of leukocytes were significantly attenuated.28,29

Accordingly, we hypothesized that hemodialysis per se may impair endothelial function and that hemodialysis using a vitamin E–coated dialyzer may restore endothelial dysfunction. To test this hypothesis, we measured flow-mediated vasodilation and plasma levels of oxidized LDL (oxLDL), an index of lipid peroxidation, before and after a single session of hemodialysis by a crossover design using vitamin E–coated or noncoated cellulose membrane dialyzer.

Methods

Subjects
Twelve hemodialyzed patients (6 men, 6 women) were enrolled in our study. All patients were receiving hemodialysis 3 times a week by cellulose membrane dialyzer, and each session lasted for 4 to 5 hours. They had been undergoing dialysis for a mean of 63.5 ± 2.9 years. Causes of end-stage renal disease were glomerulonephritis (n = 10), interstitial nephritis (n = 1), and Fancouni syndrome (n = 1). Patient mean age was 40.5 ± 3.6 years (range, 20 to 64 years). All female patients were postmenopausal. No patients had symptomatic coronary artery diseases or a family history of premature cardiovascular diseases. All subjects were nonsmokers, nonobese (body mass index, 21.1 ± 0.4 kg/m²), nondiabetic (fasting plasma glucose, 93 ± 4 mg/dL), and normolipidemic (total cholesterol, 151 ± 7 mg/dL; LDL cholesterol, 82 ± 6 mg/dL; HDL cholesterol, 47 ± 3 mg/dL; triglycerides, 106 ± 10 mg/dL), with normal plasma levels of vitamin E (0.91 ± 0.05 nmol/mL). All patients were treated with a small dose of erythropoietin (3000 U/wk). Six patients were hypertensive and were taking a calcium antagonist (n = 4), a calcium antagonist plus ACE inhibitor (n = 1), a calcium antagonist plus ACE inhibitor plus α-blocker (n = 1), and an antplatelet agent (n = 1). All medications were discontinued 12 hours before the study.

General Procedure
The protocol was explained, and written informed consent was obtained from each subject. The study was approved by the Ethical Committee for Human Study in our institution. The study was done with subjects in the supine position and in an air-conditioned room at 22°C to 23°C. We used a noncoated cellulose dialyzer (CLS-15N) or a cellulose dialyzer coated with the antioxidant vitamin E (CLS-15N) of the same membrane size. For the first experiment, all subjects underwent hemodialysis by either a cellulose or a vitamin E–coated cellulose membrane dialyzer. Endothelial function and blood chemistry measurements were done immediately before and after hemodialysis. Then they underwent hemodialysis twice by cellulose membrane. For the second experiment, the protocol of the first experiment was repeated in all patients by a crossover design. Blood samples were obtained from the contralateral shunt arm vein.

Measurements of Endothelial Function
Flow-mediated vasodilation of the brachial artery was measured by a previously described noninvasive technique to assess endothelial function.30 Briefly, after a 10-minute equilibration period, with the use of a 10-MHz linear-array transducer and the SSA-380A system (Toshiba), the brachial artery was longitudinally imaged 5 cm proximal to the antecubital crease, twice at baseline and then from 1 to 15 minutes continuously after the release of 4.5-minute upper arm arterial occlusion at 250 mm Hg of pressure with a 12.5-cm-wide cuff. Photographic images of end-diastolic frames were obtained and analyzed by 2 independent investigators blinded to the subjects and sequences. The arterial diameter was measured by a caliper at the single most equivalently imaged site with side-by-side presentation. Flow-mediated vasodilation, ie, endothelium-dependent vasodilation, was determined as the maximal percent diameter change of the postocclusion arterial diameter measurement relative to the mean of the corresponding 2 baseline measurements. The mean of the 2 measurements by independent observers was calculated. The interobserver and intraobserver variations of 2 baseline measurements were 2.8% and 1.6%, respectively. Blood flow velocity was measured by Doppler at baseline and immediately after the release of cuff occlusion. Arterial blood flow was determined as arterial cross-sectional area times mean Doppler velocity. The magnitude of reactive hyperemia was calculated as the maximum flow divided by the baseline flow. As an internal control, we measured changes in the brachial diameter, ie, endothelium-independent vasodilation, induced by sublingual glyceryl trinitrate (GTN; 300 mg; Myocor Spray, Toa Eiyo Co) given 15 minutes after the measurement of flow-mediated vasodilation. Five minutes after GTN administration, the scan was done to assess endothelium-independent vasodilation.

Oxidized LDL
Blood samples were collected in a tube containing EDTA, and plasma was stored in a refrigerator at 4°C. Plasma oxLDL was measured by a sandwich ELISA method as previously described.17,31,32 Briefly, LDL fractions were obtained from samples by sequential ultracentrifugation. The diluted LDL fractions (5 μg/well) were added to microtiter wells precoated with 0.5 μg of an anti-oxLDL monoclonal antibody (FOH1a/DLH3). After extensive washing, the remaining oxLDL was detected by use of a sheep anti-human apolipoprotein B antibody and an alkaline phosphatase–conjugated anti-sheep IgG antibody. In each ELISA plate, various concentrations of standard oxLDL, which was prepared by incubating LDL with 5 μmol/L CuSO_4 at 37°C for 3 hours, were run simultaneously to determine a standard curve.

Other Chemical Analyses
Plasma vitamin E was determined by high-performance liquid chromatography. Serum total cholesterol, HDL cholesterol, triglycerides, creatinine, blood urea nitrogen, and glucose were determined with commercial kits (Boehringer Diagnostica, Wako Chemicals Co, Daiichi Chemicals Co, and Kanto Chemical Co). LDL cholesterol was calculated by Friedewald’s formula.

Statistical Analysis
Results were expressed as mean ± SEM, and a value of P < 0.05 was considered to be statistically significant. Predialysis and postdialysis results with noncoated or vitamin E–coated dialyzer were analyzed by 2-way ANOVA for repeated measures. Linear regression analysis was performed between oxLDL and flow-mediated dilatation.

Results
A single session of dialysis with noncoated or vitamin E–coated dialyzer similarly decreased body weight, blood urea nitrogen, and creatinine and increased hematocrit (Table 1). Plasma levels of vitamin E were not different at baseline and were not altered by vitamin E–coated or noncoated dialyzer (Table 1). There were similar tendencies toward decreases in blood pressure and increases in heart rate after a session of hemodialysis by noncoated or vitamin E–coated dialyzer (Table 2).

Plasma OxLDL
Plasma levels of oxLDL before a session of hemodialysis were similar between noncoated and vitamin E–coated dialyzers (P = NS, Figure 1). After a session of hemodialysis by noncoated cellulose dialyzer, oxLDL increased by 35% from baseline (P < 0.05; Figure 1), whereas it was not altered by vitamin E–coated dialyzer (P = NS by 1-way ANOVA; P < 0.05 versus noncoated dialyzer by 2-way ANOVA; Figure 1).
Vascular Function
Before and after hemodialysis, there were no significant differences in the baseline vessel diameter, peak hyperemic response, or GTN-induced vasodilation between noncoated and vitamin E–coated dialyzers (Table 2). After a session of hemodialysis by noncoated cellulose dialyzer, flow-mediated vasodilation was significantly blunted ($P<0.05$; Figure 2). By contrast, the vitamin E–coated dialyzer did not alter flow-mediated dilation ($P=NS$ by 1-way ANOVA; $P<0.005$ versus noncoated dialyzer by 2-way ANOVA; Figure 2). Furthermore, plasma levels of oxLDL were inversely correlated with the magnitudes of flow-mediated vasodilation ($r=-0.53$, $P<0.001$; Figure 3). There were no significant differences in the basal diameters of the brachial artery, peak hyperemic responses, flow-mediated vasodilation, or GTN-induced vasodilation between hypertensives and normotensives or between men and women, and these parameters were not affected by the use of medications (data not shown). There was no correlation between GTN-induced vasodilation and plasma oxLDL.

Discussion
The salient findings of this study are that (1) endothelium-dependent vasodilation was blunted after a single session of hemodialysis by cellulose membrane dialyzer, associated with the increase in plasma oxLDL, an index of oxidative stress, and (2) with the use of a vitamin E–coated cellulose membrane dialyzer, hemodialysis-induced endothelial dys-

function and increases in plasma oxLDL were prevented. Our findings may suggest that an increase in oxidative stress caused by hemodialysis impairs endothelial function.

Effect of Hemodialysis on Endothelial Function
To assess endothelial function, we measured flow-mediated vasodilation of the brachial artery by high-resolution ultrasonography, for which the biological activity of endothelium-derived NO has been shown to be responsible.33 Although several investigators have demonstrated that flow-mediated vasodilation is impaired in patients with chronic hemodialysis,13,14 we demonstrated, for the first time, that a single session of hemodialysis per se blunted endothelium-dependent vasodilation. In the present study, the increases in blood flow during reactive hyperemia were similar before and after hemodialysis, indicating that the amount of shear stress to the brachial artery during reactive hyperemia was comparable. In addition, we found no difference in GTN-induced vasodilation, a measure of endothelium-independent vascular function. Collectively, the blunted flow-mediated vasodilation after hemodialysis could be attributed solely to endothelial dysfunction. The mechanisms of the endothelial dysfunction induced by hemodialysis remain unknown and may be multifactorial. There may be decreased endothelial NO formation by endogenous NO synthase inhibitors, which are accumulated in patients in end-stage renal disease,34–36 or by increased inactivation of NO by reactive oxygen species.15 The former possibility is unlikely, because recent findings

TABLE 1. Effects of Hemodialysis on Body Weight and Blood Analyses

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, kg</th>
<th>Hematocrit, %</th>
<th>BUN, mg/dL</th>
<th>Creatinine, mg/dL</th>
<th>Vitamin E, nmol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncoated dialyzer, before vs after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>56±3</td>
<td>31±1</td>
<td>69±4</td>
<td>11±1</td>
<td>0.95±0.05</td>
</tr>
<tr>
<td>After</td>
<td>54±3</td>
<td>36±2</td>
<td>27±2</td>
<td>5±1</td>
<td>1.04±0.08</td>
</tr>
<tr>
<td>Vitamin E–coated dialyzer, before vs after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>57±3</td>
<td>30±1</td>
<td>63±7</td>
<td>11±1</td>
<td>0.88±0.08</td>
</tr>
<tr>
<td>After</td>
<td>54±3</td>
<td>34±2</td>
<td>27±3</td>
<td>5±1</td>
<td>0.96±0.09</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM.

TABLE 2. Effects of Hemodialysis on Systemic and Local Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
<th>Basal $\phi$, mm</th>
<th>Basal Flow, mL/min</th>
<th>Peak Flow, %</th>
<th>GTN-Induced Vasodilation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncoated dialyzer, before vs after</td>
<td></td>
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</tr>
<tr>
<td>Before</td>
<td>110±8</td>
<td>72±3</td>
<td>4.7±0.2</td>
<td>190±21</td>
<td>410±28</td>
<td>12.8±1.4</td>
</tr>
<tr>
<td>After</td>
<td>97±8</td>
<td>78±8</td>
<td>4.6±0.2</td>
<td>189±22</td>
<td>344±27</td>
<td>12.9±1.7</td>
</tr>
<tr>
<td>Vitamin E–coated dialyzer, before vs after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>108±11</td>
<td>70±4</td>
<td>4.7±0.2</td>
<td>184±16</td>
<td>468±36</td>
<td>12.6±1.3</td>
</tr>
<tr>
<td>After</td>
<td>95±9</td>
<td>80±11</td>
<td>4.6±0.2</td>
<td>175±11</td>
<td>500±40</td>
<td>12.1±1.2</td>
</tr>
<tr>
<td>Noncoated vs vitamin E–coated dialyzer</td>
<td>$P=NS$</td>
<td>$P=NS$</td>
<td>$P=NS$</td>
<td>$P=NS$</td>
<td>$P=NS$</td>
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MAP indicates mean arterial pressure; HR, heart rate; and $\phi$, diameter of brachial artery. Values are mean±SEM.

$P=NS$ for all comparisons of before vs after.
have consistently advocated that NO production during hemodialysis is increased rather than decreased \(^{37-39}\) and endogenous NO synthase inhibitors are eliminated by hemodialysis.\(^{34}\) Hemodialysis may inactivate NO by generating reactive oxygen species on the surface of dialysis membranes by activation of polymorphonuclear leukocytes.\(^{19-23}\) To address this issue, we measured plasma levels of oxLDL, which were significantly elevated after a single session of hemodialysis. OxLDL is not only an index of lipid peroxidation but also causes endothelial dysfunction by itself through the impairment of the signal transduction between endothelial cell surface receptors and NO production,\(^{40}\) inhibition of NO synthase activity,\(^{40}\) and inactivation of NO released from endothelial cells.\(^{40}\) We have previously reported that rapid removal of oxLDL by a single session of apheresis improves endothelium-dependent vasodilation in hypercholesterolemic patients.\(^{32}\) Thus, it may be possible that oxidation of LDL during hemodialysis may directly impair endothelial function and decrease the biological activity of NO. Our results might be contrary to the findings by Hand et al,\(^{41}\) who demonstrated that hemodialysis significantly improved acetylcholine-stimulated vasodilation in the hand vein. Differences in vascular beds,\(^{42}\) ie, conduit artery versus vein, or differences in vasodilatory stimuli,\(^{43}\) ie, physiological shear stress versus pharmacological acetylcholine, may account for the discrepancy.

Effects of Vitamin E–Coated Dialyzer

To further examine the role of oxidative stress, we studied the effects of a vitamin E–coated hemodialysis filter on endothelial function. With this dialyzer, the impairment of endothelium-dependent vasodilation induced by hemodialysis was completely abolished. The possible mechanism(s) to account for the difference in noncoated and vitamin E–coated dialyzers can be summarized as follows: (1) systemic hemodynamic alteration, (2) removal of uremic substances, and (3) effects on vascular reactivity. The first possibility is unlikely, because hemodynamic parameters after hemodialysis, ie, blood pressure, heart rate, basal forearm blood flow, and brachial artery diameter, were similar between noncoated and vitamin E–coated dialyzers. The second possibility is also less likely, because body weight was decreased and blood urea nitrogen and creatinine were effectively removed in a similar manner. The third possibility may account for the difference in the results. In the present study, the increases in blood flow during reactive hyperemia and GTN-induced vasodilation after a session of hemodialysis were similar between noncoated and vitamin E–coated dialyzers, indicating that the differences in flow-mediated vasodilation between noncoated and vitamin E–coated dialyzers could be attributed to the distinct effects on endothelial function. As expected, this endothelial protection by the vitamin E–coated dialyzer may be due to elimination of oxidative stress, because increases in plasma levels of oxLDL by hemodialysis were prevented by the vitamin E–coated dialyzer, and endothelial function was inversely correlated with plasma oxLDL. Plasma levels of vitamin E were similar before and after hemodialysis and between the noncoated and vitamin E–coated dialyzers. These findings support the notion that the vitamin E–coated dialyzer may decrease oxidative stress through the mechanism on the
surface of the dialysis membrane but not by systemic antioxidant effects of vitamin E eluted from the dialyzer.

In summary, hemodialysis per se impairs endothelial function, possibly by increasing oxidative stress. The long-term anti-atherogenic effects of the vitamin E-coated dialyzer in patients receiving maintenance hemodialysis remain to be elucidated.

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