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Increased reduction of dimethylarginines and lowered interdialytic blood pressure by the use of biocompatible membranes

MATTHIAS SCHRÖDER, EBERHARD RIEDEL, WERNER BECK, REINHOLD M. DEPPISCH, and WOLFGANG POMMER

Krankenhaus Reinickendorf, Berlin; Institute for Biochemistry, FU Berlin; and Gambro Research, Hechingen, Germany

Increased reduction of dimethylarginines and lowered interdialytic blood pressure by the use of biocompatible membranes. Hypertension contributes to cardiac and cerebrovascular complications in HD patients. Endogenous inhibitors of nitric oxide synthase accumulate in renal failure and may interfere with the regulation of vascular tone. We investigated the elimination of asymmetric dimethylarginine (ADMA) by using biocompatible Polyamide S™ membranes in low-flux (Polyflux 6L) or high-flux (Polyflux 14S) hemodialysis or hemodiafiltration (HDF) compared with hemodialysis with cellulosic membranes. Removal rates for ADMA, symmetric dimethylarginine (SDMA), and β2-microglobulin significantly increased in HDF. The plasma total amino acid concentration and the arginine/ADMA ratio increased, and the mean 24-hour blood pressure decreased during the study. In a second study, we investigated whether plasma amino acids and interdialytic blood pressure are influenced by the use of a biocompatible membrane and HDF. Seventeen end-stage renal disease patients were treated for six weeks with hemodialysis using cellulosic membranes, six weeks with low-flux hemodialysis using Polyflux 6L, and six weeks with HDF using Polyflux 14S. Only in the diabetic patients were the hemoglobin concentration (from 10.6 ± 1.5 to 11.9 ± 0.6 mg/dL) and hematocrit (from 33.6 ± 1.9 to 36.2 ± 1.5%) increased significantly, whereas the mean 24-hour systolic blood pressure decreased (from 154 ± 22 to 129 ± 18 mm Hg). No significant changes were observed in nondiabetic patients. We conclude that primarily diabetic patients seem to benefit from the use of biocompatible membranes—most in HDF—after a period of six weeks. The regulation of nitric oxide pathways by ADMA removal and changed ADMA/arginine ratio might be contributing factors. Further prospective studies are required to show whether the long-term application of HDF or other changes of dialysis treatment modalities may help to improve well-being, morbidity, and mortality in hemodialysis patients.

Hypertension significantly contributes to cardiac and cerebrovascular complications in hemodialysis (HD) patients, and the high morbidity in this patient population is closely linked to cardiovascular complications [1–3]. The nitric oxide (NO)-arginine system plays an important role in the regulation of blood pressure and vascular biology [4, 5]. Endogenous inhibitors of NO synthase, such as dimethylarginines, are able to interfere with the regulation of vascular tone. Asymmetric dimethylarginine (ADMA) accumulates in renal failure, and elevated ADMA plasma concentrations are discussed as a possible cause of hypertension in hemodialysis patients [6]. Data about ADMA levels in patients on hemodialysis are differing widely, probably because of differences in dietary intake, treatment modalities, or the applied analysis methods [7–10].

The aim of our first study was to investigate whether the removal of dimethylarginines can be influenced by the efficiency of the treatment. We compared hemodialysis and hemodiafiltration (HDF) using membranes of different permeability and biocompatibility.

Since the first study was designed to compare the individual treatment modes and membranes, it remained open as to whether the changes observed in amino acid composition and 24-hour blood pressure were related to the improved membrane biocompatibility or to the enhanced convective elimination of uremic toxins. We addressed this question in a second study with a larger number of patients.

METHODS

First study

Six (1 female and 5 males) stable, nondiabetic hemodialysis patients [age 54 ± 13 years, time on HD 3.8 ± 2.8 years (2 months to 7.5 years), body mass index (BMI) 25 ± 2, glomerular filtration rate (GFR) 1 ± 1 mL/min] were consecutively treated for two weeks with (*1*) hemodialysis using a low-flux regenerated cellulose membrane (LunDia Alpha 600; Gambro, Lund, Sweden), (*2*) hemodialysis using a synthetic, biocompatible high-flux mem-

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brane, Polyflux 14S (1.4 m^2 Polyamide S™ membrane; Gambro, Hechingen, Germany), (*3*) online HDF (AK 100 ULTRA; Gambro, Lund, Sweden) using the same high-flux membrane and an infusion volume of 7.5 L, (*4*) online HDF using 18 L of infusion, and finally (*5*) hemodialysis using a synthetic, biocompatible low-flux membrane (Polyflux 6L, 1.4 m^2 Polyamide S™ membrane; Gambro, Hechingen, Germany). Treatment parameters (blood flow rate 250 to 300 mL/min, dialysate flow rate 500 mL/min, treatment time of 5 hours) were kept constant during all study periods. Plasma samples were taken before and exactly at the end of a treatment in each study period. Heparinized blood samples were processed to plasma within 15 minutes after sampling and were deep frozen in aliquots. Frozen plasma aliquots were lyophilized for amino acid analysis. Amino acids, including dimethylarginines, were extracted from the lyophilized samples with 80% methanol and acetate buffer. After derivatization with an o-phthalaldehyde (OPA) reagent, the amino acids were separated by reversed-phase high-performance liquid chromatography with fluorescence detection [11, 12]. For ADMA and SDMA analyses, double runs were performed. During one run, the samples were spiked with known amounts of synthetic ADMA and SDMA (Calbiochem, Bad Soden, Germany) in order to be able to correct for the limited recovery of dimethylarginines in high-performance liquid chromatography (HPLC) separation. Urea was analyzed by standard laboratory methods, and for analysis of β_2-microglobulin in plasma samples, a specific enzyme-linked immunosorbent assay method (Immundiagnostik, Bensheim, Germany) was used. Twenty-four–hour blood pressure was recorded using an ambulant blood pressure monitor (Spacelabs Medical, Kaarst, Germany) after each study period, and careful instruction was given to the patients.

Second study

Seventeen (8 females and 9 males) end-stage renal disease (ESRD) patients (6 diabetic, age 62 ± 14 years, time on HD 14 ± 14 months, body mass index 25 ± 4, GFR 4 ± 3 mL/min) were treated as follows: (*1*) six weeks with low-flux hemodialysis using membranes (LunDia Alpha 600, 1.6 m^2 regenerated cellulose flat sheet membrane; Gambro, Lund, Sweden); (*2*) six weeks with hemodialysis using a synthetic biocompatible low-flux membrane (Polyflux 6L, 1.4 m^2 Polyamide S™ hollow fiber membrane; Gambro Hechingen, Germany); and (*3*) six weeks with online HDF (AK 100 ULTRA; Gambro, Lund, Sweden) using a biocompatible high-flux membrane (Polyflux 14S, 1.4 m^2 Polyamide S™ hollow fiber membrane; Gambro, Hechingen, Germany) and 18 L infusion volume. All treatments were performed with ultrapure dialysate and sterile infusion (AK 100 ULTRA; Gambro, Lund, Sweden) fluid to exclude possible effects of bacterial contaminants in the dialysate. Treatment parameters (the same as during the first study) and medication including erythropoietin (EPO; 2400 ± 2100 IU/week) were kept constant during the study period. Student *t* test was used for statistical evaluation. Statistical significance was assumed for $P < 0.05$.

For both studies, the study protocol was approved by the local ethical committee, and informed consent of all patients was obtained prior start of the study.

RESULTS AND DISCUSSION

First study

Figure 1 shows the reduction in plasma concentration of urea (60 D), ADMA (202 D), SDMA (202 D), and β_2-microglobulin (11,800 D) during treatments performed in the different study periods. Urea reduction rates were slightly increased during HDF. The β_2-microglobulin concentration decreased significantly more during application of the two HDF modes compared with the high-flux hemodialysis treatments, reflecting increasing convective transport in HDF. As expected, no significant decrease could be observed for the two low-flux modes, independent from the membrane used.

Asymmetric dimethylarginine and especially SDMA concentrations were lowered significantly more during HDF treatments. The reduction rates for ADMA and SDMA are based on samples taken exactly at the end of the five-hour treatments (that is, not equilibrated). In contrast to the dimethylated amino acids ADMA and SDMA, the decrease in concentrations of the other amino acids (data not shown; abstract; *Artif Org* 23:655, 1999) was much less pronounced, with the exception of taurine and citrulline. Citrulline is the reaction product when NO is formed from arginine by NO synthases, and it is also an intermediate compound in the urea cycle. Taurine is an end product of the cysteine metabolism. For some amino acids, such as arginine and ornithine, an increased concentration could be observed after treatment. No systematic pattern with respect to changes in amino acid concentration could be identified when comparing the different treatment modes. From these data, it is obvious that the enhanced convective transport during HDF does not lead to more pronounced decreases in plasma free amino acid concentrations than with low- or high-flux hemodialysis.

Interestingly, the predialysis concentrations of total plasma amino acids (Fig. 2, left panel) increased continuously during the 10-week study period, and were significantly elevated at the end of the study. It is known that the Polyflux S™ membranes used during the study periods 2 to 5 had a very low complement activation (abstract; *Blood Purif* 16:236, 1998) [13]. Therefore, we speculate that the increase in plasma total amino acid concentration might be related to a less catabolic situa-

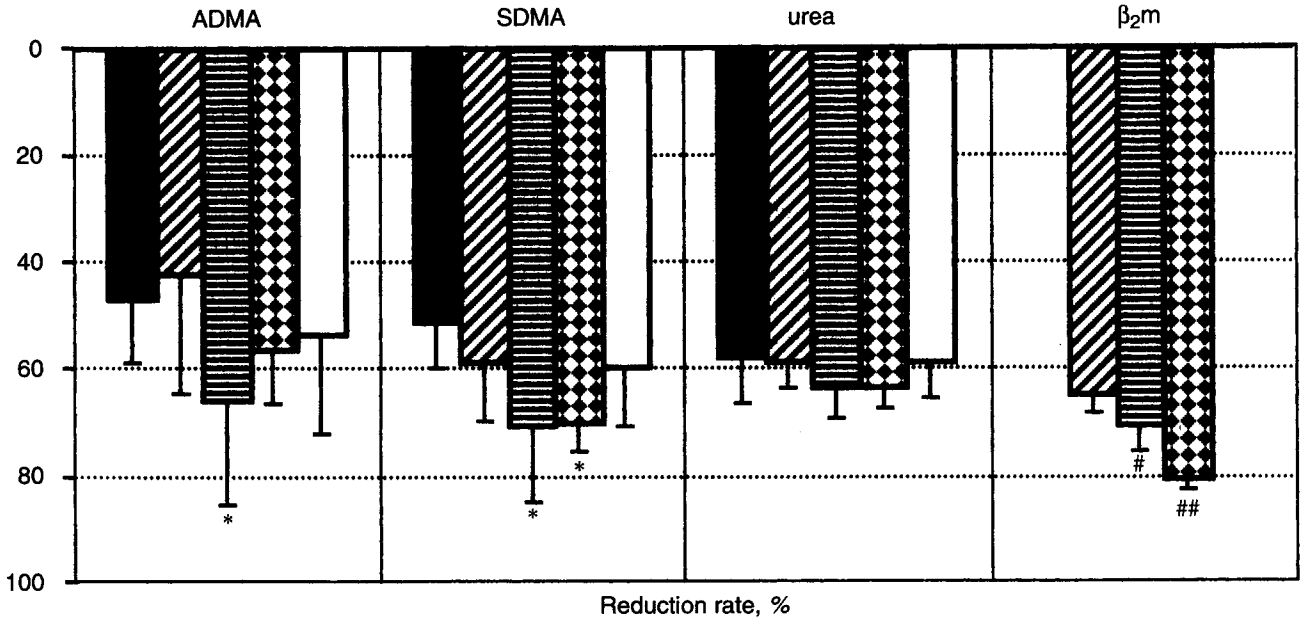

Fig. 1. Reduction rates for different solutes during the following treatments in the first study: (*1*) low-flux hemodialysis with regenerated cellulose membranes (LunDia Alpha; ■); (*2*) high-flux hemodialysis with Polyflux 14S (▨); (*3*) hemodialfiltration (HDF) 7.5 L infusion with Polyflux 14S (▤); (*4*) HDF 18 L infusion with Polyflux 14S (▩); and (*5*) low-flux hemodialysis with Polyflux 6L (□) [*$P < 0.05$ vs. (*1*); #$P < 0.05$ vs. (*2*); ##$P < 0.05$ vs. (*3*)].

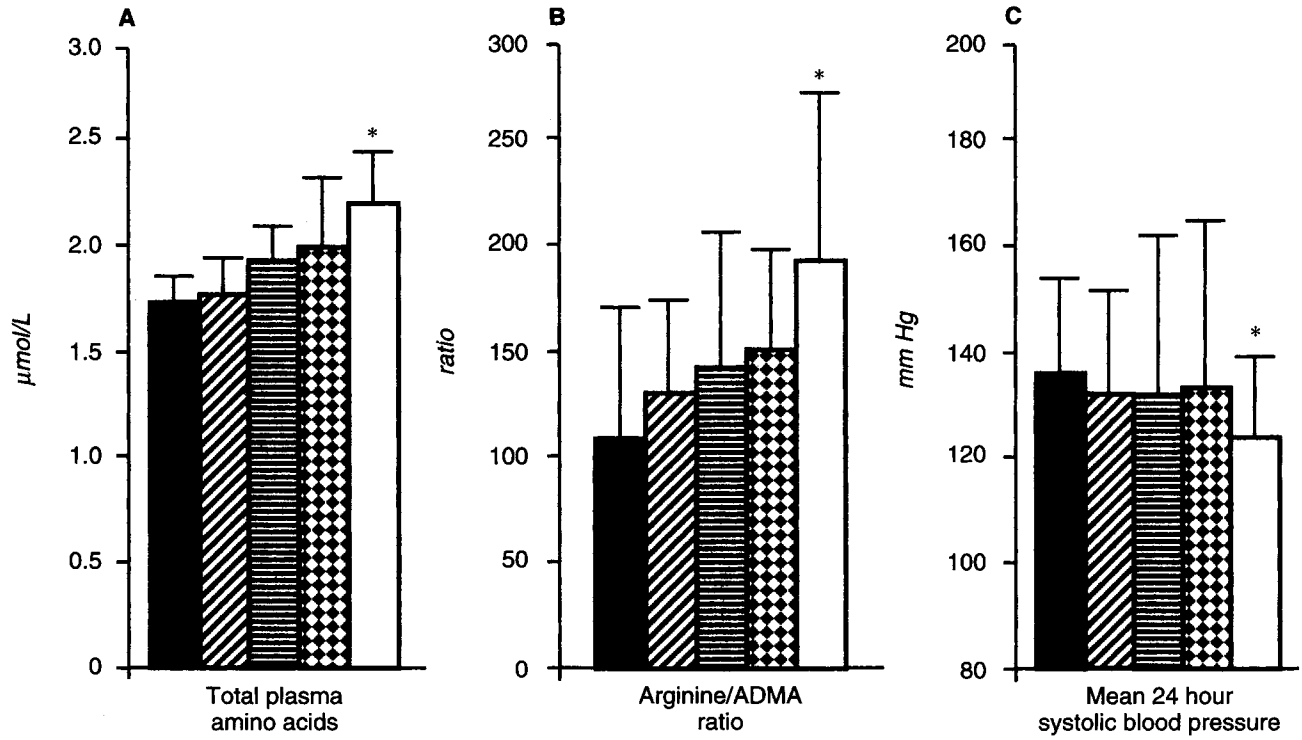

Fig. 2. Total amino acid concentration in plasma (*A*), molar ratio of arginine and ADMA (*B*) and mean systolic 24-hour blood pressure (*C*) during the first study after the study periods: (*1*) low-flux hemodialysis with regenerated cellulose membranes (LunDia Alpha; ■); (*2*) high-flux hemodialysis with Polyflux 14S (▨); (*3*) HDF 7.5 L infusion with Polyflux 14S (▤); (*4*) HDF 18 L infusion with Polyflux 14S (▩); and (*5*) low-flux hemodialysis with Polyflux 6L (□) [*$P < 0.05$ vs. (*1*)].

tion because of better biocompatibility. This interpretation would be in line with data about the influence of membrane biocompatibility on protein catabolism [14]. However, at this point, we cannot exclude the influence of changes in appetite or dietary food intake. It has been previously shown in a rat model that the middle molecular fraction (1 to 5 kD of uremic ultrafiltrates) suppresses food intake [15], and it is possible that the enhanced removal of uremic toxins in the middle molecular range by high-flux hemodialysis and even more by HDF could have a beneficial effect on the patients' appetite.

The mean systolic 24-hour blood pressure is shown in Figure 2 (right panel) for the six patients. When comparing the mean value after the first two weeks (black bars), where low-flux hemodialysis with regenerated cellulose membranes has been applied, with the mean value at the end of the study (white bar), that is, after eight weeks of use of biocompatible membranes in different treatment modes, the mean systolic blood pressure significantly decreased by 12 mm Hg. A similar decrease was found for the diastolic value (data not shown). This decrease in 24-hour blood pressure was accompanied by an increase of the ratio between arginine and ADMA concentration during these eight weeks. Although ADMA concentrations were not significantly lower at the end of the study, the increased ratio of arginine to ADMA, that is, the ratio of the substrate for NO synthesis to the inhibitor of NO synthesis, could explain an improved situation for blood pressure regulation. It has been hypothesized earlier that an imbalance between arginine and ADMA concentrations might be of interest not only with respect to blood pressure control, but also with respect to local vasoconstriction and atherogenesis [6].

Since our first study was designed to investigate the effect of the individual treatment modes and membranes (that is, low-flux HD using cellulosic membranes vs. high-flux HDF and HDF using a synthetic biocompatible membrane) on the removal of low and high molecular weight compounds, it is not possible to ascribe the changes in amino acid profile and blood pressure either to the improved biocompatibility or to the enhanced convective elimination of uremic toxins. This question was addressed in our second study.

Second study

Predialysis plasma amino acid concentrations were observed during the study (data not shown; abstract; *Artif Org* 23:655, 1999). Leucine, tryptophane, ornithine, lysine, valine, taurine, and isoleucine significantly increased after six weeks of treatment with the biocompatible low-flux membrane (Polyflux 6L) and remained elevated during the HDF phase. The total amino acid concentration tended to increase; however, the difference was not statistically significant.

Among the clinical parameters recorded during the study [that is, hemoglobin, hematocrit, interdialytic (24 h) blood pressure], no statistically significant changes could be found when analyzing all study patients together. However, in a subgroup analysis considering diabetic and nondiabetic patients separately, we found significant changes. Neither hemoglobin concentrations (10.4 ± 1.5 vs. 10.6 ± 1.2 mg/dL, P = NS) nor hematocrit (31.8 ± 3.7 vs. 32.4 ± 3.2, P = NS) nor mean 24-hour systolic blood pressure (136 ± 20 vs. 141 ± 17 mm Hg, P = NS) changed in the nondiabetic patients (Fig. 3). However, in the diabetic patients (Fig. 4), the hemoglobin levels increased during the study (10.6 ± 1.6 vs. 11.4 ± 0.7 mg/dL, P = NS), and the increase was significant after the HDF period (11.9 ± 0.6 mg/dL, $P < 0.05$). We found similar results for hematocrit levels, but now the observed increase was statistically significant already after the six weeks if low-flux hemodialysis with the biocompatible Polyflux L membrane (33.6 ± 1.9 vs. $34.5 \pm 1.9\%$, $P < 0.05$). Hematocrit levels further increased during the six-week HDF period ($36.2 \pm 1.5\%$, $P < 0.05$). When looking at the mean 24-hour systolic blood pressure values, a significant decrease could be observed after six weeks of Polyflux low-flux hemodialysis (154 ± 22 vs. 141 ± 17 mm Hg, $P < 0.05$) and a further decrease after six weeks of HDF (129 ± 18 mm Hg, $P < 0.05$). A similar decrease was found for the diastolic blood pressure value (data not shown).

SUMMARY AND CONCLUSIONS

Our first study shows that ADMA and SDMA concentrations can be significantly reduced during hemodialysis and HDF treatments, and this decrease is more pronounced in HDF. After two months use of biocompatible synthetic membranes in different treatment modes, the concentrations of total plasma amino acids and the arginine to ADMA ratio significantly increased, whereas the mean 24-hour blood pressure decreased.

In our second study after a six-week treatment period with low-flux hemodialysis using a biocompatible membrane (Polyflux 6L), mean 24-hour blood pressure decreased, and hematocrit levels increased significantly only in diabetic patients. These improvements continued during a further six weeks of HDF treatment, using a biocompatible high-flux membrane (Polyflux 14S), in addition to an increase in hemoglobin concentration. None of these changes could be observed in the nondiabetic group of patients. Although the concentration of individual amino acids increased during the study, no significant change in total amino acid concentration could be observed.

The NO system is modified in hemodialysis patients with respect to a series of factors related to dialysis treatment [16]: (*1*) up-regulation of inducible NO synthase caused by a chronic state of microinflammation, use of

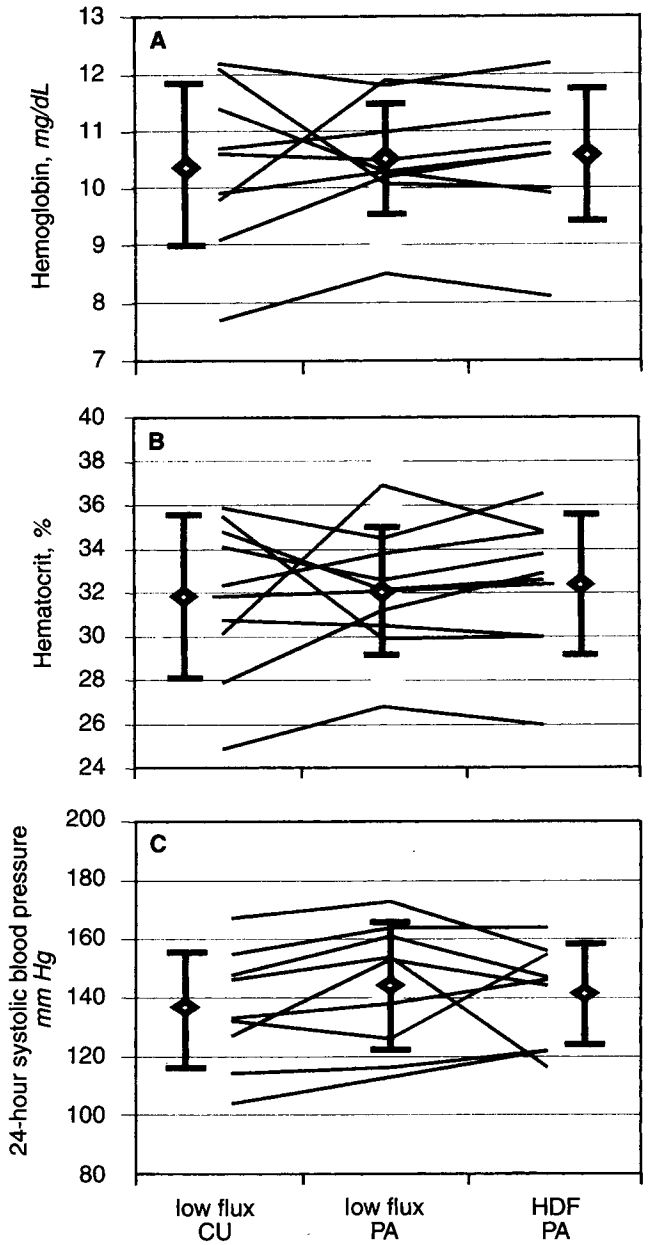

Fig. 3. Second study. Development of hemoglobin concentration (upper panel), hematocrit (middle panel), and mean systolic 24-hour blood pressure (lower panel) of nondiabetic patients after (*1*) six weeks low-flux cuprophan hemodialysis (low-flux CU), (*2*) six weeks of low-flux hemodialysis using Polyflux 6L (low flux PA), and (*3*) six weeks of HDF using Polyflux 14S and 18L infusion (HDF PA).

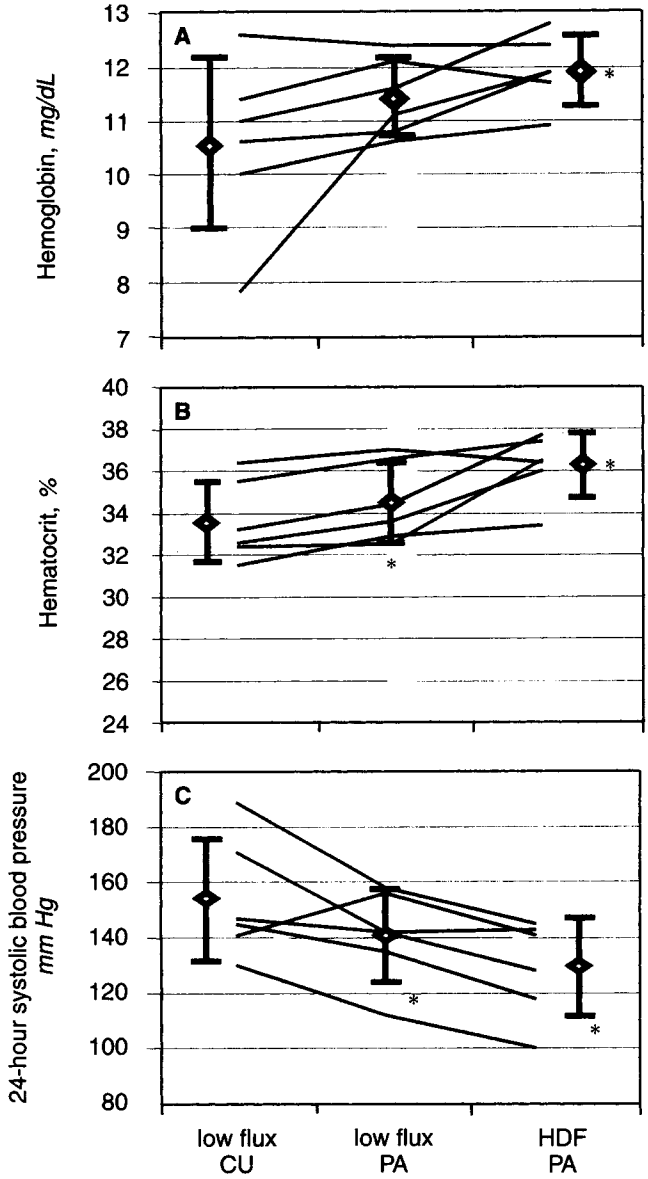

Fig. 4. Second study. Development of hemoglobin concentration (upper panel), hematocrit (middle panel), and mean systolic 24-hour blood pressure (lower panel) of diabetic patients after (*1*) six weeks of low-flux cuprophan hemodialysis (low-flux CU), (*2*) six weeks of low-flux hemodialysis using Polyflux 6L (low-flux PA), and (*3*) six weeks of HDF using Polyflux 14S and 18L infusion (HDF PA; $*P < 0.05$ vs. preceding period).

heparin, and platelet activation by extracorporeal blood circulation; (*2*) accumulation of guanidino compounds, such as ADMA, with a reduced arginine/ADMA ratio; and (*3*) chronic activation of the endothelium. To separate the different factors having an impact on NO regulation in end-stage renal disease is certainly impossible in a single in vivo study.

Based on the results of these studies, we conclude that primarily diabetic patients seem to benefit from the use of biocompatible membranes—most in HDF—in a short-term period. Our studies clearly have an observational character, but give important insights into future perspectives for the design of prospective therapy studies for specific patient groups. These types of studies must question whether the long-term application of HDF could lead to improved morbidity and mortality in hemodialysis patients.

Reprint requests to P.D. Dr. Wolfgang Pommer, Am Nordgraben 2, D-13509 Berlin, Germany.
E-mail: w.pommer@krankenhaus-reinickendorf.de

REFERENCES

1. Rahman M, Dixit A, Donley V, Gupta S, Hanslik T, Lacson E, Ogundipe A, Weigek K, Smith MC: Factors associated with inadequate blood pressure control in hypertensive hemodialysis patients. *Am J Kidney Dis* 33:498–506, 1999
2. Ritz E, Koch M: Morbidity and mortality due to hypertension in patients with renal failure. *Am J Kidney Dis* 21(Suppl 2):113–118, 1993
3. Iseki K, Kinjo K, Kimura Y, Osawa A, Fukiyama K: Evidence for high risk of cerebral hemorrhage in chronic dialysis patients. *Kidney Int* 44:1086–1090, 1993
4. Anggard E: Nitric oxide: Mediator, murderer, and medicine. *Lancet* 343:1199–1206, 1994
5. Moncada S, Higgs A: The l-arginine-nitric oxide pathway. *N Engl J Med* 329:2002–2012, 1993
6. Ritz E, Valance P, Nowicki M: The effect of malnutrition on cardiovascular mortality in dialysis patients: Is l-arginine the answer? *Nephrol Dial Transplant* 9:129–130, 1994
7. Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
8. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E: Concentration of dimethyl-l-arginine in the plasma of patients with end-stage renal failure. *Nephrol Dial Transplant* 11:2449–2452, 1996
9. Anderstam B, Ktzarski K, Bergström J: Serum levels of N^G, N^G-dimethyl-l-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. *J Am Soc Nephrol* 8:1437–1442, 1994
10. Kielstein JT, Böger RH, Bode-Böger SM, Schäffer J, Barbey M, Koch KM, Frölich JC: Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: Relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 10:594–600, 1999
11. Godel H, Graser T, Foldi P, Pfaender P, Furst P: Measurement of free amino acids in human biological fluids by high-performance liquid chromatography. *J Chromatogr* 297:49–61, 1984
12. Algermissen B, Riedel E, Nündel M: Analytik von Aminosäuren mit Fluoreszenz-HPLC. *GIT Fachz Lab* 33:783–790, 1989
13. Deppisch R, Göhl H, Smeby L: Microdomain structure of polymeric surfaces: Potential for improving blood treatment procedures. *Nephrol Dial Transplant* 13:1354–1359, 1998
14. Guiterez A, Alvestrand A, Wahren J, Bergström J: Effect of in vivo contact between blood and dialysis membranes on protein catabolism in humans. *Kidney Int* 38:487–494, 1990
15. Anderstam B, Mamoun AH, Södersten P, Bergström J: Middle-sized molecule fractions isolated from uremic ultrafiltrate and normal urine inhibit ingestive behaviour in the rat. *J Am Soc Nephrol* 7:2453–2460, 1996
16. Kang ES, Tevlin MT, Wang YB, Chiang TM, Cardenas R, Myers LK, Acchiardo SR: Hemodialysis hypotension: Interaction of inhibitors, iNOS, and the interdialytic period. *Am J Med Sci* 317:9–21, 1999