Rebound kinetics of $\beta_2$-microglobulin after hemodialysis

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Background. Evaluation of $\beta_2$-microglobulin ($\beta_2m$) removal during hemodialysis using predialysis and immediate postdialysis plasma concentrations is only valid in the absence of postdialysis rebound. Postdialysis rebound of $\beta_2m$ has not been studied extensively, and its importance in the determination of $\beta_2m$ clearance is unknown.

Methods. We evaluated the kinetics of urea and $\beta_2m$ in a crossover study of 10 chronic hemodialysis patients using dialyzers with similar urea mass transfer-area coefficients containing either low-flux cellulose acetate or high-flux cellulose triacetate membranes. Kinetics were examined during and following a 210 minute treatment by measuring plasma concentrations predialysis at regular intervals during therapy and at 0, 2, 10, 20, 30, and 60 minutes postdialysis. Clearances of urea and $\beta_2m$ were also determined directly from the arterial and venous concentration differences across the dialyzer at 60 minutes after starting dialysis.

Results. By design, urea removal was similar for both low-flux and high-flux dialyzers as assessed by the urea reduction ratio and Kt/V. Postdialysis urea rebound was similar for low- and high-flux dialyzers; the rebound in the plasma urea nitrogen concentration (expressed as a percentage of the intradialytic decrease in plasma concentration) was $9.2 \pm 1.9\%$ (mean $\pm$ SEM) at 30 minutes postdialysis and $13.0 \pm 1.4\%$ at 60 minutes postdialysis for a single pool urea Kt/V of 1.16 $\pm$ 0.05. The plasma $\beta_2m$ concentration increased by 11.1 $\pm$ 3.0$\%$ during the treatment using the low-flux dialyzer but decreased by 27.1 $\pm$ 4.0$\%$ during the treatment using the high-flux dialyzer. When using the high-flux dialyzer, the rebound of $\beta_2m$ was $44.8 \pm 21.4\%$ at 30 minute postdialysis and $45.9 \pm 15.9\%$ at 60 minutes postdialysis. The clearance of $\beta_2m$ for the high-flux dialyzer calculated from predialysis and immediate postdialysis plasma concentrations using a single-compartment model (28.2 $\pm$ 4.4 ml/min) was higher ($P < 0.05$) than that determined directly across the dialyzer (18.3 $\pm$ 2.0 ml/min). If either the 30- or 60-minute postdialysis plasma $\beta_2m$ concentration was used instead, the calculated $\beta_2m$ clearance (16.5 $\pm$ 4.8 ml/min or 15.6 $\pm$ 2.8 ml/min, respectively) was similar to that determined directly across the dialyzer.

Conclusions. Postdialysis rebound of $\beta_2m$ when using high-flux dialyzers is substantial; neglecting postdialysis rebound results in an overestimation of $\beta_2m$ clearance when calculated using a single-compartment model.

Plasma concentrations of $\beta_2$-microglobulin ($\beta_2m$) in end-stage renal disease patients have been previously shown to depend on the extent of residual renal function [1, 2], the type of blood purification therapy [3, 4], and the properties of the dialysis (or filtration) membrane [3, 5–7]. Elucidation of $\beta_2m$ kinetics is important for understanding both the pathogenesis of amyloid disease in these patients and the removal mechanisms for middle molecules during treatment. The kinetics of $\beta_2m$ during and immediately following intermittent, extracorporeal therapies are complex but may provide insights into the physiology of $\beta_2m$ transport inside the body.

Changes in the plasma $\beta_2m$ concentration during hemodialysis are often difficult to predict because they are influenced by intradialytic changes in the distribution volume of $\beta_2m$ in addition to the generation, clearance, and intercompartmental transport of $\beta_2m$. For example, the rise in plasma $\beta_2m$ concentration during hemodialysis using low-flux membranes (that is, membranes essentially impermeable to $\beta_2m$) that was originally attributed to intradialytic $\beta_2m$ generation [8, 9] was subsequently shown to result primarily from the distribution of $\beta_2m$ in the entire extracellular compartment, not only within the plasma volume [10–12]. The kinetics of $\beta_2m$ during therapies that remove significant quantities of $\beta_2m$, such as hemodialysis using high-flux membranes, hemofiltration, or hemodiafiltration, are also difficult to describe. Using a single-compartment kinetic model, Floege et al. showed that the apparent distribution volume for $\beta_2m$ during hemofiltration showed a triphasic pattern [13]; it was constant for the first 60 to 120 minutes, then increased by a factor of approximately two, and finally decreased toward the end of therapy. These investigators hypothesized that such changes in the apparent distribution volume of $\beta_2m$ could be due to fluid shifts between the extracellular and intracellular compartments. The importance of fluid shifts between compartments was indirectly verified by Höüig et al, who showed that the
reduction in plasma β2M concentration during hemodialysis using high-flux membranes was dependent on the dialysate sodium concentration [14], a parameter that had previously been shown to influence the relative amounts of fluid removed from the intracellular and extracellular compartments [15].

Although not emphasized in most previous studies, changes in the apparent distribution volume for β2M during therapy could also be due to intradialytic compartmentalization of β2M. A number of multiple compartment kinetic models describing the time dependence of plasma β2M concentration during hemodialysis have been developed and have predicted substantial compartmentalization of β2M and postdialysis rebound [16–19]. Odell et al determined β2M rebound 30 minutes after hemodialysis using a high-flux dialyzer in four patients, but the extent of postdialysis rebound was dependent on whether or not the plasma β2M concentration was evaluated from endogenous or radiolabeled β2M [17].

This study determines the extent of postdialysis rebound of plasma β2M concentration and its importance in calculating β2M clearance during hemodialysis therapy.

**METHODS**

**Patients, treatments, and study protocol**

Ten stable, chronic hemodialysis patients from the University of Utah Dialysis Program signed written informed consent; all patients completed the study. Eight of the patients were male, and two were female. The mean age of the patients was 61 (range 28 to 82) years. Prior to this study, all patients were chronically treated with dialyzers containing low-flux cellulose acetate membranes.

This study was performed during routine hemodialysis on each patient using two different dialyzers on separate occasions. On the first day of study, dialysis was performed on each patient using a new dialyzer containing 1.9 m² of high-flux cellulose triacetate membrane (CT190G; Baxter Healthcare, Deerfield, IL, USA). This dialyzer was reprocessed and used to treat the patients during the next two routine dialysis sessions. No samples were collected during the treatments using the reprocessed high-flux dialyzer. On the second study day, dialysis was performed on each patient using a new dialyzer containing 2.1 m² of low-flux cellulose acetate membrane (CA210; Baxter Healthcare). The CT190G and CA210 dialyzers have similar urea mass transfer area coefficients [20].

Centralsytem 3 dialysis machines (Gambro Healthcare, Lakewood, CO, USA) were employed for all treatments. For each patient and treatment, the blood pump speed was set to a constant value of 300 ml/min for a total treatment time of 210 minutes. The dialysate contained a constant sodium concentration of 140 mEq/liter and bicarbonate buffer, and the flow rate of dialysate was constant at 500 ml/min. The ultrafiltration rate varied from session to session in order to remove sufficient fluid to achieve the patient's prescribed dry weight.

On both days of study, blood samples were obtained from the arteriovenous fistula predialysis and from the arterial dialysis tubing at 60, 120, 180, and 210 minutes after starting dialysis. A blood sample from the venous dialysis tubing was also taken at 60 minutes of therapy to determine clearances directly across the dialyzer (discussed later in this article). At the end of the treatment, the blood pump speed was reduced to 80 ml/min, and blood samples were obtained at 20 seconds and two minutes postdialysis from the arterial dialysis tubing. Blood samples were also obtained 10, 20, 30, and 60 minutes postdialysis. An additional blood sample was obtained predialysis from the arteriovenous fistula at the next dialysis session in order to determine the urea generation rate. All samples were obtained in tubes containing ethylenediaminetetraacetic acid (EDTA) and were centrifuged to obtain the plasma for chemical assays. All plasma samples were frozen at -70°C until assay.

**Analytical**

Plasma concentrations of urea nitrogen, albumin using the bromcresol purple dye binding assay, and total protein were measured by an automated analyzer (Beckman CX7; Beckman Instruments, Fullerton, CA, USA). The interassay coefficient of variation reported by the manufacturer was 4.5% for urea nitrogen above 50 mg/dl; however, the relative variability of this assay increases with decreasing concentration. The precision of the plasma albumin and total protein concentration assays as reported by the manufacturer was ±0.3 g/dl (±1 SD) and ±0.5 g/dl, respectively. The plasma β2M concentration was measured using a radioimmunoassay (Pharmacia, Columbus, OH, USA) with a reported interassay coefficient of variation of 5.2%.

**Calculations**

Plasma urea nitrogen and β2M concentrations were reported as a function of the time after starting hemodialysis. The percentage rebound of urea and β2M was calculated from plasma concentrations in the samples obtained predialysis (Cprev), immediately postdialysis (Cpost0s), and 30- or 60-minutes postdialysis (Cpost) using the following equation:

\[
\text{Percent rebound} = \frac{(C_{\text{post}} - C_{\text{post0s}})}{(C_{\text{prev}} - C_{\text{post0s}})} \quad (\text{Eq. 1})
\]

When calculating urea rebound, all postdialysis urea nitrogen concentrations were corrected for urea generation during the postdialysis interval using the 60-minute postdialysis concentration and the predialysis concentration.
before the next dialysis session as previously described by others [21].

Blood water clearance (K) of urea across the dialyzer at 60 minutes after starting dialysis was calculated from arterial (C_a) and venous (C_v) urea nitrogen concentrations, the ultrafiltration rate (Q_uf), and the blood water flow rate (Q_a) using the following equation:

\[ K = \frac{[C_a Q_a - C_v (Q_a - Q_uf) ]}{C_a} \]  
(Eq. 2)

In this calculation, the blood water flow rate was assumed to be equal to 89.4% of the blood pump speed.

Plasma clearance of β_m across the dialyzer at 60 minutes after starting treatment was calculated using Equation 2 except that C_a and C_v now denote β_m concentrations in the arterial and venous plasma flow streams, respectively, and Q_a denotes the arterial plasma flow rate calculated from the arterial (x_a) and venous (x_v) concentrations of albumin using the following equation:

\[ Q_a = x_a Q_a / (x_v - x_a) \]  
(Eq. 3)

This approach for calculating the dialyzer clearance of β_m from arterial and venous plasma concentrations across the dialyzer has been previously described [22].

Fractional changes in plasma volume (ΔPV) during and after hemodialysis therapy were calculated using the following formula:

\[ ΔPV = |PV(t) - PV(0)|/PV(0) = x_a(0)/x_a(t) - 1 \]  
(Eq. 4)

where PV(0) and PV(t) denote the volumes of the plasma compartment at the beginning of hemodialysis and at any time during or after therapy, respectively. The values of x_a(0) and x_a(t) denote the arterial plasma concentration of albumin at the beginning and at any time during or after the hemodialysis treatment, respectively. Fractional changes in plasma volume were also computed from a corresponding equation using arterial total protein concentrations instead of those for albumin with virtually identical results. The values reported herein are the average of those calculated using plasma albumin and total protein concentrations. Note that it was not possible in this study to calculate either PV(0) or PV(t) individually, only their ratio and therefore ΔPV.

The dose of urea removal during each treatment was assessed by calculating the urea reduction ratio and urea Kt/V in three different ways. Single pool estimates of urea Kt/V (spKt/V) were calculated from urea nitrogen concentrations in the predialysis and 20-second postdialysis plasma samples using the second-generation Daugirdas formula [23]. Two estimates of equilibrated urea Kt/V (eKt/V) were also calculated. First, equilibrated urea Kt/V (eKt/Vra) was calculated from the spKt/V value and treatment time using the rate adjustment formula of Daugirdas and Schneditz [25]. Second, equilibrated urea Kt/V (eKt/V) was calculated from urea nitrogen concentrations in the predialysis and 60-minute postdialysis plasma samples using the second-generation Daugirdas formula. In this latter calculation, the 60-minute postdialysis urea nitrogen concentration was corrected for urea generation (discussed earlier in this article).

The whole body clearance of β_m (K_w) was also calculated from predialysis and postdialysis plasma concentrations assuming that β_m is distributed within a single compartment by the following equation [22]:

\[ K_w = Q_{ECV}(1 - \ln [C_{pre}/C_{post}]/\ln [1 + Q_{ECV} T/V_{ECV}(T)]) \]  
(Eq. 5)

where C_{pre} and C_{post} denote predialysis and postdialysis plasma β_m concentrations, respectively; T denotes treatment time or 210 minutes in this study, and V_{ECV}(T) denotes the distribution volume of β_m at the end of the treatment, which was assumed to be equal to extracellular volume and was evaluated as one third of total body water calculated from the anthropometric formula of Watson, Watson, and Batt [26]. In this study, the validity of using the immediate postdialysis concentration in Equation 5 to calculate dialyzer β_m clearance was compared with the same equation using postdialysis concentrations 30 and 60 minutes after the end of hemodialysis.

The value of Q_{ECV} in Equation 5 denotes the rate of fluid removal that originates from the extracellular compartment and cannot easily be measured directly. In a previous report [22], this parameter was calculated by assuming that intradialytic changes in extracellular volume were equal to either the total volume of fluid ultrafiltered from the patient during therapy or one third of that value. These assignments correspond, respectively, to assuming that fluid is removed entirely from the extracellular compartment or proportionally from the extracellular and intracellular compartments. In this study, we evaluated Q_{ECV} by assuming that the percent change in extracellular and plasma volume is the same after fluid equilibration between these compartments is complete. This assumption yields the following expression for calculating Q_{ECV}:

\[ Q_{ECV} = [ECV(T) - ECV(0)]/T = ΔPV_{eq} \times V_{ECV}(T)/(1 - ΔPV_{eq})/T \]  
(Eq. 6)

where ECV(0) and ECV(T) denote extracellular volumes at the beginning and end of hemodialysis, respectively. The value of ΔPV_{eq} denotes the change in plasma volume after fluid equilibration. Equilibration of fluid composition was assumed to occur at 60 minutes postdialysis and appeared to be reasonable in this study, as discussed later in this article.

Statistics

All parameters are reported as mean ± SEM. The significance of differences among plasma concentrations
and calculated clearances at different times during and after hemodialysis was determined using analysis of variance with repeated measures and paired Student’s t-tests using confidence limits modified by the method of Bonferroni [27].

RESULTS

Table 1 compares fluid removal and treatment characteristics when using low-flux and high-flux dialyzers. Because treatment time and blood pump speed were constant for each session, these data show that the hemodialysis sessions were essentially equivalent when using the low-flux and high-flux dialyzers, except for the clearance of βm. Although βm clearance for low-flux dialyzers at 60 minutes of therapy was numerically larger than expected, the mean value was not different from zero.

Figure 1 shows measured plasma urea nitrogen concentrations during the 210-minute hemodialysis session and 60 minutes postdialysis. When using low-flux and high-flux dialyzers, the plasma urea nitrogen concentration decreased similarly throughout the treatment. The urea reduction ratio calculated using the immediate postdialysis sample was 63.2 ± 2.3% after treatment using the low-flux dialyzer and 66.7 ± 2.3% after treatment using the high-flux dialyzer. The plasma urea nitrogen concentration increased similarly after the end of the treatment for both dialyzer types. All calculated parameters regarding urea kinetics were therefore combined for the low-flux and high-flux dialyzers. Postdialysis urea rebound corrected for interdialytic urea generation was 9.2 ± 1.9% after 30 minutes and was 13.0 ± 1.4% after 60 minutes. As expected, urea spKt/V (1.19 ± 0.05) was higher (P < 0.0001) than urea εKt/Vra (0.97 ± 0.03) and urea εKt/V (0.99 ± 0.04). The latter two estimates were not different from each other.

Figure 2 shows fractional changes in plasma volume

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**Table 1.** Fluid removal and treatment characteristics

<table>
<thead>
<tr>
<th>Dialyzer</th>
<th>Qf liter/hr</th>
<th>BWpre kg</th>
<th>BWpost kg</th>
<th>ECV/BWpost %</th>
<th>Urea K ml/min</th>
<th>βm K ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA210</td>
<td>1.12 ± 0.11</td>
<td>85.0 ± 7.5</td>
<td>81.9 ± 7.3</td>
<td>16.9 ± 0.4</td>
<td>214 ± 6</td>
<td>6.7 ± 4.0</td>
</tr>
<tr>
<td>CT190G</td>
<td>1.13 ± 0.07</td>
<td>84.8 ± 7.4</td>
<td>81.4 ± 7.2</td>
<td>17.1 ± 0.4</td>
<td>224 ± 4</td>
<td>18.3 ± 2.0</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Abbreviations are: Qf, ultrafiltration rate recorded at 60 minutes after starting dialysis; BWpre, predialysis body weight; BWpost, postdialysis body weight; ECV, volume of the extracellular compartment evaluated as one-third of total body water calculated from an anthropometric formula [26]; Urea K and βm K, the respective dialyzer clearances of urea and βm determined from arterial and venous concentrations at 60 minutes after starting dialysis.

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**Fig. 1.** Plasma urea nitrogen concentrations plotted as a function of time after starting the hemodialysis treatment. Symbols are: (■) low-flux dialyzer; (□) high-flux dialyzer. There was no difference in urea nitrogen concentration between the low-flux and high-flux dialyzers at any time point. The arrow at 210 minutes indicates the end of the hemodialysis treatment (End of HD). Error bars denote ±1 SEM.

**Fig. 2.** Fractional changes in plasma volume plotted as a function of time after starting the hemodialysis treatment. Symbols are: (■) low-flux dialyzer; (□) high-flux dialyzer. There was no difference in changes in plasma volume between the low-flux and high-flux dialyzers at any time point. The arrow at 210 minutes indicates the end of the hemodialysis treatment (End of HD). Error bars denote ±1 SEM.
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Fig. 3. Plasma β₂-microglobulin concentrations plotted as a function of time after starting the hemodialysis treatment. Symbols are: (■) low-flux dialyzer; (▲) high-flux dialyzer. Asterisks denote that the plasma β₂-microglobulin concentration was higher (*P < 0.01) during treatment with the low-flux than with the high-flux dialyzers. The arrow at 210 minutes indicates the end of the hemodialysis treatment (End of HD). Error bars denote ±1 SEM.

Figure 3 shows plasma β₂m concentrations during the 210-minute hemodialysis session and 60 minutes postdialysis for treatments using both low-flux and high-flux dialyzers. Plasma volume decreased by 10.3 ± 3.0% during treatment by the low-flux dialyzer and 14.3 ± 1.7% during treatment by the high-flux dialyzer. Postdialysis plasma volume rebound was significant after treatment with both low-flux and high-flux dialyzers. Sixty minutes after the completion of therapy, however, plasma volume remained reduced compared with predialysis values by 5.8 ± 2.2% and 7.7 ± 1.5%, respectively, as a result of treatment with the low-flux and high-flux dialyzers.

Figure 4 shows clearances of β₂m calculated from arterial and venous concentration differences directly across the dialyzer compared with those calculated using a single-compartment model using the immediate postdialysis, 30 minutes postdialysis or 60 minutes postdialysis plasma β₂m concentration. For the high-flux dialyzers, the estimates of clearance using the four different methods were different from each other when tested by analysis of variance (P = 0.02). The clearance calculated from the immediate postdialysis plasma β₂m concentration using the single-compartment model was higher (P < 0.05) than either that determined from the 60-minute postdialysis plasma concentration by 82.1 ± 38.4% or that determined directly across the dialyzer by 67.4 ± 29.5%. The similarly calculated β₂m clearance values at 30 and 60 minutes postdialysis suggest that the rebound of β₂m is complete by approximately 30 minutes after ending hemodialysis, a time period for equilibrium similar to that previously described for urea [28].

DISCUSSION

The results of this study show that postdialysis rebound of β₂m when using high-flux dialyzers is substantial and that significant overestimation of β₂m clearance can occur when it is calculated from predialysis and immediate postdialysis plasma concentrations using a single-compartment
model. An increase in plasma $\beta_{2m}$ concentration after the completion of hemodialysis using high-flux dialyzers has been reported previously [16, 17, 29], but the significance of postdialysis rebound on the assessment of $\beta_{2m}$ removal was unclear. These empirical findings are qualitatively consistent with previous theoretical predictions regarding the magnitude of the postdialysis increase in plasma $\beta_{2m}$ concentration; however, the time course of $\beta_{2m}$ rebound observed in this study differs markedly from that predicted by others [16, 17, 19, 30]. Gotch et al could not obtain a good fit between the postdialysis increase in plasma $\beta_{2m}$ concentration and predictions from a multiple compartment model unless it was assumed that $\beta_{2m}$ generation increased markedly after the end of hemodialysis [16]. Odell et al observed only a small and inconsistent rebound of $\beta_{2m}$ plasma concentration after hemodialysis treatment using a high-flux dialyzer [17]. Furthermore, the three-compartment model used by these latter investigators suggested that postdialysis rebound would continue for a time period greater than 30 minutes. The results of this study suggest that the intercompartmental mass transfer coefficient for $\beta_{2m}$ is approximately 100 ml/min, a value intermediate between those reported by others [16–18]. The similarity of postdialysis equilibration times for urea and $\beta_{2m}$ plasma concentrations suggests that their rebound kinetics may be governed by the same mechanism, possibly by regional differences in blood flow within the body [31].

The results of this study are limited by the assumptions used to derive Equations 5 and 6. Equation 5 is only an approximate solution to the single-compartment model and neglects intradialytic generation of $\beta_{2m}$ and the effects of residual renal function on intradialytic changes in plasma $\beta_{2m}$ concentrations. As discussed elsewhere, these potential errors affect the calculated clearance in different directions and may counterbalance one another [22]. The assumptions used to derive Equation 6 may also influence the calculated clearance of $\beta_{2m}$. First, it must be emphasized that this analysis would not be valid if the ultrafiltration rate during the hemodialysis treatment was not sufficiently large to result in a significant reduction in plasma volume. Under those conditions, changes in plasma protein concentrations do not accurately reflect changes in extracellular volume [10]. This analysis is only accurate when intradialytic changes in plasma volume are greater than the changes in extracellular volume [15], such that a substantial postdialysis rebound of plasma volume would occur (Fig. 2). Second, it was assumed that 60 minutes was sufficient time for equilibration of fluid between the plasma and the remainder of the extracellular compartment. Such a time scale is consistent with theoretical predictions of equilibration times for fluid within the extracellular compartment [32], and Katzarski et al have recently reported data consistent with this assumption [33]. It should also be noted that the effect of the latter concerns only influence the calculated values of $\beta_{2m}$ clearance, but do not alter our conclusions regarding the magnitude of postdialysis rebound of $\beta_{2m}$.

Previous studies have evaluated $\beta_{2m}$ removal by the reduction in plasma concentration corrected for changes in the volume of the extracellular compartment [10] or by calculating $\beta_{2m}$ clearance from the predialysis and immediate postdialysis concentrations [22]. Both of these approaches overestimate $\beta_{2m}$ removal because they do not account for postdialysis rebound of plasma $\beta_{2m}$ concentration. The significance of this study is that it shows the importance of accounting for postdialysis $\beta_{2m}$ rebound when assessing therapies that remove significant quantities of $\beta_{2m}$.

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