Hemodynamic and Hormonal Changes During Erythropoietin Therapy in Hemodialysis Patients

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Abstract. To better understand the mechanism of recombinant human erythropoietin (rhEPO)-induced increase in BP, hemodynamic parameters, body fluid volumes, and the hormones implicated in BP regulation were studied in 32 anemic hemodialysis patients before and after 3 to 4 mo of rhEPO therapy. Hemoglobin levels increased from 83 ± 1.5 to 119 ± 2.3 g/L (P < 0.01) after rhEPO therapy (25 to 43 U/kg) administered subcutaneously three times weekly. Mean 24-h systolic and diastolic ambulatory BP were significantly increased by 14 ± 3 and 10 ± 2 mmHg, respectively (P < 0.01 for both groups). Systemic vascular resistance consistently increased by 28 ± 5% (P < 0.01), whereas cardiac output was decreased by 6 ± 3% (P < 0.05). Red blood cell mass increased by 510 ± 35 ml (P < 0.01), whereas plasma volume decreased by 420 ± 66 ml (P < 0.01), which resulted in a nonsignificant increase in total blood volume. Extracellular fluid volume and exchangeable sodium were decreased by 873 ± 255 ml (P < 0.01) and 125 mmol (P < 0.01), respectively. There was a positive correlation between the changes in exchangeable sodium and in systolic BP (r = 0.41, P < 0.05). Furthermore, a greater increase in 24-h systolic BP was observed in patients in whom exchangeable sodium increased or remained unchanged (n = 10) compared with patients (n = 22) with decreased exchangeable sodium (20 ± 4 mmHg versus 8 ± 2 mmHg, respectively, P < 0.01). Plasma catecholamines, plasma renin concentration, plasma atrial natriuretic peptide, and plasma endothelin-1 did not significantly change with rhEPO treatment, whereas plasma aldosterone increased significantly (P < 0.01). Although volume-independent mechanisms may contribute to rhEPO-induced BP increase, the results presented here suggest the importance of optimally reducing extracellular fluid volume to prevent, at least in part, the development of hypertension often observed with improved uremic anemia in these patients. (J Am Soc Nephrol 9: 97–104, 1998)

Recombinant human erythropoietin (rhEPO) therapy represents a major advance in the management of anemia in patients with end-stage renal disease. It does, however, increase BP in most cases and may lead to the development of de novo hypertension or acceleration of existing arterial hypertension (1–3). The expansion of red blood cell volume without adequate readjustment of plasma and extracellular fluid volumes due to renal failure has been suggested to account for the rhEPO-induced rise in BP (4–5). Other mechanisms, such as an inappropriate increase in peripheral vascular resistance secondary to an inadequate reduction in cardiac output subsequent to anemia correction (6), an imbalance in the vasoactive hormones that modulate the BP regulatory mechanisms (7,8), or the direct vasopressor action of rhEPO (9), have also been proposed.

The objective of the present study was to investigate the variations in hemodynamic parameters, body fluid volumes, and the hormones involved in BP regulation in anemic hemodialysis patients before rhEPO therapy and 3 to 4 mo later after reaching the target hemoglobin level of 110 to 120 g/L.

Materials and Methods
This study was approved by the institutional ethics committee, and written informed consent was obtained from each patient. Thirty-seven patients were enrolled in the study. Four patients who received a renal transplant during the study and one who did not respond to rhEPO therapy were excluded. The remaining 32 patients (15 women and 17 men) completed the study and were included in the analysis. Mean age was 45 yr (range, 18 to 78 yr). At study onset, patients had been on dialysis for at least 3 mo (range, 3 to 20 mo; mean, 5.5 mo). None had received rhEPO previously. All patients had anemia due to renal disease (hemoglobin < 90 g/L) without any evidence of aluminum toxicity, hemolysis, or other causes. No patient received androgen or immunosuppressive therapy. Patients with unstable cardiovascular disease, uncontrolled hypertension, and diabetes mellitus, a disease often associated with many complications, were excluded. The underlying disease in 11 patients was interstitial nephritis; eight had glomerulonephritis, three had polycystic kidney disease, two had glomerulosclerosis, two had hereditary nephritis, and in six patients the etiology was unknown. As assessed by sequential supine BP measurements before hemodialysis, 11 patients were normotensive (<140/90 mmHg), nine had untreated borderline systolic hypertension (140 to 159 mmHg with diastolic BP < 90 mmHg), and 12 were treated and well controlled for hypertension (<140/90 mmHg) with either beta-blockers, calcium channel blockers, or angiotensin-converting enzyme inhibitors. The antihypertensive medication was not modified during the study period. Patients underwent 3- to 4-h dialysis sessions three times a week using a nonreused cellulose-acetate hollow-fiber dialyzer for each treatment (Baxter Corp., Toronto, Ontario, Canada), with a blood flow of 250 to 400 ml/min and a dialysate flow...
of 500 ml/min. All of the patients were considered to be at optimal postdialysis dry weight, which, as much as possible, was not changed during the study.

Protocol

All patients were investigated before the initiation of rhEPO replacement therapy, and again, approximately 3.5 mo later (mean, 14 wk; range, 10 to 23 wk) after reaching the target hemoglobin level of 110 to 120 g/L. rhEPO (Eprex, Janssen-Ortho, Inc., Don Mills, Ontario, Canada) was administered subcutaneously to all patients at a mean dose of 32 U/kg (range, 25 to 43 U/kg) three times weekly. BP was measured with the patient in the supine position before and after dialysis, and body weight was also recorded pre- and postdialysis. The patients continued their fluid restriction and diet recommendations throughout the study. To avoid acute changes that may be produced by the dialysis procedure, all hemodynamic parameters, body fluid volumes, and hormone measurements were determined on the same day between two regular hemodialysis sessions. Blood samples for the measurement of hormones (plasma renin concentration; plasma aldosterone; plasma norepinephrine, epinephrine, and dopamine; plasma atrial natriuretic peptide; and plasma endothelin-1) and circulating volumes (red blood cell mass, plasma volume, and total blood volume) were taken at the Clinical Research Unit at 8 a.m. after overnight recumbency to avoid postural and diurnal variation (10). The patients were then asked to empty their bladder, after which the protocol for the assessment of extracellular fluid volume and exchangeable sodium was initiated, together with the 24-h ambulatory BP monitoring. Noninvasive cardiac hemodynamic measurements were performed during the afternoon. The next morning, blood samples were again obtained for estimation of extracellular fluid volume and exchangeable sodium, and 24-h urine collection was completed for the measurement of isotopic sodium excretion.

Methods

Pre- and posthemodialysis BP were measured using standard sphygmomanometry. Mean BP was calculated as diastolic BP + 1/3 (systolic BP − diastolic BP). Twenty-four-hour ambulatory BP and heart rate were measured using an automatic noninvasive recorder (model 90207, Spacelabs, Inc., Redmond, WA), as described earlier (11). The accuracy and reproducibility of BP measurements as determined with this device have been established previously (12). Measurements were recorded every 20 min between 7 a.m. and midnight (waking period), and every 30 min between midnight and 7 a.m. (sleeping period). Patients who failed to exhibit the normal sleep-related decline in mean BP of at least 10% compared with the waking period were defined as “nondippers,” the others were defined as “dippers” (13).

Active plasma renin concentrations were determined using an immunoradiometric assay kit (sandwich technique) from ERIA Diagnostics (Pasteur, Marnes-La-Coquette, France). Reference values in healthy subjects in the recumbent position at 8 a.m. range from 7 to 25 ng/L. Plasma aldosterone was measured by a specific RIA after purification of plasma extracts by Sephadex LH-20 column chromatography (14); reference values in the recumbent position at 8 a.m. range from 90 to 290 pmol/L. Plasma atrial natriuretic peptide was determined using Amersham’s human α-atrial natriuretic peptide RIA system with Amerflex-M™ magnetic separation; reference values in the recumbent position at 8 a.m. range from 8.5 to 44.6 pg/ml. Plasma immunoreactive endothelin-1 was measured by RIA as described previously (15); reference values ranged from 2.8 to 7.5 pg/ml (mean, 5.1 ± 0.6 pg/ml). Plasma norepinephrine, epinephrine, and dopamine were measured using a radioenzymatic assay (16); reference values in the recumbent position are 600 to 1700, 0 to 300, and 0 to 1600 pmol/L, respectively. Total blood volume was measured by standard dilution procedures using sodium radiochromate (61Cr) autologous tagged red cells as described previously (17). Plasma volume was calculated from hematocrit levels corrected for trapped plasma and adjusted for large vessel hematocrit values (18). Twenty-four-hour total exchangeable sodium was determined by standard isotope dilution principles after correction for urinary loss (19). Briefly, 2 μCi of sodium-22 (Amersham, Oakville, Ontario, Canada) were injected through an in-dwelling intravenous cannula in the forearm. The technique included precise weighing of syringes to obtain the accurate amount of injected radioactivity. Total 24-h exchangeable sodium was calculated as: Serum sodium (mmol/L) × [Total counts/min received − Counts/min lost in urine] ÷ Counts/min per L of serum. Extracellular fluid volume (sodium space) was calculated during the same procedure as: (Total counts/min received − Counts/min lost in urine) ÷ Counts/min per L of serum. Interstitial fluid volume was obtained by subtracting plasma volume from extracellular fluid volume. Because each patient served as his or her own control, the volume results were expressed in milliliters without correction per square meter of body surface.

Two-dimensional and M-Mode echocardiograms were recorded on VHS tape using an HP Sonos 100 ultrasound recorder (Hewlett-Packard, Andover, MA). Standard parasternal and subcostal views were obtained. The same technician recorded all echocardiograms. Cardiac output was obtained using Doppler-echocardiography of aortic flow (Integral of the flow trace × Aortic valve area × heart rate). Peripheral vascular resistance was calculated as the ratio of mean BP during the echocardiogram [Diastolic BP + (Systolic BP − Diastolic BP) ÷ 3] and expressed as dyne.s.cm⁻⁵.

Statistical Analyses

The t test for paired measurements was used to establish statistically significant differences observed in subjects over the study period. Differences between the subgroups of patients were assessed by ANOVA. Correlations between individual parameters were evaluated by linear regression analysis. Data are reported as means ± SEM with P < 0.05 taken as the upper level of statistical significance.

Results

Hemoglobin increased from 83 ± 1.5 g/L before rhEPO therapy to 119 ± 23 g/L at the end of the study (P < 0.01). Serum creatinine and serum urea were 826 ± 39 μmol/L and 21 ± 0.8 mmol/L before rhEPO treatment and 865 ± 41 μmol/L and 21 ± 0.8 mmol/L at the end of the study (non-significant). Serum potassium, calcium, and phosphorus were comparable throughout the investigation. Standard BP measurements as well as the mean body weight loss during hemodialysis before rhEPO therapy and at the end of the study are presented in Table 1. Each value represents the mean of BP and body weight values obtained during the previous six dialysis sessions. Body weight loss during hemodialysis sessions was comparable in the two experimental conditions. A significant increase (P < 0.01) in all BP measurements was evident with rhEPO therapy. Table 1 also shows that ambulatory BP values were significantly increased by the rhEPO treatment. Mean heart rate for the full 24-h period and for each of the waking and sleeping periods was slightly, but not significantly, decreased. Figure 1 depicts the hourly mean systolic and diastolic
Table 1. Mean supine systolic and diastolic BP before and after hemodialysis as well as mean weight loss during hemodialysis, and systolic and diastolic ambulatory BP and heart rate during the 24-h, waking, and sleeping periods, before rhEPO treatment and at the end of the studya

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before rhEPO</th>
<th>End of the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-HD SBP (mmHg)</td>
<td>139 ± 3</td>
<td>151 ± 3b</td>
</tr>
<tr>
<td>Pre-HD DBP (mmHg)</td>
<td>79 ± 2</td>
<td>87 ± 2b</td>
</tr>
<tr>
<td>Post-HD SBP (mmHg)</td>
<td>139 ± 3</td>
<td>148 ± 3b</td>
</tr>
<tr>
<td>Post-HD DBP (mmHg)</td>
<td>78 ± 2</td>
<td>88 ± 2b</td>
</tr>
<tr>
<td>BW loss during HD (kg)</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>24-h S ABP (mmHg)</td>
<td>131 ± 2</td>
<td>144 ± 3b</td>
</tr>
<tr>
<td>24-h D ABP (mmHg)</td>
<td>75 ± 1</td>
<td>85 ± 2b</td>
</tr>
<tr>
<td>Waking period S ABP (mmHg)</td>
<td>133 ± 2</td>
<td>147 ± 3b</td>
</tr>
<tr>
<td>Waking period D ABP (mmHg)</td>
<td>75 ± 1</td>
<td>86 ± 2b</td>
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<tr>
<td>Sleeping period S ABP (mmHg)</td>
<td>126 ± 2</td>
<td>140 ± 3b</td>
</tr>
<tr>
<td>Sleeping period D ABP (mmHg)</td>
<td>73 ± 1</td>
<td>82 ± 2b</td>
</tr>
<tr>
<td>24-h HR (beats/min)</td>
<td>78 ± 2</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Waking period HR (beats/min)</td>
<td>80 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>Sleeping period HR (beats/min)</td>
<td>72 ± 2</td>
<td>70 ± 2</td>
</tr>
</tbody>
</table>

a rhEPO, recombinant human erythropoietin; HD, hemodialysis; SBP, systolic BP; DBP, diastolic BP; BW, body weight; S, systolic; ABP, ambulatory BP; D, diastolic; HR, heart rate.

b P < 0.01.

Figure 1. Twenty-four-hour patterns (mean ± SEM) of systolic and diastolic ambulatory BP before (closed circles) and after (open circles) recombinant human erythropoietin (rhEPO) therapy.

ambulatory BP throughout the 24-h period before and after rhEPO treatment. The mean increases in systolic and diastolic BP were comparable during the waking and the sleeping periods (13.5 ± 2.6/10.7 ± 1.6 mmHg versus 14.4 ± 2.7/7.9 ± 2.9 mmHg, NS). Ten of the 32 patients had normal circadian BP with a reduction of >10% in the mean BP while asleep (dippers), whereas the remaining 22 patients (70%) lacked this normal response (nondippers). At the beginning of the study, the mean BP of the dippers, as evaluated for the 24-h period and each of the waking and sleeping periods, were not significantly different from the nondippers (95 ± 5, 98 ± 2, and 87 ± 2 mmHg versus 94 ± 2, 94 ± 2, and 93 ± 2 mmHg). After rhEPO therapy, similar mean BP values were seen in the two groups for the full 24-h period and for the waking and sleeping periods (dippers: 105 ± 5, 106 ± 5, and 99 ± 5 mmHg versus nondippers: 106 ± 2, 107 ± 2, and 103 ± 3 mmHg, NS).

We examined the effect of rhEPO therapy on BP change relative to that established at the beginning of the study in the following patient groups: normotensive (n = 11), borderline systolic hypertensive (n = 9), and treated and well controlled hypertensive (n = 12). The increases in 24-h ambulatory mean BP in the three groups were 8 ± 3 mmHg, 15 ± 3 mmHg (nonsignificant versus normotensive), and 12 ± 4 mmHg (non-significant versus normotensive), respectively. Only one patient of the normotensive group had increased predialysis BP >160/90 mmHg and subsequently received antihypertensive medication after completion of the study. The initial BP level was higher in the two other groups, and more patients ended up with BP values >160/90 mmHg. Antihypertensive therapy had to be initiated in all patients with borderline systolic hypertension (n = 9) and had to be increased in half (n = 6) of treated and well controlled hypertensive patients after completion of the study. Thus, antihypertensive medication was either adjusted or initiated in 16 patients, whereas predialysis BP remained below 160/90 mmHg in the other 16 patients. Although initial BP were higher in the former group (148 ± 2/84 ± 2 versus 131 ± 4/75 ± 2 mmHg, P < 0.01 for systolic and diastolic values), the increase in predialysis mean BP under rhEPO therapy was comparable in the two subgroups of patients (11.4 ± 3.1 mmHg versus 9.5 ± 2.8 mmHg).

The improvement of anemia under rhEPO therapy produced a slight but significant decrease in cardiac output (5.3 ± 0.2 L/min versus 4.9 ± 0.2 L/min, P < 0.05) and a consistent increase in peripheral vascular resistance (1321 ± 65 versus 1657 ± 84 dynes.s.cm⁻², P < 0.01). Table 2 shows the mean of circulating volumes and hematocrit values. rhEPO therapy produced a mean increase of 510 ± 35 ml in red blood cell mass, whereas plasma volume was decreased by only 420 ± 6 ml, which resulted in a slight and nonsignificant increase (91 ± 72 ml) in total blood volume. Body weight, interstitial and extracellular fluid volumes, and exchangeable sodium values are also presented in Table 2. Although the body weight before rhEPO therapy and at the end of the study were comparable, there was a significant decrease (P < 0.01) in the mean values of extracellular fluid volume and exchangeable sodium. Because fluid and sodium are important determinants of BP, we analyzed the relationship between the change in BP and exchangeable sodium after rhEPO therapy. Figure 2 shows that there was a significant positive correlation (r = 0.41, P < 0.05) between these two parameters. The patients were then divided into two subgroups: those with a decrease in exchangeable sodium (n = 22) and those with no change or with an
**Table 2.** Mean hematocrit, red blood cell mass, plasma volume, total blood volume, interstitial and extracellular fluid volumes, exchangeable sodium, and body weights before therapy and at the end of the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before rhEPO</th>
<th>End of the Study</th>
<th>Δ*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>26 ± 0.5</td>
<td>39 ± 0.8b</td>
<td>+14 ± 0.9</td>
</tr>
<tr>
<td>Red blood cell mass (ml)</td>
<td>886 ± 31</td>
<td>1396 ± 46b</td>
<td>+510 ± 35</td>
</tr>
<tr>
<td>Plasma volume (ml)</td>
<td>2696 ± 93</td>
<td>2276 ± 78b</td>
<td>−420 ± 66</td>
</tr>
<tr>
<td>Total blood volume (ml)</td>
<td>3581 ± 116</td>
<td>3672 ± 109</td>
<td>+91 ± 72</td>
</tr>
<tr>
<td>Interstitial fluid volume (ml)</td>
<td>16249 ± 488</td>
<td>15807 ± 564</td>
<td>−492 ± 232</td>
</tr>
<tr>
<td>Extracellular fluid volume (ml)</td>
<td>18964 ± 555</td>
<td>18091 ± 618b</td>
<td>−873 ± 255</td>
</tr>
<tr>
<td>Exchangeable sodium (mmol)</td>
<td>2613 ± 76</td>
<td>2487 ± 86b</td>
<td>−125 ± 33</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>63.2 ± 17</td>
<td>62.9 ± 1.6</td>
<td>−0.26 ± 0.28</td>
</tr>
</tbody>
</table>

*Δ*, difference. Abbreviations as in Table 1.

*P < 0.01.

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**Figure 2.** Relationship between the changes (Δ) in systolic BP during the waking period and in exchangeable sodium (NaE) after rhEPO treatment.

**Figure 3.** Hourly changes (Δ) in ambulatory mean BP (mean ± SEM) in patients with decreased exchangeable sodium (open circles, n = 22) and in patients with no change or increased exchangeable sodium (closed circles, n = 10) after rhEPO treatment.

An increase in exchangeable sodium (n = 10) after rhEPO treatment. The latter subgroup had a significantly higher increase in mean 24-h ambulatory mean BP values compared with the former subgroup, which had a decrease in exchangeable sodium (20 ± 4 mmHg versus 8 ± 2 mmHg, P < 0.01). Figure 3 depicts the hourly changes in ambulatory mean BP for the two groups of patients. In addition, compared with the former subgroup with a decreased exchangeable sodium, a larger proportion of patients in the subgroup with no change or an increased exchangeable sodium required initiation or readjustment of their antihypertensive medication after completion of the study (7 of 10 patients, 70% versus 9 of 22 patients, 41%). Hematocrit increased by 15 ± 1% (26 ± 1 to 41 ± 1%) in patients with a decrease in exchangeable sodium and by 12 ± 1% (26 ± 1 to 38 ± 1%) in patients with no change or an increase in exchangeable sodium. Postdialysis body weights before and after rhEPO were 62.4 ± 2.9 and 62.9 ± 3.2 kg in the former group versus 64.4 ± 2.1 and 63.9 ± 1.9 kg (non-significant) in the latter group.

Plasma hormone concentrations before and after rhEPO treatment are presented in Table 3. Plasma renin concentrations were similar before and after rhEPO therapy. Plasma aldosterone values were significantly higher (P < 0.01) at the end of the study; increased plasma aldosterone was observed in 23 of 32 patients after rhEPO therapy compared with those values obtained before treatment. There was no correlation between
plasma aldosterone and plasma renin concentrations, serum potassium, plasma atrial natriuretic peptide, and exchangeable sodium. Plasma catecholamine (norepinephrine, epinephrine, and dopamine) values were comparable before and after rhEPO therapy. Plasma atrial natriuretic peptide levels were slightly but not significantly increased at the end of the study. There was a correlation between the change in plasma atrial natriuretic peptides and plasma volume after rhEPO therapy \( (r = 0.38, P < 0.05) \). Plasma endothelin-1 values were comparable before and after rhEPO treatment.

No significant correlation was observed between the increase in systolic, diastolic, or mean ambulatory BP and the rise in hematocrit \( (r = -0.27, 0.05, \) and \( -0.09, \) respectively).

**Discussion**

Evaluation of the effect of rhEPO on BP and hemodynamics in hemodialysis patients is difficult, because a large proportion of these patients require antihypertensive medication, and the variation in dialysis ultrafiltration can modify body fluid compartments and hemodynamic parameters. The merits of the present study are that 20 of the 32 patients had no antihypertensive treatment and no medication change was effected during the study in the rest of the patients with treated and well controlled hypertension. In addition, the dialysis approach and the established dry weights were maintained throughout the study. With the use of ambulatory BP monitoring, it was possible to study interdialysis BP and the effect of rhEPO therapy on circadian BP variation. The treatment of anemia with rhEPO resulted in significant increases in pre- and post-dialysis BP and in interdialysis ambulatory BP in the entire group. This increase in BP, however, was less important in nontreated normotensive patients, and only 1 of 11 patients in this subgroup had increased predialysis systolic and diastolic BP >160/90 mmHg at the completion of the study. Patients with untreated borderline systolic hypertension experienced comparable increases in BP with rhEPO therapy; because their initial BP levels were higher, they all had predialysis BP values >160/90 mmHg, which required antihypertensive treatment at the end of the study. Similarly, the antihypertensive medication had to be readjusted after completion of the study in half of the patients with treated and well controlled hypertension. These results concur with earlier studies which suggest that patients who are hypertensive or who have been treated with antihypertensive agents are more likely to develop clinically significant increases in BP during rhEPO therapy (11,20,21).

Circadian BP patterns were similar and superimposable before and after the treatment of anemia, suggesting that rhEPO has no effect on the nyctohemeral variation of BP. These findings are in agreement with our own earlier observation (11), but not that of van de Borne et al. (22), who reported in a smaller group of patients that the rhEPO-induced rise in BP was higher during the nighttime period. The reason for this discrepancy is unclear. Patients who failed to show the normal fall in sleeping BP (nondippers, a well documented phenomenon in patients with renal failure; references 23 and 24) exhibited an increase in BP while receiving rhEPO therapy similar to that seen in dippers. These results confirm our earlier observation (11) and suggest that the nondipper condition does not suggest a greater risk toward the development of hypertension during rhEPO therapy.

As recently reviewed by Mayer and Hörl (25), improvement of renal anemia with rhEPO therapy generally results in a decrease in cardiac output and an increase in peripheral vascular resistance. The results of the present study concur with the literature. Systemic vascular resistance increased by approximately 28%, which may have accounted for the rise in BP. These important changes in peripheral resistance did not seem to be adequately counterbalanced by the decrease of only 6% in cardiac output. Because body fluid volumes are an important determinant of cardiac output, analysis of these parameters in a setting in which patients are unable to regulate body fluid volumes may provide useful insights in understanding the hemodynamic changes that occur with improved anemia as a result of rhEPO therapy.

The red blood cell mass in this study increased by half a liter, or approximately 15%, of the total blood volume. Theoretically, if patients with normal renal function were to receive a comparable transfusion of red blood cells, the kidney would reduce the extracellular fluid volume (plasma and interstitial volumes) proportionally to maintain stable circulating volumes. In uremic predialysis patients treated with rhEPO, Anastasiades et al. (4) showed that the expansion of red cell volume was not compensated by an adequate contraction of plasma volume. In contrast, a second group of patients on peritoneal dialysis had a comparable expansion of red cell volume but an equivalent reduction of their plasma volume, resulting in unchanged total blood volume and a smaller increase in BP (4). In the present study, the ultrafiltration was not modified throughout the protocol. It is therefore noteworthy that, despite a comparable body weight at the end of the study, the extracellular fluid volume was significantly decreased by \( -873 ± 255 \) ml. Abraham et al. (5) observed a similar phenomenon in a smaller group of eight patients; the extracellular fluid volume significantly decreased after rhEPO treatment despite a slight increase in body weight. It is possible that these patients, as well as ours, had an increase in tissue mass or dry weight resulting from an increase in exercise capacity (26,27).
or an improvement in appetite and nutritional status (28), which are known to be indirect effects of rhEPO therapy. The major finding of the present study was that patients who experienced a decrease in extracellular fluid volume or exchangeable sodium with the improvement of anemia associated with rhEPO therapy had a lower increase in BP. These data emphasize the importance of readjusting body fluid volume by dialysis ultrafiltration when correcting anemia in hemodialysis patients. It is common in daily practice to use ultrafiltration to compensate for the volume of red cell transfusions. To our knowledge, there are no published reports that provide guidelines for modification of the dialysis prescription during improvement of anemia with rhEPO replacement therapy. The data obtained at the end of our study (Table 2) suggest that, to achieve a plasma volume reduction proportional to the increase in red blood cell volume, the interstitial volume (which is a larger compartment in equilibrium with plasma volume) should be contracted by at least a similar or probably a larger volume. In practical terms, it appears that ultrafiltration should be adjusted to reduce the extracellular fluid volume (plasma and interstitial volumes) by at least twice the new red blood cell mass. We calculated that an increase of 1% in hematocrit corresponds to an increase of 38 ml in red blood cells; alternatively, an increase of 1 g/L in hemoglobin corresponds to an increase of 14 ml in red blood cells. Thus, in the present study, whereas hematocrit increased by a mean of 14%, the red blood cell volume increased by a mean of 510 ml. Ideally, the extracellular fluid volume should probably have been reduced progressively during the course of rhEPO therapy by at least 1 L. Prospective controlled studies should be performed to provide more precise guidelines to control the “volume-dependent” increase of BP during rhEPO therapy.

There is now substantial clinical and experimental evidence to suggest that systemic vascular resistance and BP may also increase during the reversal of anemia associated with rhEPO therapy due to the activation of “volume-independent” factors of BP control. In this respect, the observed correlation between the changes in exchangeable sodium and in systolic BP (Figure 2) has a squared correlation coefficient \( r^2 \) of 0.16, which suggests that the rise in BP could be attributed mainly to factors other than body fluid expansion. Although several mechanisms related to an increase in hematocrit and whole blood viscosity have been postulated (29,30), recent data suggest that rhEPO-induced hypertension is most likely unrelated to these hemorheological changes (11,31,32), which is further supported by the results of the present study. Alternatively, it has been shown that rhEPO therapy increases the pressor response to noradrenaline and angiotensin II and improves the anemia-mediated disturbance of alpha-2 receptor function (33). These alterations may or may not translate into increased plasma levels of catecholamines. Our results do not show any significant change in plasma levels of norepinephrine, epinephrine, and dopamine as reported previously by others (11,34). Plasma renin concentration values were comparable in our patients before and after rhEPO therapy. These results concur with other studies, in which circulating plasma renin levels were evaluated (5,11,34), but do not rule out a potential role for renin in specific tissues. In rats treated with rhEPO, Eggena et al. (35) reported normal plasma renin values in the presence of increased renin substrate mRNA in the kidney and aorta, which correlated with an elevation in BP; the administration of an angiotensin-converting enzyme inhibitor prevented the BP rise in these animals. In the present human study, despite the absence of changes in plasma renin concentration, plasma aldosterone levels were significantly increased after rhEPO therapy; more than two-thirds of the patients exhibited an increase in plasma aldosterone at the end of the study. There was no correlation between changes in plasma aldosterone and plasma renin concentration, plasma atrial natriuretic peptide, and serum potassium. There are few reports of aldosterone measurements during rhEPO therapy. Abraham et al. (5) reported a nonsignificant increase in eight hemodialysis patients, whereas Kokot et al. (36) observed a decrease in plasma aldosterone in five hemodialysed patients after 3 mo of rhEPO treatment. The difference in experimental conditions and the modification in antihypertensive medication during these studies may account for the discrepancy observed with the present study; however, our data do not address the mechanism of the rhEPO-induced increase in aldosterone.

Plasma atrial natriuretic peptide levels in our hemodialysis patients before rhEPO therapy were about twice the reference values as reported by Hasegawa et al. (37). After rhEPO therapy, the values were slightly but not significantly increased as observed in earlier reports (34,38), and these changes correlated with plasma volume. The variations in plasma atrial natriuretic peptide concentrations may be a result of a compensatory mechanism related to volume fluctuations in these patients.

Recent studies have suggested possible links between rhEPO-associated hypertension and endothelium-derived vasoconstrictor autacoids, mainly because erythropoietin receptors are present on the surface of vascular endothelial cells (39) and also because these cells are capable of releasing endothelin-1 under the influence of rhEPO (40). Carlini et al. (7) reported that intravenous, but not subcutaneous, injection of rhEPO in hemodialysis patients increased plasma levels of endothelin-1. In the present study as well as in other reports (7,15) using the subcutaneous route with delayed absorption of the hormone, plasma endothelin-1 levels were not modified by rhEPO therapy. However, an unchanged plasma concentration of endothelin-1 does not necessarily reflect unchanged endothelial endothelin-1 production rate, because 80% of endothelin-1 released by endothelial cells is directed abluminally (toward the vascular smooth muscle cells), and may therefore exert local effects without apparent increases in the plasma (41).

In summary, the improvement of anemia with rhEPO therapy in hemodialysis patients is associated with significant changes in BP, cardiac hemodynamics, and body fluid volumes. Patients who exhibited a decrease in exchangeable sodium had lower increases in BP, suggesting the importance of optimally reducing extracellular fluid volume to prevent the “volume-dependent” increase in BP seen with improvement of anemia associated with rhEPO therapy in hemodialysis patients. Additional studies are necessary to determine the con-
tribution of other “volume-independent” factors in this clinical setting.

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References
32. Kaupke CJ, Kim S, Vaziri ND: Effect of erythrocyte mass on
34. Ono K, Hisahue Y: The rate of increase in hematocrit, humoral vasoactive substances and blood pressure changes in hemodialysis patients treated with recombinant human erythropoietin or blood transfusion. Clin Nephrol 37: 23–27, 1992