

Acetylcysteine Reduces Plasma Homocysteine Concentration and Improves Pulse Pressure and Endothelial Function in Patients With End-Stage Renal Failure

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Background—Increased oxidative stress, elevated plasma homocysteine concentration, increased pulse pressure, and impaired endothelial function constitute risk factors for increased mortality in patients with end-stage renal failure.

Methods and Results—We investigated the metabolic and hemodynamic effects of intravenous administration of acetylcysteine, a thiol-containing antioxidant, during a hemodialysis session in a prospective, randomized, placebo-controlled crossover study in 20 patients with end-stage renal failure. Under control conditions, a hemodialysis session reduced plasma homocysteine concentration to $58 \pm 22\%$ predialysis (mean \pm SD), whereas in the presence of acetylcysteine, the plasma homocysteine concentration was significantly more reduced to $12 \pm 7\%$ predialysis ($P < 0.01$). The reduction of plasma homocysteine concentration was significantly correlated with a reduction of pulse pressure. A 10% decrease in plasma homocysteine concentration was associated with a decrease of pulse pressure by 2.5 mm Hg. Analysis of the second derivative of photoplethysmogram waveform showed changes of arterial wave reflectance during hemodialysis in the presence of acetylcysteine, indicating improved endothelial function.

Conclusions—Acetylcysteine-dependent increase of homocysteine removal during a hemodialysis session improves plasma homocysteine concentration, pulse pressure, and endothelial function in patients with end-stage renal failure. (*Circulation*. 2004;109:369-374.)

Key Words: antioxidants ■ drugs ■ endothelium ■ risk factors

Patients with end-stage renal failure show increased cardiovascular mortality attributable to accumulation of several risk factors, including hypertension, anemia, oxidative stress, and elevated plasma homocysteine concentration. A significant increase of plasma homocysteine levels in patients with end-stage renal failure has been shown by several groups and correlated with arteriosclerotic vascular diseases and mortality.¹⁻⁵

See p 294

A recent analysis showed that an increase of plasma homocysteine concentration is associated with increased blood pressure.⁶ Increased plasma homocysteine concentration is associated with increased oxidative stress, which is in part responsible for the endothelial dysfunction observed in patients with end-stage renal failure.⁷ Impaired endothelial function is associated with abnormalities of arterial wave reflectance, contributing to increased pulse pressure. Pulse pressure itself is known to be an important independent predictor of cardiovascular mortality in patients with end-stage renal failure.⁸ Therefore, hyperhomocysteinemia, in-

creased oxidative stress, endothelial dysfunction, abnormalities in arterial wave reflectance, and increased pulse pressure all contribute to the increased cardiovascular morbidity in patients with end-stage renal failure.

Several attempts have been made to reduce elevated plasma homocysteine concentration. In the general population, supplementation with folic acid and vitamin B12 reduces plasma homocysteine concentration by approximately one quarter to one third.⁹ In patients with end-stage renal failure, the administration of folic acid or folinic acid decreased total homocysteine concentration between 32% and 46%.^{10,11}

Our group recently showed that acetylcysteine, a thiol-containing antioxidant, is able to significantly reduce composite cardiovascular end points in patients with end-stage renal failure.¹² Intravenous administration of acetylcysteine in healthy subjects significantly reduced plasma homocysteine concentration.¹³ Because during a standard hemodialysis session, plasma homocysteine concentration decreases by only approximately 28%, we reasoned that the administration of acetylcysteine during a hemodialysis session can drasti-

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cally improve elevated plasma homocysteine concentration in patients with end-stage renal failure.¹⁴ Usually, patients with end-stage renal failure already receive extensive oral medication. We therefore reasoned that the intravenous administration of acetylcysteine during hemodialysis is superior compared with oral administration because of better patient compliance.

We investigated the effects of intravenous administration of acetylcysteine during a single hemodialysis session on plasma homocysteine concentration in a prospective, randomized, placebo-controlled crossover design. Our results showed that the administration of acetylcysteine was able to completely normalize plasma homocysteine concentration in hemodialysis patients and was significantly associated with a reduction of pulse pressure and an improvement in endothelial function, both indicating an improvement of vascular function.

Methods

Patients

Twenty patients with end-stage renal failure were enrolled in this study. None of the patients received any supplementation with folic acid, folinic acid, vitamin B6, or vitamin B12. All of the patients were routinely dialyzed for 4 hours 3 times weekly using a biocompatible polysulfone membrane (F8, Fresenius Medical Care) with no dialyzer reuse. The dialysis solutions used were bicarbonate-based. Kt/V values (the amount of plasma cleared of urea divided by the urea distribution volume) was measured according to Daugirdas' formula.¹⁵ In a crossover design, each patient received acetylcysteine (5 g in 5% glucose solution for 4 hours) during a single hemodialysis session and 5% glucose solution alone for placebo control during another hemodialysis session. Acetylcysteine or glucose for placebo control was given on 2 consecutive regular hemodialysis sessions. Patients were randomly assigned to the order of administration of acetylcysteine or placebo. The dosage of acetylcysteine is in the range that has been reported to efficiently reduce plasma homocysteine concentration in healthy subjects.¹³ In addition, a dosage of 150 mg/kg acetylcysteine has been given intravenously to prevent contrast-induced nephropathy.¹⁶ No serial administration of acetylcysteine was intended in this study. The local ethics committee approved the study protocol, and all patients gave written informed consent.

Biochemical Measurements

Samples of venous blood were drawn from the arterial-venous fistula immediately before and at the end of the hemodialysis session. Plasma was separated from blood cells by immediate centrifugation. Plasma homocysteine concentration was measured by high-performance liquid chromatography method with fluorescence detection.^{17,18}

Fingertip Photoplethysmography

Analysis of the second derivative of photoplethysmogram waveform was used to characterize pulse waves during the hemodialysis session. In contrast to other hemodynamic methods, the use of fingertip photoplethysmography is advantageous, because pulse waves can be continuously measured during the hemodialysis without affecting the dialysis procedure or the patient. Photoplethysmography was conducted using a Vitaguard VG3000 monitor (Getemed) with the sensor located at the cuticle of the third digit of the hand contralateral to the location of vascular hemodialysis access. Raw data were collected at a rate of 32 per second, transferred to a personal computer, and additionally analyzed with self-made software. For each measurement point, data from 10 beats were averaged and differentiated 2 times (GraphPad prism 3.0, Graph Pad Software). The second derivative of photoplethysmogram

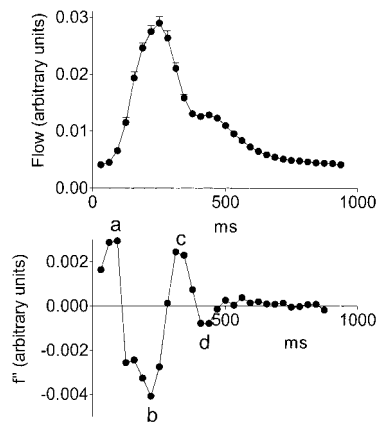


Figure 1. Representative photoplethysmogram waveform (top) and its second derivative of waveform (f' , bottom) obtained from a healthy control subject. For each measurement point, data from 10 beats were averaged and presented as mean \pm SEM. The peaks of the second derivative of photoplethysmogram waveform are labeled as follows: a, first peak; b, second peak; c, third peak; and d, fourth peak.

waveform consisted of several separate peaks, as shown in Figure 1. The ratios of the first peak (a) and the second peak (b, creating the ab ratio) or the fourth peak (d, creating the ad ratio) were calculated. The absolute values were used for the calculations. The initial ab ratios or ad ratios measured before the start of the hemodialysis session were set to 100% (control values).

To show the characteristics of endothelium-dependent flow-mediated vasodilation by fingertip photoplethysmography, healthy control subjects were studied during reactive hyperemia, an established method to investigate endothelial function.¹⁹ Pulse waves obtained on the ipsilateral arm during reactive hyperemia evoked by the release of a cuff on the upper arm were analyzed. The sphygmomanometric cuff was placed above the antecubital fossa. The cuff was inflated to 240 mm Hg for 5 minutes to evoke transient ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state, increases shear stress, and increases endothelium-dependent nitric oxide production and hence endothelium-dependent vasodilation. Fingertip photoplethysmography was conducted in 7 healthy control subjects, who did not take any medication and were free of intercurrent illness, and ab ratios and ad ratios were derived from analysis of second derivative of photoplethysmogram waveform. Healthy control subjects did not receive acetylcysteine. To assess endothelium-independent vasodilation, 0.4 mg of nitroglycerin was administered sublingually.

Statistics

Data are given as mean \pm SD if not indicated otherwise. An estimate of the sample size had been made beforehand (GraphPad Instat, GraphPad Software). To determine the sample size, a standard deviation of 8 μ mol/L for plasma homocysteine was expected in patients with end-stage renal failure. Assuming a mean difference of 9 μ mol/L, at least 17 patients were needed to obtain a 90% power of the study. Differences between groups were analyzed using Wilcoxon Mann-Whitney test (GraphPad prism 3.0). Multivariate regression analysis was conducted using SPSS for windows, version 11.5. Two-sided P values below 0.05 were considered to indicate statistical significance.

Results

The clinical and biochemical characteristics of 20 patients with end-stage renal failure are shown in Table 1. Hemodialysis was performed in the absence (placebo control) and presence of acetylcysteine in a prospective, randomized,

TABLE 1. Clinical and Biochemical Characteristics of 20 Patients With End-Stage Renal Failure

Age, y	70±11
Female	6 (30)
Body weight, kg	66±14
Renal disease (%)	
Diabetic nephropathy	6 (30)
Nephrosclerosis	3 (15)
Tubulointerstitial nephritis	1 (5)
Polycystic kidney disease	2 (10)
Other/unknown	8 (40)
Months on hemodialysis	28±54
Hemodialysis adequacy, Kt/V	1.1±0.3
Leukocytes, ×10 ⁹ /L	10±6
Hemoglobin, g/dL	9±2
Platelets, ×10 ⁹ /L	282±152
Serum creatinine, μmol/L	541±168
Blood urea nitrogen, mmol/L	17±6
Total protein, g/L	64±7
Serum calcium, mmol/L	2.3±0.2
Serum phosphate, mmol/L	1.3±0.5

Continuous data are shown as mean±SD. Systolic and diastolic blood pressure and heart rate were measured immediately before the hemodialysis session.

crossover design. Under control conditions and in the presence of acetylcysteine, hemodialysis sessions were performed after an essentially equal procedure. In both groups (placebo control versus acetylcysteine), there were similar Kt/V values (placebo, 1.1±0.3; acetylcysteine, 1.2±0.2; $P=0.38$), ultrafiltration volumes (placebo, 2.2±1.2 L; acetylcysteine, 2.5±1.1 L; $P=0.50$), hematocrit (placebo, 102±9% predialysis; acetylcysteine, 105±13% predialysis; $P=0.32$), and serum protein concentrations (placebo, 104±10% predialysis; acetylcysteine, 107±17% predialysis; $P=0.62$).

The intravenous administration of acetylcysteine during the hemodialysis session did not show any clinical side effects in the 20 patients studied. This is in accordance with the results of a previous study by our group, where acetylcysteine was administered orally for a median of 14.5 months in patients with end-stage renal failure.¹⁸ In that study, 5 patients (8%) reported gastrointestinal discomfort during treatment with acetylcysteine, and no major side effects were observed. Hypotension may be a side effect of acetylcysteine administration; however, in the present study, none of the patients receiving acetylcysteine showed hypotension during hemodialysis, probably because of the slow continuous administration during the hemodialysis session.

First, we evaluated the effect of acetylcysteine on plasma homocysteine concentration. Under control conditions, the plasma homocysteine concentration was reduced from 19.8±9.2 to 11.9±7.8 μmol/L after a hemodialysis session. In the presence of acetylcysteine, the plasma homocysteine concentration was reduced from 20.1±8.5 to 2.2±1.2 μmol/L after a hemodialysis session. The reduction of plasma homocysteine concentration during a hemodialysis session

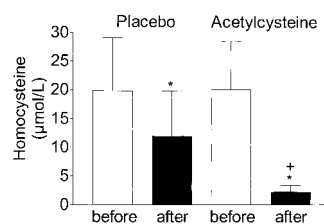


Figure 2. Bar graph showing plasma homocysteine concentration in patients with end-stage renal failure before (open bars) and after (black bars) a hemodialysis session under control conditions and in the presence of acetylcysteine. In a crossover design, each patient received acetylcysteine intravenously during a hemodialysis session and placebo for control during another hemodialysis session. Data are mean±SD from n=20 patients. * $P<0.01$ for the comparison before versus after hemodialysis. + $P<0.01$ for the comparison of acetylcysteine versus control.

was significantly more pronounced in the presence of acetylcysteine (Figure 2). Under control conditions, a hemodialysis session reduced plasma homocysteine concentration to 58±22% predialysis, whereas in the presence of acetylcysteine, a hemodialysis session reduced plasma homocysteine concentration to 12±7% predialysis ($P<0.01$ acetylcysteine versus placebo control). We also analyzed the data in 15 patients showing kt/V values above 1.2. In these patients, under control conditions, a hemodialysis session reduced plasma homocysteine concentration to 53±18% predialysis, whereas in the presence of acetylcysteine, a hemodialysis session reduced plasma homocysteine concentration to 11±7% predialysis ($P<0.01$ acetylcysteine versus placebo control).

There was a significant correlation between the ratio of urea after and before hemodialysis and the ratio of homocysteine after and before hemodialysis ($y=0.14x+26.8$; $r^2=0.20$; $P=0.01$), confirming that removal of homocysteine is related to dialysis dose. However, the ratio of urea after and before hemodialysis was not significantly different between the 2 groups (placebo control versus acetylcysteine).

The beneficial effect of acetylcysteine could be detected even 2 days after its administration. At this time, the plasma homocysteine concentration under control conditions was 134±65% predialysis. In contrast, 2 days after administration of acetylcysteine, the plasma homocysteine concentration was 88±36% predialysis.

Next, we investigated whether normalization of plasma homocysteine concentration influenced hemodynamics in patients with end-stage renal failure. The changes of heart rate, systolic and diastolic blood pressure, and pulse pressure are shown in Table 2. Under placebo control conditions, pulse pressure did not significantly change during a hemodialysis session (77±20 versus 80±24 mm Hg), whereas in the presence of acetylcysteine, the pulse pressure was significantly reduced from 86±17 to 76±22 mm Hg at the end of the hemodialysis session ($P<0.05$). As indicated in Figure 3, the reduction of plasma homocysteine concentration from the acetylcysteine group and the placebo group was significantly correlated with a reduction of pulse pressure. A 10% decrease in plasma homocysteine concentration was associated with a decrease of pulse pressure by 2.5 mm Hg. Multivariate

TABLE 2. Hemodynamic Parameters in 20 Patients With End-Stage Renal Failure Before and After a Hemodialysis Session Under Control Conditions (Placebo) and in the Presence of Acetylcysteine (ACC)

Characteristic/Treatment	Before Hemodialysis	After Hemodialysis	<i>P</i>
Heart rate, bpm			
Placebo	73±11	82±18	0.028
ACC	80±22	87±21	0.052
Systolic blood pressure, mm Hg			
Placebo	149±20	150±28	0.948
ACC	154±21	148±27	0.500
Diastolic blood pressure, mm Hg			
Placebo	72±14	70±14	0.291
ACC	68±15	72±19	0.188
Pulse pressure, mm Hg			
Placebo	77±20	80±24	0.507
ACC	86±17	76±22	0.030

Data are mean±SD.

analysis showed that these changes of pulse pressure were only dependent on changes of plasma homocysteine concentration (regression coefficient, 0.504; 95% CI, 0.238 to 0.771), whereas Kt/V, ultrafiltration volume, and changes of hematocrit or serum protein concentration had no effect.

To evaluate the hemodynamic effects of increased homocysteine removal during hemodialysis in the presence of acetylcysteine, we investigated pulse waves during the hemodialysis session. Figure 4 shows summary data for ab ratios and ad ratios derived from analysis of the second derivative of photoplethysmogram waveforms. The ab ratio decreased during the hemodialysis session under control conditions, whereas in the presence of acetylcysteine, the ab ratio increased (*P* for trend <0.01). The ad ratio, mainly characterizing peripheral arterial wave reflectance, decreased during

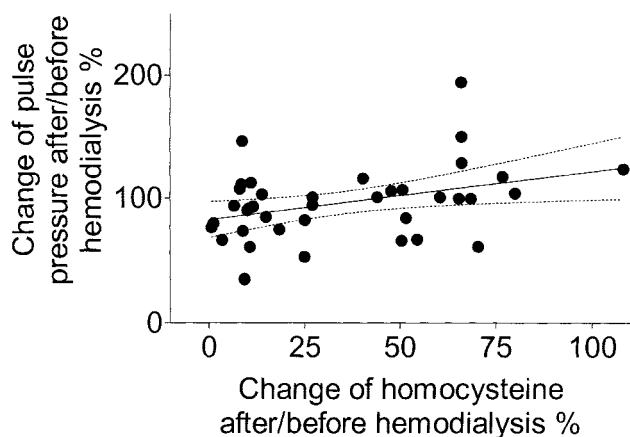


Figure 3. Correlation between changes of plasma homocysteine concentration and pulse pressure changes. Regression analysis of changes of plasma homocysteine concentration and changes of pulse pressure in patients with end-stage renal failure. The ratio of plasma homocysteine concentration after and before a hemodialysis session was plotted against the ratio of pulse pressure after and before a hemodialysis session. Linear regression line and 95% CI are shown ($r^2=0.14$, $P=0.019$).

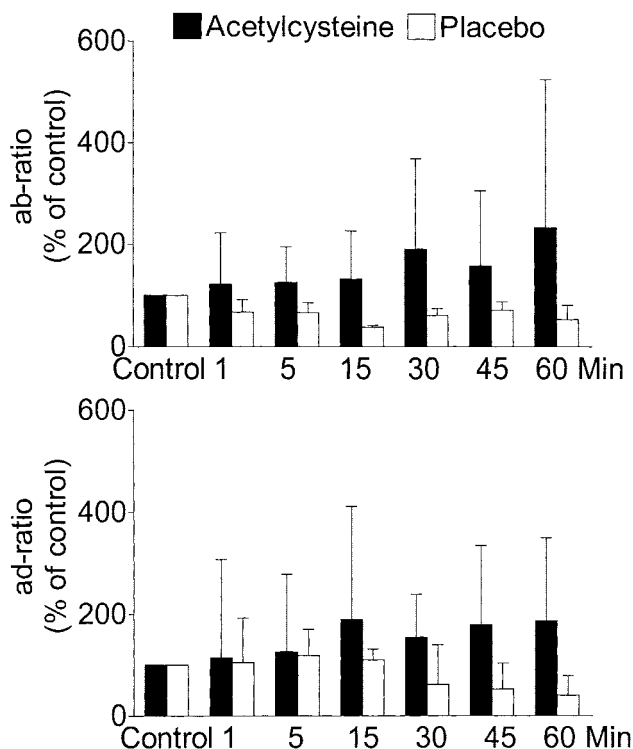


Figure 4. Summary data showing changes of photoplethysmogram waveform characteristics in patients with end-stage renal failure during a hemodialysis session. The ab ratios (top) and ad ratios (bottom) were calculated from a second derivative of photoplethysmogram waveform. Both ratios were measured at different points during a hemodialysis session under control conditions (placebo) and in the presence of acetylcysteine and are given as percent of initial control value obtained before hemodialysis. Data are mean±SD.

the hemodialysis session under control conditions, whereas in the presence of acetylcysteine, the ad ratio increased (*P* for trend <0.01).

To evaluate whether these changes of pulse waves in the presence of acetylcysteine could reflect improvement in endothelial function, we tested the endothelium-dependent flow-mediated vasodilation during reactive hyperemia and endothelium-independent vasodilation during nitroglycerine administration on ab ratio and ad ratio in healthy subjects. As shown in Figure 5, endothelium-dependent flow-mediated vasodilation, but not endothelium-independent vasodilation, significantly increased both ab ratio and ad ratio, respectively. This means that an increase in ab ratio and ad ratio, which was observed in patients with end-stage renal failure in the presence of acetylcysteine, is in accordance with an improvement of endothelial function.

Discussion

The present study shows that in the presence of acetylcysteine, plasma homocysteine concentration was markedly lowered beyond the effects of hemodialysis alone. Additionally, in the presence of the antioxidant acetylcysteine, improved endothelial function could be observed.

Several attempts have been made to reduce elevated plasma homocysteine concentration in patients with end-

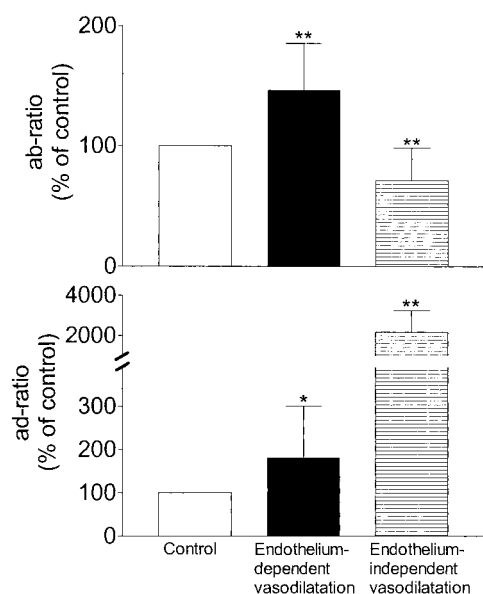


Figure 5. Characteristics of endothelium-dependent and endothelium-independent vasodilation using fingertip photoplethysmography. The ab ratios and ad ratios were calculated from a second derivative of photoplethysmogram waveform in healthy control subjects. The ab ratios and ad ratios (percent of initial control value obtained under resting conditions) were measured at endothelium-dependent flow-mediated vasodilation during reactive hyperemia and at endothelium-independent vasodilation during nitroglycerine administration. Data are mean \pm SD. * $P < 0.05$ and ** $P < 0.01$ compared with control.

stage renal failure. However, even high concentrations of folic acid did not normalize elevated plasma homocysteine concentration in these patients.¹⁰ The present study now shows that the intravenous administration of acetylcysteine during a hemodialysis session is able to completely normalize plasma homocysteine concentration in patients with end-stage renal failure.

The effects of oral acetylcysteine administration on plasma homocysteine concentration have been reported previously. Oral administration of acetylcysteine reduced plasma homocysteine concentration in healthy women.²⁰ A recent study additionally showed that hemodialysis patients treated with oral acetylcysteine for 4 weeks had a significant 19% reduction of plasma homocysteine concentration, whereas in the placebo group, no significant reduction occurred. The differences between the acetylcysteine group and the placebo group showed a P value of 0.07.²¹ The intravenous administration of acetylcysteine was also shown to reduce plasma homocysteine concentration in healthy subjects.¹³ In completion of these findings, the investigation presented here is the first report on intravenous administration of acetylcysteine in patients with end-stage renal failure. The increased reduction of homocysteine after intravenous administration of acetylcysteine that we found is presumably related to a quick displacement of homocysteine from protein-binding sites, allowing an increased proportion of homocysteine to be available for plasma clearance by hemodialysis, considering the small size of homocysteine. It has been suggested that the intradialytic decline of plasma homocysteine concentration resulted from the removal of unbound homocysteine, whereas

the sustained reduction of plasma homocysteine after hemodialysis had been attributed to the elimination of uremic toxins with inhibitory activity against enzymes involved in the metabolism of homocysteine.²² However, additional effects of acetylcysteine on homocysteine metabolism may contribute to its beneficial properties.

There is strong evidence that the reduction of elevated plasma homocysteine concentration in patients with end-stage renal failure is of clinical importance. First, a recent study showed an association of quartiles of plasma homocysteine concentration with mortality in patients with end-stage renal failure.²³ Second, a prospective study showed that the relative risk for cardiovascular events, including death, increased 1% per micromolar increase in plasma homocysteine concentration.⁵ The present study now shows that the reduction of plasma homocysteine concentration was convincingly associated with improved endothelial function and improvement, and hence reduction, of pulse pressure.

The finding of our study that a 10% decrease in plasma homocysteine concentration in patients with end-stage renal failure was associated with a decrease of pulse pressure by 2.5 mm Hg constitutes an important observation and is in accordance with a recent study showing that acute hyperhomocysteinemia in healthy subjects increases pulse pressure.²⁴ Moreover, elevated pulse pressure has been shown to be an independent predictor of mortality in patients with end-stage renal failure, a fact that underlines the importance of reducing pulse pressure.²⁵ Increased central arterial stiffness and alteration of pulse wave reflections generated from peripheral arteries contribute to the increase in pulse pressure observed with age, hypertension, or end-stage renal failure. Analysis of the second derivative of photoplethysmogram waveform has repeatedly been used to evaluate peripheral arterial wave reflectance.²⁶ Using an analysis of pulse waves obtained from fingertip photoplethysmography, Chowienczyk et al²⁷ showed that endothelial-dependent vasodilation induced by albuterol was attenuated with N^G -monomethyl-L-arginine and blunted in diabetic patients with endothelial dysfunction. We used characteristic ratios derived from the second derivative of photoplethysmogram waveforms to describe the hemodynamic changes during a hemodialysis session. This approach demonstrated an improvement in endothelial function in patients with end-stage renal failure, because the same pattern was observed during endothelium-dependent flow-mediated vasodilation in healthy subjects. The fact that patients with end-stage renal failure largely suffer from endothelial dysfunction underlines the importance of these findings.

Several mechanisms may be responsible for the improved endothelial function under acetylcysteine treatment. First, there are several lines of evidence that increased oxidative stress is responsible for endothelial dysfunction in patients with end-stage renal failure.⁷ Because it has been repeatedly shown that acetylcysteine has antioxidative properties, it thus may improve endothelial function. Second, homocysteine, which itself is associated with oxidative stress, was shown to induce programmed cell death in endothelial cells.²⁸ Third, homocysteine increases asymmetric dimethylarginine, an uremic toxin, that impairs endothelium-dependent vasodilation.^{29,30} In accordance with these results, regression analysis

showed that improvement of endothelium-dependent vasodilation correlated closely with the reduction in free plasma homocysteine concentration.³¹ Therefore, increased removal of homocysteine should be a causative factor for the shown improvement of endothelial function in the present study.

In conclusion, the intravenous administration of acetylcysteine normalizes plasma homocysteine concentration. This was significantly associated with an improvement of pulse pressure and endothelial function. Because the effects described here are correlative, additional outcome studies with a different study design may be warranted. However, these effects may explain the published beneficial effects of acetylcysteine on cardiovascular morbidity in patients with end-stage renal failure, and, therefore, intravenous administration of acetylcysteine during the hemodialysis session might be a novel, promising approach to reduce atherosclerotic risk in patients with end-stage renal failure.

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