Enhanced long-term reduction of plasma leptin concentrations by super-flux polysulfone dialysers

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Abstract

Background. Hyperleptinaemia in chronic haemodialysis (CHD) patients has been associated with malnutrition, which is an independent predictor of morbidity and mortality in this patient group.

Methods. To assess the influence of HD on plasma leptin, 10 CHD patients were crossover randomized to low-flux polysulfone (PS: F 6HPS), high-flux PS (F 60S), super-flux PS (F 500S) or super-flux cellulose-tri-acetate (CTA: Tricea 150G) for 12 weeks each. Blood samples were collected at the start of the study and each 12-week period. In addition, the relationship between patient characteristics, inflammation and leptin was analysed.

Results. At baseline, all groups showed similar leptin concentrations (mean 33.6±21.7 ng/ml). After a single HD session, a significant (P<0.01) decrease was observed with all three high permeable devices (Tricea 150G C0 52.7±6.4%; F 60S C0 63.1±5.7%; F 500S C0 68.7±8.2%), whereas leptin remained stable with low-flux PS. After 12 weeks, a marked increase was observed with low-flux PS (week 1, 30.4±23.0; week 12, 40.5±5.4 ng/ml, P=0.05), no change with super-flux CTA and high-flux PS (Tricea 150G week 1, 29.4±23.7; week 12, 32.0±27.9 ng/ml, P=ns; F 60S week 1, 36.0±31.8; week 12, 33.0±31.2 ng/ml, P=ns), and a significant decrease with super-flux PS (week 1, 38.3±33.0; week 12, 29.5±31.9 ng/ml, P=0.02). The change in leptin after 12 weeks was significantly different between super-flux PS, and both low-flux PS (P=0.009) and super-flux CTA (P=0.01). Besides interleukin-6 (IL-6) at the start of the study (P=0.006), no correlations were observed between patient characteristics, parameters of inflammation and plasma leptin levels.

Conclusions. Apart from low-flux PS, plasma leptin decreased considerably with all three high permeable dialysers after a single HD session. In the long run, leptin levels were lower with high-flux PS than with low-flux PS. Moreover, after switching from high-flux PS to super-flux PS (but not super-flux CTA), an additional decrease in leptin was observed. Apart from IL-6 at the start of the study, neither patient characteristics nor inflammatory parameters correlated with plasma leptin levels in this patient group.

Keywords: CRP; dialyser; haemodialysis; IL-6; leptin; polysulfone; super-flux

Introduction

Malnutrition is a major clinical problem in chronic haemodialysis (CHD) patients and a powerful independent predictor of morbidity and mortality in this population [1]. Various factors may contribute to the pathogenesis of malnutrition, including age, co-morbidity, dialysis vintage, inadequacy of dialysis therapy, uraemic toxicity and inflammation [2]. The identification of leptin (molecular weight 16 kDa), encoded by the ob gene [3], has markedly increased the understanding of the complex physiological system that regulates satiety and eating behaviour. A major action of leptin on the hypothalamus is to inhibit the production of neuropeptide Y, resulting in a decreased appetite and increased energy expenditure [4]. Recent studies have demonstrated that end-stage renal disease (ESRD) patients have inappropriately high leptin levels [5] and it has been speculated that leptin may be a uraemic toxin that mediates anorexia and weight loss, which are commonly observed in...
chronic HD (CHD) patients [6,7]. The pathophysiological mechanism of hyperleptinaemia in ESRD is not well understood. As the kidney eliminates a variety of polypeptide hormones, leptin might accumulate in ESRD due to reduced renal clearance. In humans, the kidney appeared to be a major site of leptin removal [5]. Moreover, an inverse correlation between leptin and glomerular filtration rate has been demonstrated in patients with various degrees of renal failure [8]. The important role of the kidney in leptin metabolism is further underscored by the fact that leptin levels are reduced after transplantation [9]. In line with the data in ESRD patients, elevated leptin levels have been described, although low values have been reported as well [10]. In this respect, various factors might play a role, including removal by splanchnic organs, gender, body fat mass and insulin levels [11,12]. Finally, it has been suggested that chronic inflammation plays an important role in the elevation of plasma leptin concentrations. Indeed, an association between pro-inflammatory cytokines and leptin synthesis in adipocytes has been demonstrated recently [13]. Therefore, it is tempting to speculate that the chronic micro-inflammatory state, commonly observed in ESRD patients, may contribute to the relatively high leptin levels in this patient group.

So far, the effect of HD on plasma leptin concentrations has only been investigated in a small number of studies, generally showing a marked intra-dialytical reduction during HD with high-flux devices [14,15]. Only one sequential analysis demonstrated a long-term benefit in eight CHD patients with exceptionally high leptin (> 70 mg/l) levels, who were treated with biocompatible high-flux dialysers [16]. In the present prospective randomized crossover study, we compared the effectiveness of four types of dialysers, differing in pore size and/or membrane material, on the elimination of plasma leptin, both during a single dialysis session and in the long-term, in 10 CHD patients.

**Subjects and methods**

**Patients**

Ten stable patients participated in the study after giving written informed consent. Patient characteristics are shown in Table 1. Causes of renal insufficiency were hypertension (n = 4), diabetic nephropathy (n = 1), polycystic kidney disease (n = 1), focal glomerulosclerosis (n = 1), chronic glomerulonephritis (n = 1), acute tubular necrosis (n = 1) and renal failure of unknown origin (n = 1). Exclusion criteria at the entry of the study were co-morbidity (malignancy, renal failure of unknown origin (glomerulonephritis (n = 1), acute tubular necrosis (n = 1) and renal failure of unknown origin (n = 1)). Exclusion criteria at the entry of the study were co-morbidity (malignancy, renal failure of unknown origin (glomerulonephritis (n = 1), acute tubular necrosis (n = 1) and renal failure of unknown origin (n = 1)).

**Design of the study**

Before starting the study, all patients were dialysed with high-flux polysulfone (PS) dialysers. Patients were randomized to HD with a low-flux PS (F 6HPS), a high-flux PS (F 60S), a super-flux PS (F 500S) or a super-flux cellulose-tri-acetate (CTA, Tricea 150G) dialyser for 12 consecutive weeks in a crossover study design. To exclude carry-over effects, a wash-out period of 4 weeks with the high-flux PS dialyser was instituted between each study period. The present study consisted of two parts. In the first part, blood samples were collected during a single dialysis session in the first week of each study period from the afferent line both before (t0) and after 3 h of HD (t180). In the second part, blood samples were drawn from the afferent line at t0, both at the start of the study and after 12 weeks. Samples were analysed for leptin, interleukin-6 (IL-6) and C-reactive protein.

**Analytical methods**

**Total leptin.** Plasma samples were stored at −25°C until required for testing. Total leptin concentrations were analysed with a commercially available enzyme-linked immuno-sorbent (ELISA) kit (Diagnostic Systems Laboratories, Inc., TX, USA) based on anti-human leptin antibodies. The lower limit of detection was 0.05 ng/ml. The intra- and inter-assay coefficients of variation were, respectively, 4.4% at a standard concentration of 4.9 ng/ml and 4.9% at a standard concentration of 4.7 ng/ml. The reference value was <16 ng/ml.

**IL-6.** IL-6 was determined in K3-EDTA plasma by a sandwich-type immunoassay, according to the manufacturers’ procedures (Central Laboratory of the Netherlands
Red Cross Blood Transfusion Service, Amsterdam, The Netherlands [17]. After centrifugation (10 min, 1500 g), the plasma samples were stored immediately at −70°C until required for testing. The lower limit of detection for IL-6 was 0.3 pg/ml. The IL-6 reference value of healthy individuals was below 10 pg/ml.

**C-reactive protein (CRP).** Serum CRP concentrations were determined by nephelometry (BN II, Dade Behring B.V., Leusden, The Netherlands). The lower limit of detection of CRP was 2 mg/l. CRP reference values for healthy individuals were below 5 mg/l.

**Correction for haematocrit (Ht).** Plasma leptin concentrations at t180 were corrected for changes in Ht: corrected value \( \text{value}_{180} = (\text{Ht}_{180}/\text{Ht}_{t180}) \times \text{value}_{t180} \).

**Body mass index (BMI).** The BMI was defined as the weight in kilograms divided by the square of the height in meters.

**Adequacy of dialysis.** Kt/V \( \text{urea} \) was measured using a urea monitor (Biostat, Baxter, Utrecht, The Netherlands). This measurement was performed by means of a membrane-bound urease and an ammonium-ion-selective electrode, which measures the ammonium generated by the reaction: urea \( \rightarrow \) (urease) ammonia (upper and lower detection levels urea: 1.0–28.5 mmol/l and precision 1.8%). Correction for the two-compartment model was performed by the formula of Daugirdas and Maduell: eKt/V = Kt/V \[ 1 - \left( 0.06/T \right) \] + 0.03.

**Endotoxin assay.** Dialysate samples for the determination of lipopolysaccharide (LPS) concentrations were collected at \( t_{180} \) in pyrogen-sterile FALCON 2063 polypropylene tubes (Becton Dickinson, Franklin Lakes, USA). LPS activity in dialysate was quantified by a kinetic chromogenic method based on the LAL-assay (Bio Whittaker, Wakersville, USA). Standard series of purified *Escherichia coli* 055:B5 LPS were made in the range 0.005–50.0 EU/ml. Inhibition and interference testing was performed on each sample by an endotoxin spike. To overcome inhibition/enhancement, all dialysate samples were diluted 10 times. All determinations were performed in duplicate and recoveries of spikes between 50 and 150% were accepted. Limit of determination was 0.05 EU/ml.

**Statistical analysis**

Data are expressed as mean ± SD, or median and range when appropriate. Analysis was performed with the Statistical Package for Social Sciences/PC+ software system using paired \( T \)-tests. Correlation coefficients were calculated with the Pearson method. Differences were considered statistically significant at \( P < 0.05 \).

**Results**

**Leptin, single dialysis session**

At baseline, while patients were maintained on high-flux PS dialysers, all groups showed similar leptin concentrations (mean 33.6 ± 21.7 ng/ml). A significant decrease was observed after HD with the super-flux CTA membrane (mean 29.4 ± 23.7, \( t_{180} \) 13.9 ± 11.1 mg/l, \( P < 0.01 \)), the high-flux PS (mean 36.0 ± 31.8, \( t_{180} \) 13.3 ± 11.6 mg/l, \( P < 0.01 \)) and the super-flux PS (mean 38.3 ± 33.0, \( t_{180} \) 12.0 ± 9.9 mg/l, \( P < 0.01 \)), whereas plasma leptin concentrations did not change after HD with the low-flux PS dialyser (mean 30.4 ± 23.0, \( t_{180} \) 28.1 ± 20.8 ng/ml). As shown in Figure 1, marked differences were observed between the low-flux PS dialyser and the other three modalities separately (F 6HPS −7.6 ± 2.8 vs Tricea 150G −52.7 ± 6.4%, \( P < 0.01 \); vs F 60S −63.1 ± 5.7%, \( P = 0.01 \); vs F 500S −68.7 ± 8.2%, \( P = 0.01 \)). After HD with the low-flux PS dialyser, a reduction to reference values for leptin (< 16 ng/ml) was observed in only one patient (10%), in contrast to 70% of the patients after HD with the other three modalities.

**Leptin, long-term**

After 12 weeks of HD with the low-flux PS dialyser, a significant increase in plasma leptin concentrations was observed (week 1, 30.4 ± 23.0; week 12, 40.5 ± 25.4 mg/ml, \( P = 0.05 \)). During HD with both the super-flux CTA and the high-flux PS modality, plasma leptin concentrations remained unchanged (Tricea 150G week 1, 29.4 ± 23.7; week 12, 32.0 ± 27.9 mg/ml; F 60S week 1, 36.0 ± 31.8; week 12, 33.0 ± 31.2 ng/ml). In contrast, during HD with the super-flux PS dialyser, plasma leptin concentrations decreased significantly (week 1, 36.0 ± 31.8; week 12, 29.5 ± 31.9 ng/ml, \( P = 0.02 \); see Figure 2). Significant differences were observed between the super-flux PS dialyser and both the low-flux PS as well as the super-flux CTA modality (F 500S −23.0 ± 2.8% vs F 6HPS +33.2 ± 4.6%, \( P = 0.01 \); F 500S −23.0 ± 2.8% vs Tricea 150G +8.8 ± 1.1%, \( P = 0.01 \)). In addition, a marked difference was observed between the high-flux and the low-flux PS dialyser (F 60S −8.3 ± 0.6% vs F 6HPS +33.2 ± 4.6%, \( P = 0.02 \).)
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Plasma leptin (% change)

Fig. 1. Change of plasma leptin (%) after one single dialysis session with all four modalities. A significant decrease was observed after HD with super-flux CTA ($P < 0.01$), high-flux PS ($P < 0.01$) and super-flux PS ($P < 0.01$), whereas plasma leptin concentrations did not change after HD with low-flux PS. Marked differences were observed between the low-flux PS dialyser and the other modalities separately.

Plasma leptin (ng/ml)

Fig. 2. Change of plasma leptin concentration (ng/ml) for each individual patient after long-term HD with all four modalities (black lines). As for the mean plasma concentrations (dotted lines), after 12 weeks of HD with low-flux PS (F 6HPS), a significant increase was observed ($P = 0.05$), whereas levels remained stable with super-flux CTA (Tricea 150G) and high-flux PS (F 60S). A marked decrease was observed with super-flux PS (F 500S; $P = 0.02$).
After 12 weeks of HD with the low-flux PS and the super-flux CTA dialysers, the percentages of patients with plasma leptin concentrations within the reference range was only 10 and 20%, respectively, whereas in case of high-flux and super-flux PS these percentages were 50 and 60%, respectively.

LPS content of dialysate

During the study period, mean LPS concentration in unfiltered dialysate was 0.051 ± 0.005 EU/ml [18].

IL-6

At baseline, mean values were within the reference range. IL-6 levels remained stable after 12 weeks of HD with all four modalities (F 6HPS week 1, 2.4 ± 0.9 pg/ml and week 12, 2.5 ± 1.1 pg/ml; F 60S week 1, 2.6 ± 0.9 pg/ml and week 12, 2.6 ± 1.0 pg/ml; F 500S week 1, 2.5 ± 1.3 pg/ml and week 12, 2.3 ± 1.2 pg/ml; Tricea 150G week 1, 2.8 ± 1.4 pg/ml and week 12, 3.0 ± 1.3 pg/ml). Marked fluctuations were not observed after 12 weeks of HD.

Relationship between patient-related characteristics, inflammation and plasma leptin concentrations

Significant correlations were not found between age, sex, BMI, time on dialysis, residual kidney function, dialysis dose (Kt/V) and plasma leptin concentrations. At the start of the study, a strong correlation was observed between IL-6 and leptin levels (r = 0.59, P = 0.006), whereas CRP and leptin concentrations were not significantly correlated. As mentioned before, neither IL-6 nor CRP changed over time in any of the four modalities. In contrast, during HD with super-flux PS plasma leptin concentrations decreased significantly. Consequently, any correlation between the change in plasma leptin and fluctuations in the two inflammatory parameters was absent (data not shown).

Discussion

The present study was designed to evaluate four types of dialysers, differing in pore size and/or membrane material, with respect to both intra-dialytical and long-term effects on plasma leptin concentrations in CHD patients. In contrast to low-flux PS, plasma leptin decreased considerably during a single HD session with high permeable materials, including high-flux PS, super-flux PS and high-flux CTA. As the molecular weights of leptin is ~16 kDa and low-flux PS has a cut-off point for the transfer of substances in the range 6–8 kDa, these results are in line with our expectations beforehand and previous uncontrolled observations [14,15]. As for the long term, a marked increase in plasma leptin was observed after 12 weeks of HD with low-flux PS, which was significantly different from both high-flux PS and the super-flux PS modality. Only super-flux PS was capable of reducing plasma leptin in the long-term in a group of patients who were dialysed routinely and during washout periods with high-flux devices. Therefore, it appears that plasma leptin levels in CHD patients are lower during long-term treatment with high-flux PS, when compared with low-flux PS. Moreover, our data suggest that after switching from high-flux PS to super-flux PS (but not super-flux CTA), an additional decrease in plasma leptin levels can be obtained. This material, having an UF-coefficient of > 60 ml/mmHg/h, has been designed to maximize convective transport by increasing the pressure drop along the fibres of the membrane [19]. Consequently, these characteristics allow the transfer of relatively large uraemic toxins across the membrane (Table 2). Of interest, apart from convective transport, adsorption of proteins to the internal pore structure of high permeable PS dialysers has been recognized recently as an important mechanism for the removal of relatively large uraemic toxins. As mentioned, plasma leptin decreased to a similar extent during a single HD session with both high-flux and super-flux dialysers, whereas in the long term super-flux PS was considerably more effective than high-flux PS and super-flux CTA. Therefore, it is intriguing to speculate that in the case of super-flux PS increased removal of unidentified uraemic toxins, which are involved in the metabolism of leptin, is responsible for these findings.

Recent findings suggest that leptin levels are influenced by inflammation. In animal studies elevated leptin levels appeared to be responsible for the anorexia that was observed during inflammatory conditions [20]. Data regarding the effect of inflammation on plasma leptin concentrations in humans, however, are not unanimous [8,13]. In healthy controls, the administration of cytokines, such as tumour necrosis factor α (TNFα) and IL-1α, led to an increase in plasma leptin levels [21]. In ESRD patients, both negative [22] and positive [6] correlations with inflammation have been described.

From a prior long-term sequential analysis on the influence of low-flux cellulose, low-flux PS and high-flux PS on plasma leptin levels in eight CHD patients with exceptional high leptin (>70 mg/l) levels [16], it appeared that high-flux PS reduced plasma leptin levels significantly over a period of 8 weeks, when compared with the two low-flux devices. Of note, in the latter analysis no correlations were found between TNFα and leptin concentrations, despite lower TNFα levels in both PS groups. In the present study a highly
significant correlation was found between plasma leptin concentrations and IL-6, but remarkably, only at the start. As mentioned, leptin levels decreased after 12 weeks of HD with super-flux devices, whereas parameters of inflammation did not. In a concurrent study [23], intercurrent clinical events were predictive of changes in both plasma IL-6 and CRP, whereas neither the bacteriological quality of the dialysate nor the type of dialyser had any influence on these parameters. Hence, our combined data suggest that in a group of patients with only mild inflammation (mean CRP: 6.0 ± 4.8 mg/l) baseline leptin levels can be modified by HD, depending on the flux characteristics of the dialysers used. Whether HD with high permeable dialysers can lower leptin values in more serious inflammatory states, warrants further investigation.

With respect to the present study, at baseline none of the patients had plasma leptin concentrations within the reference range, in contrast to 60% of the patients after 12 weeks of HD with super-flux PS. As elevated leptin concentrations have been associated with a low protein intake and loss of lean body mass in peritoneal dialysis [6] and HD patients [7], and positive correlations have been described in HD patients between leptin levels and markers of malnutrition, including hypo-albuminaemia [2] and the protein catabolic rate, our findings may have important consequences for clinical practice.

To summarize, with the exception of low-flux PS, plasma leptin was effectively reduced during a single HD session with high-flux PS, super-flux PS and super-flux CTA dialysers, suggesting enhanced convective removal. However, marked differences were observed in the long-term, super-flux PS being more effective than both high-flux PS and super-flux CTA, whereas an increase was observed with low-flux PD. Whether a decline in plasma leptin concentrations results in an improvement of the nutritional state and a reduced cardiovascular risk in CHD patients deserves further study.

Conflict of interest statement. None declared.

References


Received for publication: 22.7.03
Accepted in revised form: 17.12.03