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Determinants of the serum concentrations of low molecular weight proteins in patients on maintenance hemodialysis

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Determinants of the serum concentrations of low molecular weight proteins in patients on maintenance hemodialysis. Factors influencing the serum concentrations of low molecular weight proteins (LMWP) during long-term hemodialysis were studied in 112 patients undergoing dialysis for an average of 61.1 months (range 1 to 243). These patients were treated with AN69, cellulose acetate, cuprophan or polysulfone membranes. The following proteins were measured in serum before and after a four hour dialysis session: cystatin C (CYST C), β_2-microglobulin (β_2m), Clara cell protein (CC16) and retinol-binding protein (RBP). Pre-dialysis levels of the four proteins were markedly elevated. In simple regression analysis, pre-dialysis serum concentrations of β_2m and CC16 weakly correlated with the duration of dialysis treatment, but these relations completely disappeared when a stepwise regression analysis was performed using as predictors age, sex, residual diuresis, body weight loss (BWL), duration of hemodialysis and the type or ultrafiltration coefficient (UFC) of the membranes. The only significant determinants which emerged from this analysis were the residual diuresis and age which negatively correlated with CYST C, β_2m and CC16 (residual diuresis only), and sex which influenced CYST C. During the dialysis session, the microproteins underwent changes that were related to their molecular radius, the membrane UFC and the BWL. After adjustment for the latter, high flux membranes (UFC $\geq$ 15 ml/h $\cdot$ m^2 $\cdot$ mm Hg) allowed up to 50% of CYST C and 25% of β_2m to be removed. No significant elimination of CC16 and RBP was evident. On the basis of these results, we estimated the effective pore radius of high flux membranes between 1.5 and 1.7 nm and that of low flux membranes as below 1.5 nm. In conclusion, we demonstrated a marked elevation of the pre-dialysis serum levels of four LMWP. These levels are determined, before dialysis, by residual diuresis, age and sex, and during the dialysis sessions, by the size of the protein, the BWL and the UFC of the membrane. The latter had no significant impact on the pre-dialysis serum levels of LMWP.

Low molecular weight proteins (LMWP) are readily eliminated from plasma by glomerular filtration followed by reabsorption and catabolism in proximal tubule cells. Their plasma level thus rises as renal function fails. These LMWP include a great variety of proteins with different biological functions. Most studies carried out in patients with chronic renal failure have focused on β_2-microglobulin (β_2m, molecular wt 11.8 kDa) identified as a major amyloidogenic component in long-term hemodialysis patients [1–3]. Other microproteins such as retinol-binding protein (RBP, molecular wt 21.2 kDa) [4, 5], cystatin C (CYST C, molecular wt 13.3 kDa) [6], α_1-microglobulin (molecular wt 33 kDa) [7] accumulate in the serum of uremic patients and might also contribute to some clinical disturbances in these patients.

The determinants of the plasma levels of LMWP in hemodialysis patients are not yet clearly identified. Studies conducted to date suggest that the residual renal function and the permeability of dialysis membrane are the main factors involved in the elimination of β_2m. The contribution of other factors such as age, duration of hemodialysis treatment, extrarenal catabolism, biocompatibilty of dialysis materials and the formation of amyloid deposits is still debated [8–16]. Whether the influence of these factors extends to other microproteins remains largely unknown. We have attempted to answer these questions by studying the behavior in serum of patients on maintenance dialysis of four LMWP: (1) cystatin C, a cystein-proteinase inhibitor secreted by all nucleated cells; (2) β_2m which is a subunit of class I HLA antigens; (3) protein 1 or Clara cell protein (CC16, molecular wt 15.8 kDa), an α-microprotein secreted at the surface of respiratory airways where it seems to play an immunosuppressive role [17–21]; and (4) RBP, the carrier protein of vitamin A in plasma. The results show that pre-dialysis serum levels of these four microproteins are elevated in hemodialyzed patients. These levels are independent of the duration of hemodialysis treatment or the type of membrane used but are determined only by patient-related factors (age, sex and residual diuresis).

Methods

Patients

One hundred and twelve patients (59 males and 53 females) with a mean age of 60.6 ± 13.0 (males) and 59.5 ± 14.4 (females) years on stable maintenance hemodialysis for an average of 61.1 months (range 1 to 243) were recruited from two dialysis units. The underlying causes of renal failure were chronic interstitial nephritis (N = 35), chronic glomerulonephritis (N = 21), polycystic kidney disease (N = 17), diabetes mellitus (N = 8), nephroangiosclerosis (N = 6), vasculitis (N = 6) and others (N = 19). Fifty-seven patients were anuric, 22 had a residual diuresis less than 500 ml/24 hr and 33 had a residual diuresis

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Table 1. Characteristics of dialyzers

Dialyzer	Manufacturer	Membrane	Surface area m^2	UFC *ml/hr · mm Hg*
H1210	Hospal	AN69	1	20
H1207	Hospal	AN69	0.7	18
S3000	Hospal	AN69	1.2	36
GA 4H	Gambro	Cuprophan	1.0	6.4
GFS Plus 12	Gambro	Cuprophan	1.3	6.8
GFS Plus 16	Gambro	Cuprophan	1.7	8.4
GA 6F 120H	Gambro	Cuprophan	1.3	3.8
DUO FLUX	CD Medical	Cellulose acetate	1.5	15
CA 130	Baxter	Cellulose acetate	1.3	6.1
CA 210	Baxter	Cellulose acetate	2.1	10
CORDIS 4000	Cordis	Cellulose acetate	1.4	4.5
RAPIDO BL643	Belco	Polysulfone	1.4	12.5
F 40	Fresenius	Polysulfone	0.7	20
F 50	Fresenius	Polysulfone	1.1	30

Abbreviation is UFC, ultrafiltration coefficient. Values are provided by the manufacturer.

higher than 500 ml/24 hr. None of these patients at the time of the study had evidence of nonrenal diseases likely to interfere with the microproteins under study, such as chronic liver or lung disease, malignancy or inflammatory disorders.

Dialyzer membranes

Four types of membranes were studied: AN69 ($N = 72$), cellulose acetate ($N = 9$), cuprophan ($N = 22$) and polysulfone ($N = 9$). The ultrafiltration coefficient (UFC), which can be regarded as an estimate of the permeability of the membrane, ranged from 3.2 to 30 ml/hr · m^2 · mm Hg (Table 1). All patients had been dialyzed with the same type of dialysis membrane from the onset of dialysis treatment or at least since twelve months, except seven patients who switched from low (cuprophan or cellulose acetate) to high flux (AN69, $N = 6$ or polysulfone, $N = 1$) membrane at least one month prior to the study (3 at 1 month and 4 between months 6 and 12). Each patient underwent three dialysis sessions of four hours duration per week. Reused dialyzers were sterilized with sodium hypochlorite [22]. The blood flow was 250 to 350 ml/min, and the mean body weight loss (BWL) during a dialysis session was 1.95 ± 0.98 kg.

Analytical methods

Blood samples were taken immediately before and after the dialysis session. Residual diuresis was assessed on a 24-hour urine sample obtained the day before dialysis. Creatinine in serum and urine was measured by the Jaffé's technique. The serum or urinary concentrations of the four proteins studied (Table 2 lists their characteristics) were determined by a sensitive immunoassay relying on the agglutination of latex particles (latex immunoassay) [23, 24]. The effective hydrodynamic or molecular radius (Mr) of the proteins was estimated by fractionating pooled sera from 10 hemodialysis patients on a Sephacryl S-200 column by fast protein liquid chromatography (Pharmacia-LKB Biotechnology, Uppsala, Sweden). The concentrations of the proteins in the eluted fractions were determined by latex immunoassay. As shown in Figure 1, CC16, CYST C and β_2m eluted as a single component with a molecular size corresponding to the free protein. RBP, however, eluted in two distinct peaks: a major peak containing about 80% of the protein and corresponding to the free unbound protein, and a smaller peak coeluting with albumin and composed of the transthyretin-bound RBP.

Statistical analysis

Results were expressed as the arithmetic mean ± SD (SEM in figures). Statistical tests were done by using the Statview SE software (Abacus Concepts, 1988). The determinants of the serum concentrations of the proteins before dialysis were traced by stepwise regression analysis using as the dependent variable the logarithm of the serum concentration of the protein, and as independent variables age, sex, residual diuresis, BWL, duration of hemodialysis treatment, UFC or type of dialysis membrane. The determinants of the variations of the protein concentration in serum during the dialysis sessions were identified by using as the dependent variable the logarithm of the protein serum concentration ratio after/before dialysis, and as predictors the BWL, the UFC or the type of dialysis membrane, and the number of utilizations of the dialyzers. Sex, UFC and residual diuresis were introduced in the model categorized as follows: female 0, male 1; UFC < 15, ≥ 15–20, > 20 ml/hr · m^2 · mm Hg and residual diuresis ≤ 50, > 50–250, > 250–500, > 500–750, > 750 ml/24 hr. Comparisons between the groups were performed by one-way analysis of variance followed by the Scheffé's multiple comparison test with $P < 0.05$ considered as statistically significant.

Results

Determinants of pre-dialysis serum levels

The pre- and post-dialysis serum concentrations of the four microproteins were markedly increased compared to the normal values (Table 2). In simple regression analysis, the concentrations of β_2m and CC16 were positively correlated with the duration of hemodialysis treatment (Fig. 2), but these relations completely disappeared when a stepwise regression analysis was performed using as predictors age, sex, residual diuresis, BWL, duration of hemodialysis and the type or UFC of the membranes (Fig. 2, Table 3). The stepwise regression analysis showed indeed that the pre-dialysis serum concentrations of CYST C, β_2m and CC16 were inversely related to the residual diuresis, the best correlated protein being β_2m, followed by CC16 and CYST C. It can be estimated that for each fraction of 250 ml/24 hr of preserved residual diuresis, the pre-dialysis serum levels of β_2m and CC16 decrease on an average by 15% and that of CYST C by 3%. The pre-dialysis serum levels of β_2m and CYST C also declined with advancing age, on the average by 7 and 9% per decade, respectively. A significant influence of sex was apparent for serum CYST C whose values were higher in men than in women. It is interesting to note that, after adjustment for other contributors, none of the proteins studied was significantly associated with the duration of hemodialysis treatment, as illustrated in Figure 2, or the type of dialysis membrane, even when anuric patients are considered separately (results not shown). No determinant of RBP in serum emerged from the stepwise regression analysis.

Table 2. Characteristics of the four microproteins studied and their concentrations in serum of patients before and after dialysis

Characteristics	Cystain C (CYST C)	β_2-microglobulin (β_2m)	Clara cell protein (CC16)	Retinol binding protein (RBP)
Origin	Nucleated cells	Nucleated cells	Respiratory tract	Liver
Function	Cystein proteinase inhibitor	HLA antigen class I	Immunosuppressive?	Transport of vitamin A
Molecular weight *kDa*	13.3	11.8	15.8	21.2
Molecular radius *nm*	1.51	1.6	1.9	1.75
Isoelectric point *pI*	9.30	5.4–5.7	4.7	4.4–4.8
Normal range in serum *mg/liter*	0.6–1.6	1–2	0.05–0.1	50–80
Pre-dialysis values *mg/liter*[a]	11.8 ± 3.0	38.9 ± 13.4	2.9 ± 1.7	166 ± 68
Post-dialysis values *mg/liter*[a]	8.3 ± 4.4[b]	37.0 ± 15.1[b]	3.3 ± 2.0[b]	192 ± 78[b]

[a] Data are mean ± SD.
[b] $P < 0.02$, compared to pre-dialysis values

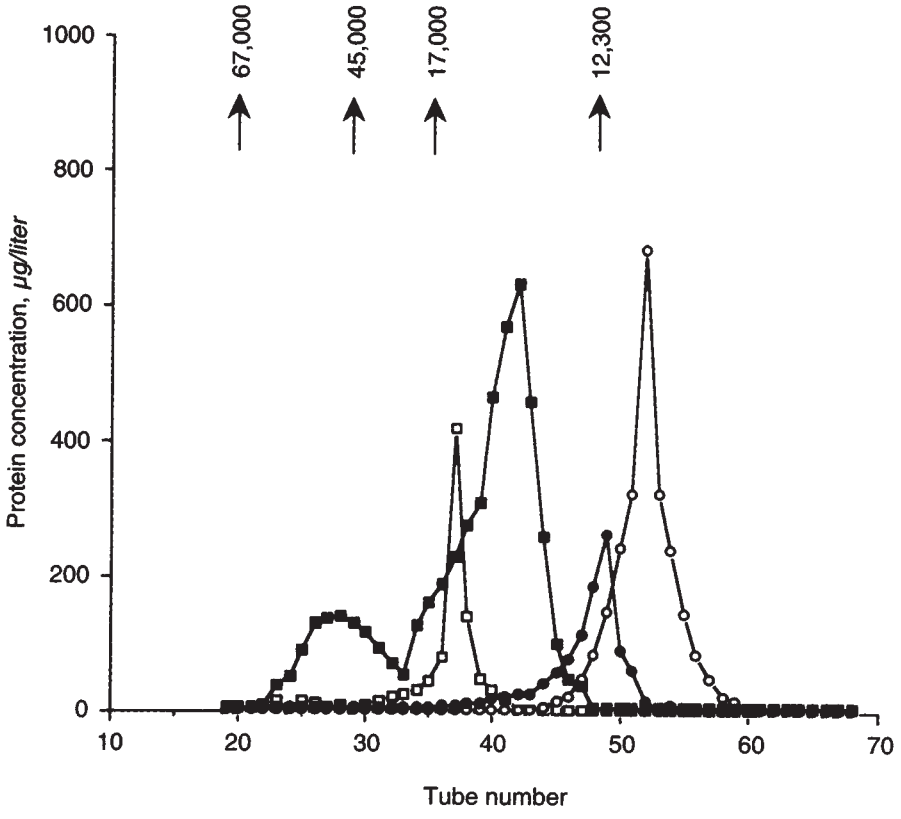

Fig. 1. *Distribution of Clara cell protein (□), cystatin C (○), β_2-microglobulin (●) and retinol-binding protein (■) in the fractions obtained by chromatographing a pooled specimen of serum from 10 hemodialyzed patients on Sephacryl S-200.* Arrows indicate markers of relative molecular mass.

Determinants of post-dialysis serum levels

Proteins examined separately. During the dialysis session, the serum concentrations of the four microproteins underwent changes that could be related to the UFC and the BWL (Table 4). The dialyzer's reuse and the type of dialysis membrane did not emerge as significant contributors of their serum concentration changes during dialysis. As expected, the elimination of the proteins during dialysis increased with the UFC and apparently decreased with the BWL, the latter being an index of the hemoconcentration.

Figure 3 shows the changes of protein concentrations in serum during dialysis by stratifying the dialyzers according to the UFC. This figure also compares two modes of correction for hemoconcentration, one based on the partial regression coefficient derived from the stepwise regression analysis (Table 4) and the other using the formula proposed for β_2m by Bergström and Wehle [25]. The post-dialysis serum levels of CYST C and β_2m were significantly decreased at UFC ≥ 15 ml/hr · m^2 · mm Hg. Below this threshold the values were significantly increased as a result of hemoconcentration. Post-dialysis serum levels of CC16 and RBP were significantly increased for all UFC categories. Adjustment for BWL abolished almost all post-dialysis elevations, and accentuated the decreases observed with CYST C and β_2m which reached 50 and 25%, respectively at UFC ≥ 15 ml/hr · m^2 · mm Hg. It is of interest to note that the adjustment for hemoconcentration was almost the same whether it was based on the partial regression coefficient or on the formula of Bergström and Wehle [25] (Fig. 3).

All proteins combined. When a stepwise regression analysis was performed by combining data obtained with all proteins and

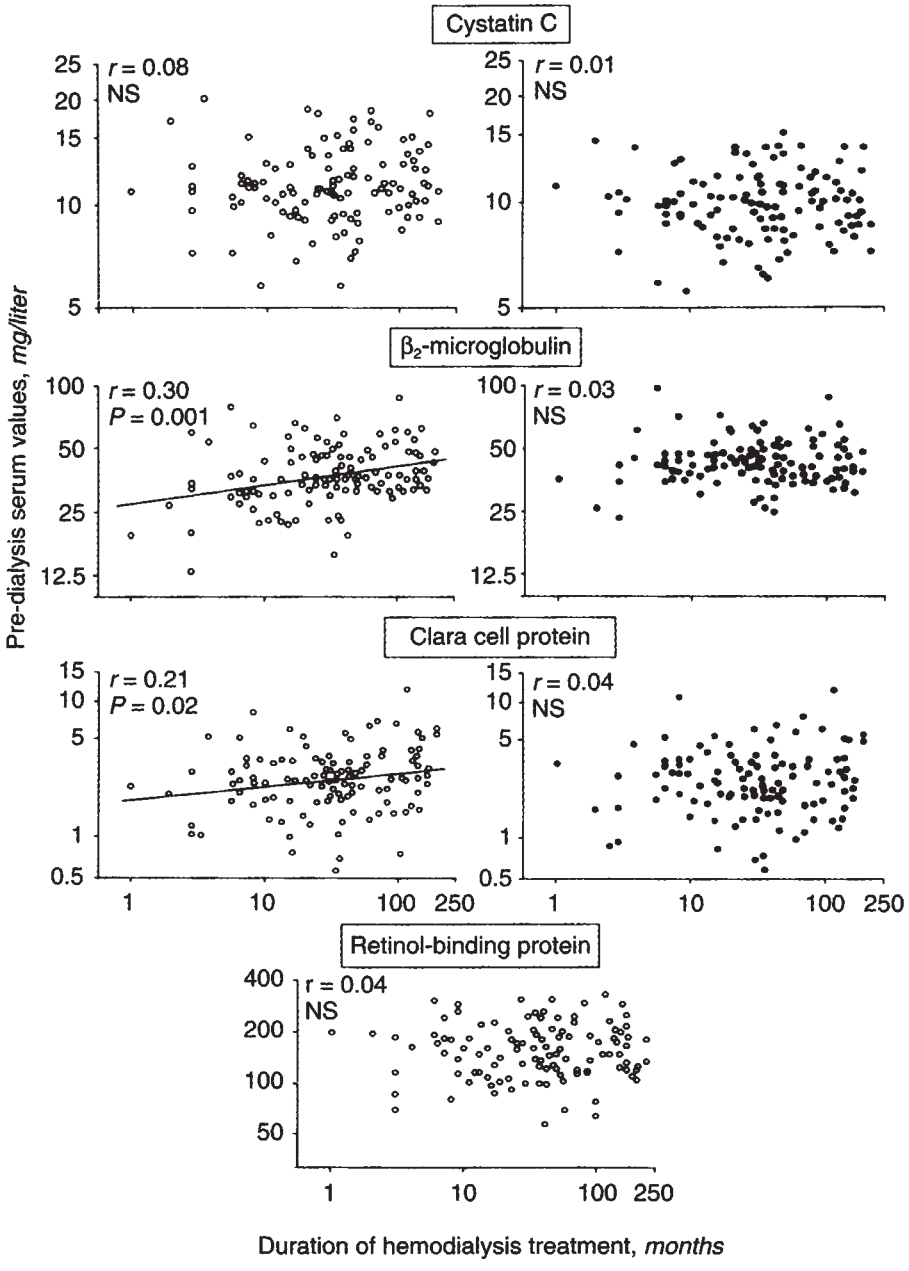

Fig. 2. *Pre-dialysis serum concentrations of cystatin C (CYST C), β_2-microglobulin (β_2m), Clara cell protein (CC16) and retinol-binding protein (RBP) as a function of duration of hemodialysis treatment.* Values are shown unadjusted (○) or adjusted (●) for residual diuresis (CYST C, β_2m, CC16), age (CYST C, β_2m) and sex (CYST C) on the basis of the partial regression coefficients. Data are mean ± SE.

introducing the Mr as independent variable, the latter emerged as the major contributor of their serum concentration changes during dialysis (partial r^2: 0.29, $P = 0.0001$), followed by the UFC (partial $r^2 = 0.11$, $P = 0.0001$) and the BWL (partial $r^2 = 0.03$, $P = 0.03$).

Figure 4 illustrates the influence of the UFC and the Mr on the BWL-adjusted changes of protein concentration in serum during the hemodialysis session. In both membrane categories with UFC values higher than 15 ml/hr · m^2 · mm Hg, protein removal clearly depended on the Mr. This figure also shows the changes of serum creatinine concentration for the three UFC categories. It appears that the high flux membranes (UFC > 15 ml/hr · m^2 · mm Hg) allowed an unrestricted passage of CYST C almost identical to that of creatinine. On the basis of these data, the effective pore radius could be estimated between 1.5 and 1.7 nm for high flux membranes and below 1.5 nm for low flux membranes.

Discussion

It is well established that the kidney plays a major role in the catabolism of small proteins [26, 27] and that renal failure results in a drastic elevation of their concentration in plasma [4–7, 28–30]. The fate of these proteins during long-term hemodialysis, in particular their time course as a function of treatment duration, is still unclear. We studied four microproteins with distinct functions and molecular features in patients undergoing chronic hemodialysis on different types of membranes. With a molecular weight ranging from 11.8 kDa (β_2m) to

Table 3. Determinants of the pre-dialysis serum levels of cystatin C (CYST C), β_2-microglobulin (β_2m), Clara cell protein (CC16) and retinol-binding protein (RBP) in patients on maintenance hemodialysis

Dependent variable	Independent variables	Partial r^2	Partial regression coefficient
Log CYST C	Age	0.19[b]	−0.0025
	Sex	0.05[a]	0.043
	Residual diuresis	0.07[a]	−0.019
	Membrane	0.0006	0.008
	Body weight loss	0.00013	−0.002
	Ultrafiltration coefficient	0.0014	−0.0006
	Hemodialysis duration	0.0005	−0.00005
Log β_2m	Age	0.13[b]	−0.004
	Sex	0.0043	0.012
	Residual diuresis	0.36[b]	−0.06
	Membrane	0.0004	0.0021
	Body weight loss	0.0025	0.0078
	Ultrafiltration coefficient	0.00001	0.00003
	Hemodialysis duration	0.00001	0.00001
Log CC16	Age	0.033	−0.004
	Sex	0.035	0.084
	Residual diuresis	0.13[b]	−0.055
	Membrane	0.005	0.08
	Body weight loss	0.001	−0.004
	Ultrafiltration coefficient	0.04	−0.009
	Hemodialysis duration	0.012	0.0005
Log RBP	Age	0.024	−0.002
	Sex	0.0001	−0.015
	Residual diuresis	0.01	−0.013
	Membrane	0.0001	0.009
	Body weight loss	0.043	0.035
	Ultrafiltration coefficient	0.0034	−0.002
	Hemodialysis duration	0.00001	−0.00001

[a] $P < 0.05$, [b] $P < 0.001$; otherwise statistically not significant.

Table 4. Factors influencing the changes of low molecular weight serum proteins during a dialysis session

Dependent variable	Independent variables	Partial r^2	Partial regression coefficient
Log CYST C[a/b]	UFC	0.45[d]	−0.20
	BWL	0.14[c]	0.031
	Membrane	0.01	0.06
	Dialyzer reuse	0.003	−0.01
Log β_2m[a/b]	UFC	0.34[d]	−0.09
	BWL	0.12[c]	0.024
	Membrane	0.008	0.001
	Dialyzer reuse	0.007	−0.0005
Log CC16[a/b]	UFC	0.08[c]	−0.04
	BWL	0.13[d]	0.031
	Membrane	0.03	−0.0006
	Dialyzer reuse	0.002	0.003
Log RBP[a/b]	UFC	0.06[c]	−0.026
	BWL	0.07[c]	0.021
	Membrane	0.02	−0.003
	Dialyzer reuse	0.02	−0.0045

Abbreviations are: UFC, ultrafiltration coefficient (ml/hr·m^2·mm Hg); BWL, body weight loss.

[a] and [b] post- and pre-dialysis value, respectively [c] $P < 0.05$, [d] $P < 0.001$; otherwise statistically not significant.

21 kDa (RBP) and a pI from 4.4 to 9.3, the four proteins studied can be considered as representative of the low molecular weight protein (LMWP) class. In addition, two of these proteins (CC16 and CYST C) appear to have biological activities [20, 21, 31, 32] which might contribute to some immune dysregulation or other disturbances in hemodialysis patients.

Compared to normal values, the pre-dialysis serum levels of RBP, CYST C, β_2m and CC16 were multiplied on the average by factors of 3, 20, 26 and 39, respectively. For the three last proteins, these relative increases correspond well to the elevation of the free intact protein which is the only form detected by our immunoassay. For RBP, by contrast, we could not specifically measure the free form of the protein and the values reported here correspond to the sum of the free plus the transthyretin-bound protein. However, since 80% of RBP in the serum of these patients occur as a free form (Fig. 1) and the concentration of free RBP in normal serum averages 6 mg/liter [5], it can be estimated that the concentration of free RBP is multiplied by a factor of about 20 which is comparable to that observed for the three other proteins. The elevation of RBP [4, 5], CYST C [6] and β_2m [28–30] in the serum of patients on long-term hemodialysis has already been reported in previous studies. The present study is the first to demonstrate a pronounced increase of CC16 in the serum of these patients. In view of the immunosuppressive and anti-inflammatory properties of this protei [17, 20–21], its role in the physiopathology of uremic syndrome certainly deserves further study.

The determinants of the pre-dialysis serum levels of these proteins during long-term hemodialysis have been examined by stepwise regression analysis using as possible predictors age, sex, residual diuresis, BWL, duration of hemodialysis treatment and the type or UFC of the dialysis membrane. Only the three first factors emerged as significant determinants for at least one of the measured proteins. The residual diuresis, which is used here as a surrogate of the residual renal function [33], is negatively correlated to the pre-dialysis values of CYST C, β_2m and CC16. These results are in agreement with those obtained by Brown et al [8] for β_2m and α_1-microglobulin. The contribution of residual diuresis to the elimination of these proteins is, however, variable: it is more important for β_2m to be followed by CC16 and CYST C and it exerts no influence on RBP. The reason for such a variation is unclear but one might conceive that the contribution of residual diuresis becomes significant only for those proteins which have limited possibilities of extra-renal elimination and therefore can reach sufficiently high concentrations in plasma. The fact that proteins significantly influenced by residual diuresis are precisely those with the highest relative increase in serum (β_2m and CC16) is consistent with this hypothesis.

Age appears as the second determinant of the pre-dialysis values of CYST C and β_2m which decrease with advancing age. This observation is of special interest because age is considered as a risk factor in the development of β_2m amyloid deposits in dialyzed patients [11, 15]. The decreased levels of β_2m in aged patients could reflect an enhanced removal of this protein from plasma as a result of its deposition in synovial tissues. This explanation might also apply to CYST C which could be present in synovial tissues in view of its fibrillar structure and ability to form amyloid deposits in the central nervous system [34–36]. Alternatively the synthesis of these LMWP might decrease with age but to date no evidence supports this hypothesis. Whatever their underlying mechanisms, the opposite effects of age on the serum levels of β_2m and on the propensity of hemodialyzed patients to develop amyloid deposits reinforce the hypothesis [12] that the elevation of β_2m in plasma is not the only factor

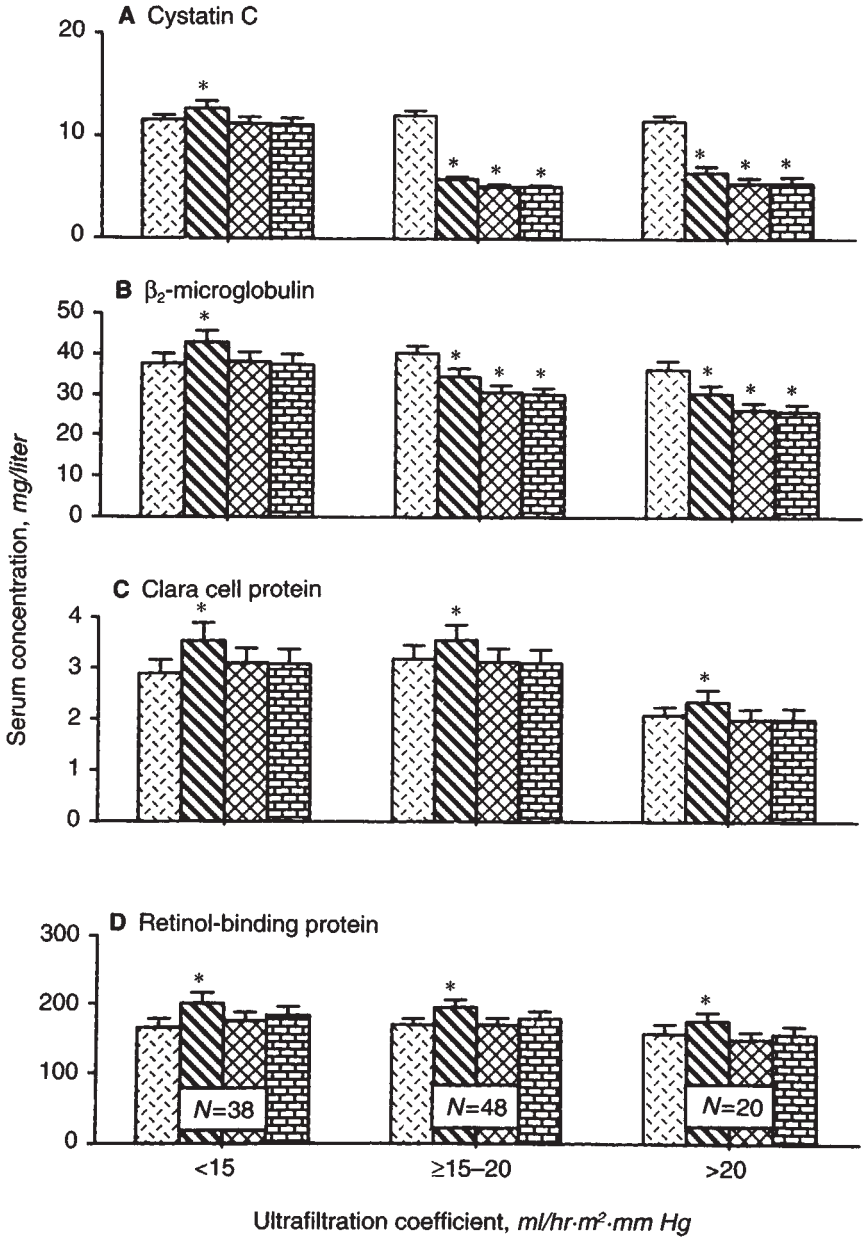

Fig. 3. *Pre-, post-dialysis and body weight loss adjusted serum values of cystatin C (CYST C), β_2-microglobulin (β_2m), Clara cell protein (CC16) and retinol-binding protein (RBP) as a function of the ultrafiltration coefficient (UFC).* Symbols are: (▨) pre-dialysis; (▧) crude values post-dialysis; (▩) post-dialysis adjusted for weight loss; (▤) post-dialysis values corrected according to [25]. Data are mean ± SE. * Significantly different from pre-dialysis values.

determining the development of β_2m amyloidosis in long-term hemodialyzed patients.

It is quite remarkable that neither the duration of hemodialysis therapy, nor the UFC or the type of dialysis membrane influenced the pre-dialysis values of LMWP and this even when only anuric patients are considered. These observations are at variance with those of Charra, Calemard and Laurent [13] who observed a negative correlation between plasma β_2m and duration of hemodialysis treatment. This discrepancy might arise from the fact that Charra et al [13] have not taken into account the influence of age. Our findings also stand in contrast with several reports claiming that patients treated with synthetic membranes have lower pre-dialysis levels of β_2m than those treated with cuprophan membranes [37, 38]. Again, these studies failed to take into account the effect of age and residual diuresis, factors whose importance is well highlighted by our analysis. Whatever the reality of this difference, it remains evident that the steady state levels of LMWP in the serum of patients on maintenance dialysis are markedly elevated and on average the same regardless the clearing capability of the dialysis membranes.

In contrast to determinants of the pre-dialysis levels of LMWP in patients on chronic hemodialysis, those governing their changes during the dialysis sessions are much more straightforward. For each LMWP examined the UFC and BWL emerge as significant determinants whereas the type, the surface area and the reuse of dialysis membrane exert no influence. For the latter, the explanation probably lies in the use of sodium hypochlorite which efficiently eliminates proteins adsorbed onto the dialyzer [39]. The use of a less effective cleaning agent might have led to a different conclusion. BWL results either in an increase of the serum levels of LMWP (such as low flux

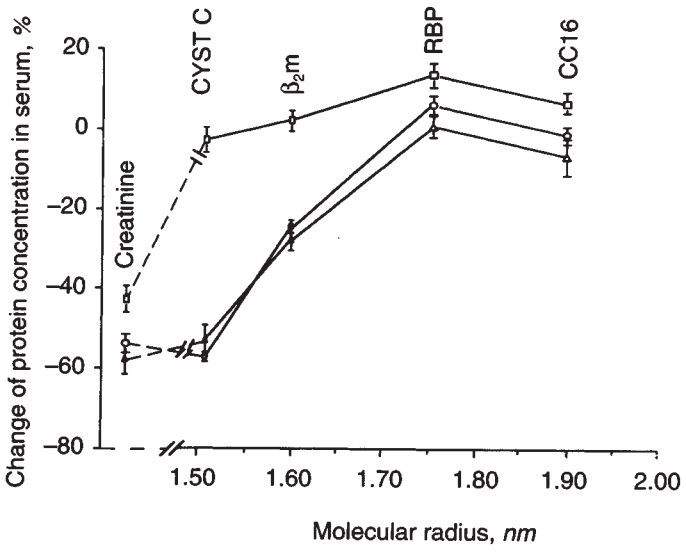

Fig. 4. *Influence of the ultrafiltration coefficient (UFC) and of the effective hydrodynamic radius or molecular radius (Mr) on the removal of low molecular weight plasma proteins during hemodialysis.* Symbols are: (□) UFC <15; (○) ≥15–20; (△) >20 ml/hr · m² · mm Hg. Values have been adjusted for body weight loss on the basis of the partial regression coefficient.

membranes) or an attenuation of their post-dialysis fall (high flux membranes). It is noteworthy that virtually identical adjustments for BWL are calculated for the four microproteins on the basis either of the partial regression coefficient or of the formula proposed by Bergström and Wehle [25]. The validity of the assumptions underlying the latter formula is thus corroborated. The fact that all proteins studied show a post-dialysis elevation of a comparable magnitude (between 10 and 25%) in low flux membranes (cuprophan, cellulose acetate and polysulfone) and that this rise disappears after adjustment for BWL argues strongly against the hypothesis of a stimulation of their synthesis as a predominant mechanism for their elevation during hemodialysis. This means that the enhanced synthesis or release of β_2m from circulating blood cells observed by some authors [40, 41] is insufficient to significantly increase the serum levels of β_2m during a four-hour dialysis session. As expected, the elimination rate of the proteins is related to the membrane permeability (UFC). For CYST C and β_2m, the influence of UFC is characterized by a threshold value around 15 ml/hr · m² · mm Hg above which the proteins can be eliminated. These results confirm the previous observations made with β_2m [9, 10, 42].

The availability of information on four simultaneously tested microproteins of various molecular size provides a unique opportunity to analyze the physical determinants of protein clearance by the membranes. When data obtained with the four microproteins are combined, the stepwise regression demonstrates that changes in serum protein concentration during dialysis are determined primarily by their Mr followed by the UFC of the membrane and the BWL. Knowing the Mr of the various proteins and their percentage change (normalized for BWL) during dialysis for each category of UFC, we were able to estimate the effective pore radius of the various membranes. As illustrated in Figure 4, the effective pore radius of high flux membranes lies apparently between 1.5 and 1.7 nm, whereas that of low flux membranes appears smaller than 1.5 nm. It is important to stress that these molecular radii are only apparent for they do not take into account the possible electrostatic interactions between microproteins and albumin or other plasma proteins adsorbed onto the dialyzer membranes. These proteins which are mostly negatively charged generate an electrical field which can hinder or facilitate the passage of microproteins depending on their charge. It is thus possible that the facilitated passage of CYST C (strongly cationic) compared to β_2m (weakly anionic) does not result only from the difference in molecular size between both proteins but also from the difference in molecular charge. The close correlation between the Mr and the pI ($r = 0.90$, $P < 0.0001$) of the studied proteins did not allow us to study the influence of the pI in the stepwise regression analysis.

The fact that pre-dialysis serum levels of LMWP show no correlation with the duration of hemodialysis treatment clearly points at factors other than residual diuresis and the dialysis clearance in the disposal of these microproteins in long-term hemodialyzed patients. The deposition in bone joints certainly plays a role for proteins that have a fibrillar configuration (β_2m and perhaps CYST C) [34–36], although the exact contribution of these deposits is difficult to quantify. With respect to RBP the liver appears as a probable site of catabolism. Experimental studies indicate that in normal rats liver can account for up to 30% of the catabolism of plasma RBP [43], and one can logically postulate that this contribution increases during renal failure. The removal of plasma RBP by the liver seems, however, to involve specific surface cell receptors, and it is unlikely that such receptors exist for all microproteins. The possibility of a feedback control in the synthesis of microproteins cannot be formally excluded, but seems improbable considering the diversity of their sources. These considerations lead us to postulate the existence of other routes for the disposal of LMWP in hemodialysis patients, especially in anuric patients, in addition to the amyloid deposits and liver uptake.

In conclusion, the variations of serum concentrations of low molecular weight proteins in long-term hemodialysis patients are determined, before dialysis, by residual diuresis, age and sex, and during the dialysis session, by the size of the protein, the ultrafiltration coefficient of the dialysis membrane and the body weight loss. The duration of hemodialysis treatment and the permeability of the membranes did not influence the pre-dialysis serum levels of microproteins.

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