Low-Molecular Weight Proteins in End-Stage Renal Disease: Potential Toxicity and Dialytic Removal Mechanisms

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Abstract. Low-molecular-weight proteins (LMWP) are now recognized as a distinct class of uremic toxins, and numerous compounds in this category have been identified. Dr. Henderson has spent much of his career investigating ways to enhance the removal of intermediate- and large-sized uremic retention molecules. As LMWP clearly fall under this category, it is fitting to provide a review of several aspects of this molecular class. Normal renal metabolism of LMWP is discussed along with the changes that occur during chronic renal insufficiency. The effect of end-stage renal disease on plasma LMWP concentrations is assessed. As examples of the potential uremic toxicity of this molecular class, leptin, adrenomedullin, and the compounds associated with increased susceptibility to infection are highlighted. Finally, an overview of LMWP removal mechanisms for both hemodialysis and the convective therapies is provided.

With the identification of β2-microglobulin (β2M) as an active participant in dialysis-related amyloid (DRA) fibril formation (1), low-molecular-weight proteins (LMWP) became a distinct class of uremic toxins. Since this discovery, a number of other putative uremic toxins from this same category have been identified (2–6), some of which appear in Table 1. Dr. Henderson has spent much of his career investigating ways to enhance the removal of intermediate- and large-sized uremic retention molecules. As LMWP clearly fall under this category (7), it is fitting to provide a review of several aspects of this molecular class. Specifically, LMWP handling under conditions of renal health and disease, LMWP of potential clinical significance in uremic patients, and dialytic removal of LMWP are discussed.

Renal Handling of LMWP

Normal Renal Elimination

Although not precisely defined, LMWP as a class have a molecular weight spectrum ranging from approximately 1000 to 50,000 daltons. Thus, peptides with as few as 10 amino acids and proteins nearly as large as albumin compose this group (8). Although the initial step in solute removal by the kidney is glomerular filtration, the net elimination of uremic toxins is primarily determined by processes that occur in distal portions of the kidney. The renal handling of LMWP was discussed in two comprehensive reviews published by Carone et al. (8) and Maack et al. (9) and can be summarized as follows. The kidney is responsible for a significant fraction of the metabolic clearance (30% to 80%) of these compounds. The sequential renal metabolic processes are glomerular filtration and luminal resorption at the proximal tubule, with the former as the rate-limiting step. Hydrolysis of the resorbed protein to its constituent amino acids happens within the proximal tubular cell, after which antiluminal resorption by the peritubular capillaries occurs.

Effect of Chronic Renal Insufficiency and End-Stage Renal Disease

A prototypical LMWP for which the metabolism in patients with normal renal function and varying degrees of renal insufficiency has been characterized is complement Factor D, a 23.5-kD upregulator of the alternative complement pathway (10). A significant direct correlation was observed between serum Factor D concentration and serum creatinine, whereas the relationship between serum Factor D concentration and creatinine clearance was very similar to that between serum creatinine and creatinine clearance. In patients with intact glomerular and proximal tubule function, despite a glomerular sieving coefficient of 0.36, <0.2% of filtered Factor D was measured in the final urine. In this study, serum Factor D concentrations in patients with end-stage renal disease (ESRD) were 10- to 20-fold higher than those with normal kidney function.

Kabanda et al. (11) also assessed the effect of ESRD on plasma concentrations of several LMWP in a group of chronic hemodialysis (HD) patients. The specific proteins investigated and some of their physiochemical characteristics are shown in Table 2. The four proteins, cystatin C, β2M, clara cell protein, and retinal binding protein, differ not only in molecular weight but also in their source of generation, molecular dimension, acidity at physiologic pH, and degree of protein binding. As such, they are representative of the diversity of the LMWP...
class in its entirety. The mean age of the study group was approximately 60 yr, and the average dialysis vintage was approximately 5 yr, with nearly 50% of the patients being anuric. The patients were treated with dialyzers that had membranes of varying water permeability, surface area, and composition. Relative to normal serum concentrations, pre-HD serum LMWP concentrations were elevated from approximately threefold (retinol binding protein) to 40-fold (β2M). In a multivariate regression analysis, residual diuresis, age, and gender were found to be significant predictors of pre-HD serum LMWP concentration. A similar type of analysis revealed molecular radius, dialyzer ultrafiltration coefficient, and ultrafiltration-related weight loss to be significant predictors of HD-induced changes in LMWP serum concentration.

**Specific Uremic Toxins in the LMWP Class**

As Table 1 indicates, a considerable number of putative uremic toxins in the LMWP category have been identified. Two molecules and a group of related molecules from this group, all relatively recently identified, are discussed here. These molecules have been chosen because they may play a role in three of the major clinical problems that uremic patients face: malnutrition, cardiovascular disease, and increased susceptibility to infection. A discussion of β2M’s role as a uremic toxin is not possible here, as a comprehensive review of this issue constitutes a full manuscript. For such a review, the reader is referred to an excellent summary published recently by Drueke (12).

**Leptin**

Leptin, a 16-kD protein secreted by adipocytes, regulates body composition by lowering food intake and increasing metabolic rate (13,14). It circulates in the plasma as both a free and a bound compound. Although percentage body fat is a direct determinant of plasma leptin concentration, obesity is associated with resistance to the protein’s weight-reducing effects (15). Sharma et al. (5) observed an inverse linear correlation between serum creatinine and net leptin extraction by the renal vascular bed, and several studies (5,16–23) in ESRD patients have demonstrated an increase either in the absolute plasma leptin level or in the ratio of plasma leptin to some measure of body fat. The increased leptin concentrations in uremic patients seem to be due entirely to increases in the unbound form (23).

**Table 1. Potential uremic toxins**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Molecular Weight (kD)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenomedullin</td>
<td>6</td>
<td>Hypotension</td>
</tr>
<tr>
<td>CIP</td>
<td>8.5</td>
<td>Chemotaxis inhibition</td>
</tr>
<tr>
<td>C3a</td>
<td>8.9</td>
<td>Anaphylatoid reactions and leukocyte stimulation</td>
</tr>
<tr>
<td>PTH</td>
<td>9</td>
<td>Osteodystrophy; other effects</td>
</tr>
<tr>
<td>AGE peptides</td>
<td>&lt;10</td>
<td>Various</td>
</tr>
<tr>
<td>AGE proteins</td>
<td>&gt;10</td>
<td>Various</td>
</tr>
<tr>
<td>C5a</td>
<td>11</td>
<td>Anaphylactoid reactions and leukocyte stimulation</td>
</tr>
<tr>
<td>β2M</td>
<td>11.8</td>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Leptin</td>
<td>16</td>
<td>Malnutrition</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17</td>
<td>Tubular damage</td>
</tr>
<tr>
<td>Factor D</td>
<td>23</td>
<td>Complement activation</td>
</tr>
<tr>
<td>GIP I</td>
<td>28</td>
<td>Granulocyte inhibition</td>
</tr>
<tr>
<td>Carbamylated proteins</td>
<td>various</td>
<td>? anemia</td>
</tr>
</tbody>
</table>

* CIP, chemotaxis-inhibiting protein; PTH, parathyroid hormone; AGE, advanced glycation endproduct; β2M, β2-microglobulin.

**Table 2. Low-molecular-weight protein characteristics**

<table>
<thead>
<tr>
<th></th>
<th>CYST C</th>
<th>β2M</th>
<th>CC16</th>
<th>RBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (kD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular radius (nm)</td>
<td>1.51</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>9.30</td>
<td>~5.5</td>
<td>4.7</td>
<td>~4.6</td>
</tr>
<tr>
<td>Normal serum range (mg/L)</td>
<td>0.6–1.6</td>
<td>1–2</td>
<td>0.05–0.1</td>
<td>50–80</td>
</tr>
<tr>
<td>Predialysis valueb (mg/L)</td>
<td>12 ± 3</td>
<td>39 ± 13</td>
<td>2.9 ± 1.7</td>
<td>166 ± 68</td>
</tr>
<tr>
<td>Postdialysis valuéc (mg/L)</td>
<td>8.3 ± 4.4</td>
<td>37 ± 15</td>
<td>3.3 ± 2.0</td>
<td>192 ± 78</td>
</tr>
</tbody>
</table>

* CYST C, cystatin C; β2M, β2-microglobulin; CC16, Clara cell protein; RBP, retinol binding protein.

*b Data are presented as mean ± SD.

*c P < 0.02 versus predialysis value.

Adapted with permission from Kabanda et al. (11).
Leptin’s effects coincide closely with the major clinical findings in malnourished uremic patients (i.e., anorexia and weight loss), and indirect evidence indicates that elevated leptin (or leptin/fat mass) levels are a contributory factor. Young et al. (17) reported a significant inverse relationship between dietary protein intake and the plasma leptin/total fat ratio in an ESRD population. Johansen et al. (18) observed a significant negative correlation between serum leptin levels and several nutritional parameters, including serum albumin, protein catabolic rate, and transferrin. In a cross-sectional analysis of chronic HD patients during a 17-mo period, Odamaki et al. (20) found a significant negative correlation between weight loss and the leptin/body fat mass ratio (Figure 1). Finally, in a longitudinal study, Stenvinkel et al. (22) correlated lean body mass (LBM) changes with plasma leptin and markers of inflammation in incident peritoneal dialysis patients during a 1-yr period. Patients who were losing LBM had higher initial C-reactive protein levels and demonstrated an increase in serum leptin during this time, relative to patients who gained LBM. A significant association between initial C-reactive protein and change in serum leptin during the study period was also observed, suggesting a contributory role for inflammation in the elevation of serum leptin in uremic patients or vice versa. However, both this group of investigators and Arkouche et al. (24) emphasized that there remains no direct proof that increases in serum leptin (or leptin/fat ratio) cause loss of appetite in uremic patients, and further investigation is needed.

Adrenomedullin

Adrenomedullin (ADM) is a 6-kD peptide isolated initially from human pheochromocytoma tissue and bears some structural resemblance to calcitonin gene-related peptide (4,25–27). The compound is a potent vasodilator, and its production seems to be modulated by sympathetic nervous activity, body fluid volume, and BP. Increased ADM plasma concentrations have been found in a broad variety of disorders, including hypertension, congestive heart failure, cirrhosis, chronic obstructive pulmonary disease, and renal failure (27). It is generally assumed that the increased plasma levels observed in renal failure patients are at least partially due to impaired elimination, although this has not been demonstrated definitively.

Several studies have assessed plasma ADM levels in chronic HD patients. Washimine et al. (28) found mean pre-HD plasma ADM to be elevated approximately fourfold relative to that of normal control subjects. However, pre-HD mean arterial pressure and plasma ADM were not correlated significantly, and HD did not influence plasma ADM significantly. Toepfer et al. (29) also found no significant effect of HD on plasma ADM, based on the immediate post-HD ADM levels. However, a significant decrease (relative to pre-HD) was observed 14 to 20 h post-HD, as was a significant direct correlation between BP and plasma ADM. Conversely, Mallamaci et al. (30) reported a nearly 50% decrease in plasma ADM (hemoconcentration-corrected) during isolated ultrafiltration, with no change occurring during sham ultrafiltration. It should be noted that patients in each of these studies were treated with low-flux dialyzers. Therefore, the plasma ADM changes described above could not have been related to significant convective dialytic removal of the compound. Finally, Cases et al. (31) explored the possible role of ADM as a mediator of sustained hypotension, defined as a systolic BP lower than 100 mmHg between HD sessions. (The type of dialyzer used in this study was not specified.) A significant inverse correlation was observed between pre-HD mean arterial pressure and plasma ADM (Figure 2), suggesting a role for ADM in the blunted response to pressor stimuli that uremic patients in general are known to have.

The above studies do not provide a straightforward explanation of ADM’s potential role in the cardiovascular pathophysiology of ESRD patients. It is not clear what factors, other than decreased or absent renal clearance, contribute to the significantly elevated ADM levels in ESRD. The data of Mallamaci et al. (30) suggest that fluid overload and hypertension provoke a vasodilatory response in the form of increased ADM.

![Figure 1](image1.png)  
*Figure 1. Relationship between body weight change during 17 mo and serum leptin/body mass index ratio in hemodialysis (HD) patients. Reprinted with permission from Odamaki et al. (20).*

![Figure 2](image2.png)  
*Figure 2. Inverse relationship between mean arterial pressure and plasma adrenomedullin in HD patients. Reprinted with permission from Cases et al. (31).*
generation. As such, elevated ADM plasma levels are simply an epiphenomenon. Conversely, Case’s data (31) indicate that the potent vasodilatory properties of ADM per se play a pathophysiologic role in hypotension-prone HD patients. If the validity of this latter scenario is confirmed, then efforts to enhance the dialytic removal of this compound in hypotension-prone patients may be useful. Validation of this concept would also lend credence to the hypothesis, posited by Dr. Henderson, that enhanced cardiovascular stability observed during hemofiltration (relative to diffusive HD) is related to the convective removal of a relatively high-molecular-weight vasodepressor substance (32).

Uremic Peptides Involved in Increased Susceptibility to Infection

Hörö and colleagues (2,6,33–36) identified a series of compounds that may contribute to the enhanced susceptibility of uremic patients to infection. These compounds include granulocyte-inhibiting protein (GIP) I, a 28-kD compound that has significant homology with light chains; GIP II, a 12-kD compound consistent with a modified form of β2M; and degranulation-inhibiting protein I, a 14.4-kD compound that has an amino acid sequence identical to that of angiogenin. All of these compounds were isolated either from the ultrafiltrate of patients undergoing high-flux dialysis or hemofiltration or from peritoneal dialysate. A more recently identified molecule is the 8.5-kD chemotaxis-inhibiting protein (CIP), also isolated from peritoneal effluent (36). This compound was found to be a modified (more acidic) form of ubiquitin, a putative mediator of muscle wasting in uremia (37). The chemotactic inhibition of both CIP and unmodified ubiquitin was evaluated by measuring the chemotaxis migration distance of polymorphonuclear cells toward a bacteria-derived chemotactic attractant (N-formyl-leucyl-phenylalanine; Figure 3). Polymorphonuclear cells displayed defective chemotaxis in the presence of CIP (group 1), as did cells that were preincubated only with CIP (group 1r), suggesting an irreversible effect of the peptide on cellular function. Conversely, normal chemotaxis was observed in the presence of unmodified ubiquitin. The authors hypothesized that glycation or carbamylation, two processes known to alter the structure and function of proteins in uremia (3,7,38), is responsible for the modification of ubiquitin to CIP.

Figure 3. Chemotactic movement toward N-formyl-leucyl-phenylalanine of polymorphonuclear leukocytes from healthy subjects in the presence of chemotaxis-inhibitory peptide (CIP) purified from a patient (1), commercially available ubiquitin (U), or after preincubation with and subsequent removal of CIP from a patient (1r). Mean ± SEM. *P < 0.05; **P < 0.01. Reprinted with permission from Cohen et al. (6).

LMWP Removal during High-Flux HD

Although not representative of all LMWP (39), β2M acts as surrogate for this class of molecules, mostly because of the large number of studies that have characterized its dialytic removal. On the basis of these studies (40–44), it is clear that the primary mechanism of β2M removal is membrane specific. For certain membranes, such as sulfonated polyacrylonitrile (AN69; Hospal, Lyon, France) and particularly polymethylmethacrylate, removal is achieved predominantly or solely by adsorption. Membrane composition and structure (pore size) influence this process both qualitatively (i.e., the specific plasma proteins adsorbed) and quantitatively (45,46). Although the adsorptive removal of LMWP is regarded generally as being beneficial, plasma protein adsorption in general may also influence dialyzer performance in unfavorable ways (47,48). A layer of proteins adsorbed nonspecifically (i.e., the secondary membrane) reduces a dialyzer’s effective water and solute permeability. In general, this layer of proteins is composed predominantly of proteins found in high concentration in the plasma, such as albumin and fibrinogen. This phenomenon may have a particularly significant effect in the convective therapies, as discussed below.

For most membranes used in the diffusive HD mode, however, LMWP are removed by a combination of diffusion and convection. Attempts have been made to quantify the individual contributions of these two processes during HD (49,50). These attempts, however, have been fraught with difficulty for several reasons, not the least of which is the inability to quantify the degree to which internal filtration (51) contributes to convective solute removal. Under normal operating conditions of high-flux HD, fluid flow within the dialyzer is characterized by (forward) filtration (blood to dialysate directionality) in the proximal part of the dialyzer and “backfiltration” (dialysate to blood directionality) in the distal portion of the dialyzer. Although concerning from the perspective that it may result in the transfer of bioactive dialysate contaminants to the bloodstream (52), this internal filtration mechanism is beneficial with respect to the removal of relatively large-sized uremic compounds. In fact, blood-side (decreased hollow fiber inner diameter) (53,54) and dialysate-side (flow path restriction) (55) modifications designed to exploit this internal filtration mechanism were proposed recently to enhance middle molecule removal.

Factors that Influence LMWP Removal in Hemofiltration and Hemodiafiltration

Because of the relatively low absolute ultrafiltration rate used in diffusive HD, plasma proteins that eventually comprise
the secondary membrane are transported to the dialyzer membrane surface relatively slowly. However, the transport rate of these proteins is much higher in the convective therapies, and, in general, secondary membrane effects are a more important consideration for the convective therapies than for diffusive HD. (The terms “gel layer” and “protein cake” are frequently used to refer to the secondary membrane.) This adsorbed layer effectively represents an additional mass transfer resistance that reduces the effective solute and water permeability of a high-flux dialyzer. In vitro data (56–58) indicate that both hydraulic permeability and large molecule sieving coefficients decrease substantially when highly permeable membranes are exposed to protein-containing fluids (relative to membranes exposed to non–protein-containing fluids).

Röckel et al. (59) investigated the effect of secondary membrane formation on LMWP sieving coefficient (SC) values during clinical hemodiafiltration with a high-flux polysulfone hemofilter. These investigators measured in vivo sieving coefficients for several LMWP ranging in molecular weight from 12 kD (β2,M) to 55 kD (prealbumin). Sieving coefficient determinations were made in the first 10 min (peak SC) and at treatment times of 20 and 180 min. For each LMWP, a significant decrease was observed between the peak SC and the 20-min value. This effect was directly proportional to protein molecular weight such that after 20 min of treatment, no measurable filtration of solutes of molecular weight >30 kD was evident.

As Kim (60) has shown, the removal of LMWP during convective treatments is also influenced greatly by the phenomenon of concentration polarization. This process relates to the accumulation of a rejected solute at the membrane surface during filtration. Note that concentration polarization is distinct from secondary membrane formation, as the former essentially represents the boundary layer effect for an individual protein, whereas the latter is a structural mass transfer resistance. This boundary layer reaches steady state when the rate at which a protein is convected toward the membrane surface from the bulk bloodstream region is equal to the rate at which it diffuses away back into the bulk flow, as recently discussed by Dr. Henderson himself (61) (Figure 4). The ultimate thickness of the polarized layer is determined by several factors, especially blood flow rate, ultrafiltration rate, hematocrit, plasma protein concentration, and the mode of substitution fluid administration.

For equivalent ultrafiltration rates up to 110 ml/min (with $Q_b = 300$ ml/min), several investigators have shown that postdilution hemodiafiltration is superior to predilution hemodiafiltration with respect to removal of LMWP in the 12- to 33-kD range (60,62,63). This benefit of the postdilution mode is explained by the thicker polarized boundary layer (relative to the predilution mode) and the associated high-concentration “submembranous” protein on which the convective forces act. Because the degree of polarization is directly proportional to the degree of rejection, the relative benefit of the postdilution mode generally increases as LMWP molecular weight increases. Unfortunately, these same considerations also apply to albumin removal. As such, from the perspective of large solute removal, postdilution hemodiafiltration requires a balance to be struck between optimized LMWP and minimized albumin losses.

### Summary and Conclusions

Dr. Henderson has spent much of his illustrious career investigating ways to extend the spectrum of solute removal in dialysis therapies, and he has had a primary focus on middle molecules. In this article, several aspects of the middle molecule uremic toxin class of LMWP have been discussed. I close by taking this opportunity to salute Dr. Henderson for the many seminal contributions he has made to the field of uremia therapy. In addition, I extend my sincere appreciation to him for being such a great mentor and friend during our tenure together at Baxter during the past seven years.

### References


22. Stenvinkel P, Lindholm B, Lonqviqst F, Katzarski K, Heim-


25. Sato K, Hirata Y, Imai T, Iwashina M, Marumo F: Character-


34. Haag-Weber M, Mai B, Horl WH: Isolation of a granulocyte-
inhibitory protein from ureamic patients with homology to β2-microglobulin. *Nephrol Dial Transplant* 9: 382–388, 1994


38. Mun KC, Golper TA: Impaired biological activity by erythro-

39. Leypooldt JK, Cheung AK: Characterization of molecular trans-