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Elevated leptin fragments in renal failure correlate with BMI and haematopoiesis and are normalized by haemodialysis

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Summary

OBJECTIVE Leptin is an adipocyte hormone important in appetite, energy homeostasis, neuroendocrine and haematopoietic function. Patients with renal failure often have elevated total and free leptin levels. Biologically active leptin fragments (leptin$_{22–56}$ and leptin$_{57–92}$) have been identified, but whether these fragments are affected by renal failure and/or haemodialysis (HD) is not known.

RESEARCH METHODS Leptin, leptin$_{22–56}$ and leptin$_{57–92}$ levels were measured in 28 HD patients [14 men, 14 women, age 45·5 ± 16·4 years, body mass index (BMI) 23·8 ± 3·6 kg/m^2] and 25 healthy controls with similar age and BMI. In 18 HD patients, leptin and fragment levels were measured before and after 2 consecutive dialysis treatments and on the intermediate, dialysis-free day.

RESULTS Baseline leptin levels were higher in HD patients *vs.* controls (69·3 ± 62·2 ng/ml *vs.* 30·4 ± 32·7, $P < 0·02$) as were leptin$_{22–56}$ (7·14 ± 7·04 ng/ml *vs.* 1·86 ± 1·84, $P < 0·02$) and leptin$_{57–92}$ (5·94 ± 6·08 ng/ml *vs.* 1·58 ± 1·98, $P < 0·02$). Baseline leptin and fragment levels correlated significantly and independently with BMI in HD patients ($r = 0·70$, $r = 0·59$, $r = 0·72$, respectively, $P < 0·001$). After each HD session, leptin levels were reduced to levels not different from controls, but increased on the intermediate, dialysis-free day. Similar to and independently from total leptin, both leptin fragments were reduced after the first HD session to levels not different from controls. The reduction in leptin levels was higher with synthetic *vs.* cellulosic membrane types (37·7 ± 23·5% *vs.* 18·1 ± 21·8%, $P < 0·03$). Leptin correlated weakly with the erythropoietin to haematocrit ratio ($T = −0·20$, $P = 0·14$), while leptin$_{22–56}$ had a significant negative correlation with this index ($T = −0·42$, $P < 0·01$), suggesting that this fragment may favour haematopoiesis.

DISCUSSION Leptin fragments are detected in human serum, and both leptin and leptin fragments correlate with BMI, are significantly elevated in HD patients compared to controls, and are significantly decreased by haemodialysis. The elevated leptin fragments may have important physiological significance for the anorexia, hypogonadism, and anaemia commonly seen in HD patients, but this remains to be conclusively shown by interventional trials.

Leptin is a 16 kDa protein hormone secreted by adipocytes which plays an important role not only in regulating appetite and energy expenditure, but also in the function of other systems including neuroendocrine, haematopoietic and immune systems (Ahima *et al*., 1996; Lord *et al*., 1998; Mantzoros, 1999). Malnutrition occurs commonly in haemodialysis (HD) patients and has been linked to increased morbidity and mortality (Bergstrom, 1995). It has been hypothesized that elevated leptin levels may contribute to poor food intake and subsequent malnutrition, and patients with end-stage renal disease have been reported to have elevated total and free serum leptin levels (Heimburger *et al*., 1997; Howard *et al*., 1997; Merabet *et al*., 1997; Sharma *et al*., 1997; Nordfors *et al*., 1998; Widjaja *et al*., 2000) or elevated leptin to body fat mass ratios (Young *et al*., 1997; Nishikawa *et al*., 1999). Like other protein hormones elevated in renal failure, the mechanism for elevated leptin levels may be due to decreased clearance (consistent with the evidence that the kidneys can clear leptin from the circulation; Meyer *et al*., 1997; Sharma *et al*., 1997), increased leptin production, or the effect of immune and metabolic factors (Heimburger *et al*., 1997; Stenvinkel *et al*., 1997).

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Biologically active leptin fragments have been identified and found in animal studies to have both similar ($leptin_{22–56}$ and $leptin_{116–130}$) and/or different effects ($leptin_{116–130}$) as the native molecule on body weight (Samson *et al*., 1996) and other physiological functions (Gonzalez *et al*., 1999, 2000; Tena-Sempere *et al*., 2000). Because leptin fragments have not been directly measured in HD or renal failure patients before, it is not known whether they are elevated in renal failure similar to fragments of other hormones such as parathyroid hormone. Furthermore, given that leptin fragments may affect appetite, evaluating whether they are elevated in renal failure patients may provide insight into a potential mechanism for the cachexia of renal failure.

Materials and methods

Design

We measured total leptin levels and the leptin fragments $leptin_{22–56}$ (N-terminal end) and $leptin_{57–92}$ (mid-molecule) in 28 stable HD patients and compared their levels with controls matched for body mass index (BMI) and age. In 18 of these patients, we assessed the acute effect of HD on leptin and leptin fragments by measuring these levels before and after 2 consecutive dialysis treatments and on the intermediate, dialysis-free day.

Subjects

For the evaluation of leptin and leptin fragment levels in chronic renal failure, 28 stable HD patients (14 males and 14 females, age 45·5 ± 16·4 years old, BMI 23·8 ± 3·6 kg/m^2) and 25 healthy age- and BMI-matched controls were included. All HD patients were on a three times per week treatment programme for at least the previous 2 years. Exclusion criteria included: diabetes, malignancy, collagen vascular disease, recent infection or surgical procedures, acute cardiopulmonary incidents and use of corticosteroids, lipid-lowering drugs, or nonsteroidal anti-inflammatory agents. All patients were on a recommended diet of 1·2 g/kg/day of protein and on their usual medications as prescribed by the attending nephrologists. Primary renal disease was chronic glomerulonephritis (14 patients), chronic pyelonephritis (five), polycystic disease (three), analgesic nephropathy (one) and unknown (five). All patients had residual urine volume < 200 ml/48 h. Blood sampling was performed after an overnight fast (prior to initiation of the midweek dialysis session for the HD patients).

For the investigation of the acute effect of HD treatment on leptin and leptin fragment levels in 18 of the above patients (11 males and seven females, age 48·0 ± 17·5 years old, BMI 22·7 ± 2·8 kg/m^2), leptin, $leptin_{22–56}$ and $leptin_{57–92}$ were measured before and after 2 consecutive dialysis treatments and on the intermediate, dialysis-free day. All patients received 12 h/week of dialysis treatment.

Dialyser membranes used included: haemophane (11 patients), low flux polysulphone (two), PMMA (two), cuprophane (one), polyethylenoglycol (one), cellulose acetate (one), with membrane surface area ranging from 0·9 m^2 to 1·3 m^2 and ultrafiltration coefficient ranging from 3·4 ml/min/mmHg to 8·5 ml/min/mmHg. All sessions were carried out with 500 ml/min dialysate flow against a standard glucose-free bicarbonate dialysate, and no glucose-containing parenteral fluids were used for priming or during the session. Blood flow for all sessions was 250–300 ml/min, and none of the dialysers were reused. Venous blood samples were obtained immediately before the midweek dialysis, upon completion of the session, the next morning (20 h after the end of dialysis), and before and after the last dialysis of the same week. The post-dialysis samples were drawn with the low (50 ml/min for 2 min) technique (Depner, 1994). The two predialysis samples and the sample on the dialysis-free day were after an overnight fast. The two postdialysis samples were after a light, sugar-free meal that the patients took during the treatment session. Usual meals have no effect on serum leptin levels (Considine *et al*., 1996).

The ethics committee of Laiko Hospital approved the study. All patients and healthy controls were adults of Greek origin and consented to participate in the study.

Measurements

Leptin was measured by radioimmunoassay (RIA) using a commercially available kit (Linco Research, Inc., St Charles, MO, USA). The limit of sensitivity was 0·5 ng/ml, the intra-assay percentage coefficient of variation (CV) was 6–7%. $Leptin_{22–56}$ and $leptin_{57–92}$ were measured by RIA using a commercially available kit with specific antibodies directed towards the N-terminal and mid-portion of the leptin molecule, respectively (Phoenix Pharmaceuticals, Inc., Belmont, CA). For $leptin_{22–56}$ and $leptin_{57–92}$, the limit of detection was 0·1 ng/ml, with intra-assay %CV < 10%. Leptin, $leptin_{22–56}$ and $leptin_{57–92}$ values were corrected for extracellular volume differences as previously described (Bergstrom & Wehle, 1987).

To evaluate for possible interference of circulating leptin levels with the leptin fragment assay, concentrations of 0·20 ng/ml and 3·20 ng/ml of $leptin_{22–56}$ and $leptin_{57–92}$ were incubated with known concentrations of leptin (0, 2, 5, 10, 20, 50 and 100 ng/ml). There was evidence of some interference of leptin only at a level of 50 ng/ml and 100 ng/ml with $leptin_{22–56}$ at 0·20 ng/ml (~1·5 and five times higher, respectively), but no evidence of interference with total leptin levels when high concentrations (3·20 ng/ml) of $leptin_{22–56}$ or any concentration of $leptin_{57–92}$ were considered. Similar independent experiments performed by the manufacturer of the RIA using physiologic leptin concentrations (less than 50) gave similar results, showing 1% cross-reactivity of $leptin_{22–56}$ with total leptin but no cross-reactivity of $leptin_{57–92}$ with total leptin. In addition, immunoprecipitation experiments

were performed to assess whether the antibodies for leptin$_{22–56}$ and leptin$_{57–92}$ would cross-react with total leptin in solution and/or nonspecific endogenous proteins in serum. Highly purified rhLeptin$_{22–56}$, rhLeptin$_{57–92}$, rhLeptin (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA), or serum was incubated at ambient temperature for 30 min with rabbit antileptin$_{22–56}$ antibody or rabbit antileptin$_{57–92}$ antibody in the presence of bovine serum albumin (BSA; 0·5 mg/ml), 150 mM NaCl, 1 mM EDTA and 50 mM HEPES buffer at pH 7·4. The resulting immunoprecipitates were denatured using 0·1% sodium dodecyl sulphate (SDS) and 1·0 µl 2-mercaptoethanol, then separated by gel electrophoresis. Protein bands were viewed by staining with Coomassie blue.

Statistical analysis

All sets of values involved in the analysis were investigated for normality of distribution with or without logarithmic and square root transformation using the Shapiro-Wilk's test. Parametric or nonparametric comparison tests were used as appropriate. Correlations for leptin were investigated using Pearson's (R) or Kendal's Tau (T). To adjust for leptin, leptin$_{22–56}$ and leptin$_{57–92}$, correlation with the various parameters under investigation was sought using multiple regression or Kendal's partial Tau ($T_{partial}$). To investigate the acute effect of HD on total leptin changes, univariate repeated measures of logarithmic transformed leptin values ANCOVA with BMI as covariate was used. Univariate repeated measures of logarithmic-transformed values ANCOVA with the respective total leptin as moving covariate for each set of leptin$_{22–56}$ and leptin$_{57–92}$ evaluations was used to analyse the effect of dialysis treatment on leptin fragments. Multiple comparisons were performed with Scheffe's test. Level of statistical significance was ≥ 95% ($P \leq 0{\cdot}05$). Values are expressed as mean ± SD, with the median and range of values indicated when necessary.

Results

We first evaluated the specificity of the leptin fragment assays using immunoprecipitation experiments. Results demonstrate that only one band corresponding to a product of ~4 K_d (i.e. the expected molecular weight of each leptin fragment) was immunoprecipitated with the corresponding leptin fragment antibody (Fig. 1a). To assess for possible interference of total leptin with

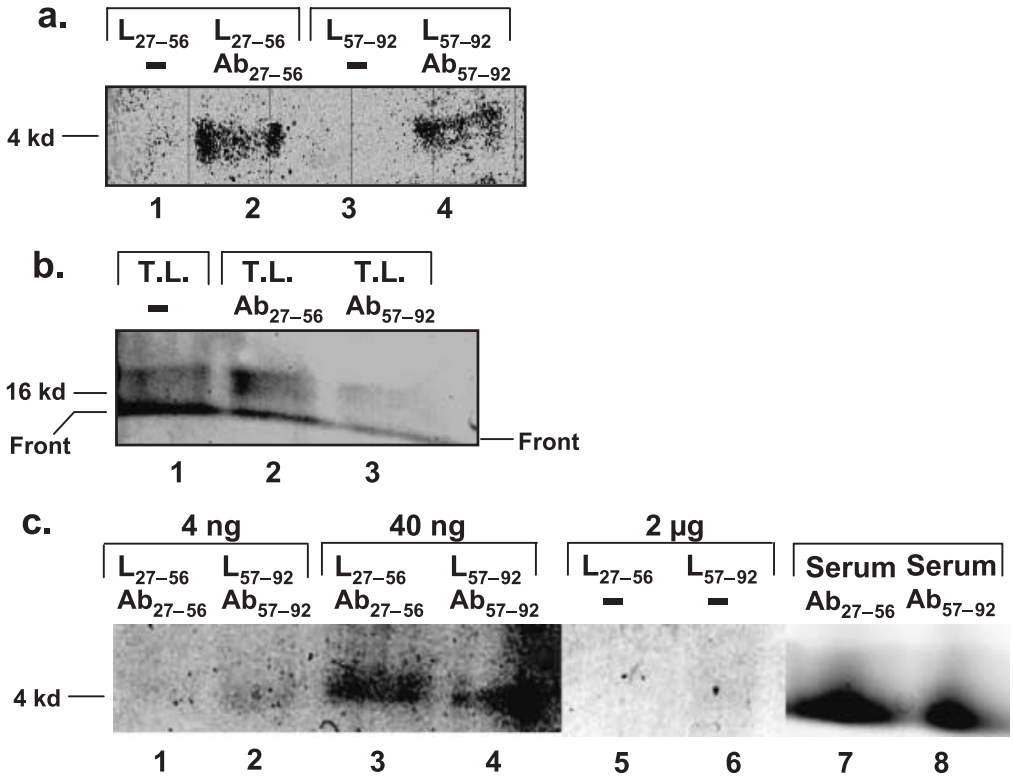

Fig. 1 Immunoprecipitation experiments for validation of the leptin$_{27–56}$ and leptin$_{57–92}$ fragment radioimmunoassays. (a) Lane 1, rhLeptin$_{27–56}$ (1 µg); lane 2, rhLeptin$_{27–56}$ (1 µg) and rabbit antileptin$_{27–56}$ antibody (Ab27–56); lane 3, rhLeptin$_{57–92}$ (1 µg); lane 4, rhLeptin$_{57–92}$ (1 µg) and rabbit antileptin$_{57–92}$ antibody (Ab$_{57–92}$). (b) Lane 1, rhLeptin (1 µg); lane 2, rhLeptin (1 µg) and Ab$_{27–56}$; lane 3, rhLeptin (1 µg) and Ab$_{57–92}$. (c) Lanes 1 and 2, 4 ng of each fragment with their respective antibody; lanes 3 and 4, 40 ng of each fragment with their respective antibody; lanes 5 and 6, 2 µg of each respective fragment alone (negative control); lane 7, serum and Ab$_{27–56}$; lane 8, serum and Ab$_{57–92}$.

Table 1 Clinical characteristics, leptin, $leptin_{22–56}$ and $leptin_{57–92}$ for controls and haemodialysis patients

	n	AGE (years) Mean ± SD	BMI (kg/m^2) Mean ± SD	Leptin (ng/ml) Mean ± SD (Median)	$Leptin_{22–56}$ (ng/ml) Mean ± SD (Median)	$Leptin_{57–92}$ (ng/ml) Mean ± SD (Median)
Controls	25	45·4 ± 13·1	24·0 ± 3·1	30·4 ± 32·7 (19·5)	1·86 ± 1·84 (1·52)	1·58 ± 1·98 (0·68)
Male	11	45·5 ± 9·2	24·9 ± 2·8	13·5 ± 8·5* (11·2)	1·21 ± 1·55† (0·42)	0·85 ± 0·85* (0·51)
Female	14	45·5 ± 15·0	23·2 ± 3·3	43·8 ± 38·4 (25·0)	2·41 ± 1·93 (2·23)	2·14 ± 2·42 (0·84)
HD patients	28	45·5 ± 16·4	23·8 ± 3·6	69·3 ± 62·2‡ (46·1)	7·14 ± 7·04‡ (4·04)	5·94 ± 6·08‡ (3·61)
Male	14	45·6 ± 17·6	23·8 ± 3·8	50·1 ± 49·1 (28·4)	5·16 ± 5·39‡ (3·11)	4·22 ± 4·97‡ (1·28)
Female	14	45·4 ± 15·2	23·9 ± 3·6	88·6 ± 69·5 (66·6)	9·11 ± 8·09‡ (6·59)	7·97 ± 6·86‡ (8·04)

*$P < 0·04$ *vs.* female controls, †$P < 0·07$ *vs.* female controls, ‡$P < 0·02$ *vs.* controls.

the leptin fragments, total leptin was incubated with each leptin fragment antibody. One band of ~30 kDa was observed in the negative control (total leptin alone without antibody) and thus is likely of minimal importance (Fig. 1b). An additional band of ~16 kDa is seen when total leptin is incubated with $antileptin_{27–56}$ antibody (lane 2 in Fig. 1b), indicating a small amount of cross-reactivity between total leptin and the $leptin_{22–56}$ fragment, but no cross-reactivity was observed between total leptin and $leptin_{57–92}$ (lane 3 in Fig. 1b). Importantly, there were no additional bands and thus no significant cross-reactivity of either leptin fragment antibody with nonspecific serum proteins when compared to a negative control containing neither of the respective antibodies (Fig. 1c). Similar results were obtained when these experiments were performed with concentrations of each leptin fragment ranging from levels similar to that measured in serum up to 1000 times higher (Fig. 1c and data not shown). These experiments were performed using concentrations of antibody 13 times higher than what is used in RIA, making the possibility of cross-reactivity using an RIA even more unlikely.

Table 1 shows the clinical characteristics (age and BMI), leptin, $leptin_{22–56}$ and $leptin_{57–92}$ levels for controls and HD patients. Mean age and BMI did not differ significantly between HD patients and controls, as well as after gender breakdown. Leptin levels of all HD patients were significantly higher than those of the controls (69·3 ± 62·2 ng/ml *vs.* 30·4 ± 32·7 ng/ml, $P < 0·001$, unpaired *t*-test performed after log transformation; Table 1). Male controls had significantly lower leptin compared to female controls ($P < 0·001$ unpaired *t*-test), but the difference of leptin between genders in the HD patients did not reach statistical significance (unpaired *t*-test performed after log transformation). Mean $leptin_{22–56}$ and $leptin_{57–92}$ levels were significantly higher for all HD patients compared to controls ($P < 0·01$ Wilcoxon/Mann–Whitney test), as well as after breakdown by gender ($P < 0·02$ unpaired *t*-test after log transformation except for $leptin_{22–56}$ between male controls *vs.* male patients where the Wilcoxon/Mann–Whitney test was used; Table 1). For the HD patients, neither $leptin_{22–56}$ nor $leptin_{57–92}$ differed significantly between genders ($P < 0·08$ and $P < 0·13$, respectively, Wilcoxon/Mann–Whitney test for $leptin_{22–56}$ and unpaired *t*-test after log transformation for $leptin_{57–92}$). Mean serum albumin for the HD patients was 3·9 ± 0·5 g/dl (range 3·0–4·5 g/dl), and mean haematocrit was 34·2 ± 4·7% (range 25·6–47·4).

Leptin exhibited a positive correlation with BMI for controls ($r = 0·40$, $P < 0·05$) and HD patients ($r = 0·70$, $P < 0·001$) as did $leptin_{57–92}$ for controls ($r = 0·46$, $P = 0·05$) and HD patients ($r = 0·72$, $P < 0·001$), whereas $leptin_{22–56}$ correlated with BMI only in HD patients ($r = 0·59$, $P < 0·001$). $Leptin_{22–56}$ and $leptin_{57–92}$ were significantly correlated with leptin in both the controls ($leptin_{22–56}$: T = 0·39, $P < 0·01$; $leptin_{57–92}$: $r = 0·78$, $P < 0·001$) and the HD patients ($leptin_{22–56}$: $r = 0·51$, $P < 0·01$; $leptin_{57–92}$: T = 0·61, $P < 0·001$). Leptin and leptin fragments were not significantly correlated with age.

Although the antibodies used for measuring leptin fragments are specific, there was evidence in the specificity experiments that leptin at a concentration of 50 ng/ml and 100 ng/ml had an effect to elevate the $leptin_{22–56}$ fragment level by approximately 1·5 and 3·5 times higher, respectively, than the actual value. Univariate analysis of subjects with high total leptin and low $leptin_{22–56}$ levels might have been inappropriately elevated. Thus, although we would expect that measurements of $leptin_{22–56}$ levels for only a minority of subjects (those with $leptin_{22–56}$ in the range of 0·20 ng/ml and total leptin higher than 50 ng/ml) would have been affected by circulating leptin levels, and given that variables of interest are inter-related, we used multiple regression models to assess whether the associations of interest are independent of BMI and total leptin levels. In multiple regression models with $leptin_{22–56}$ and $leptin_{57–92}$ as dependent variables and BMI and total leptin as independent variables, we found that $leptin_{57–92}$ (adjusted for leptin) correlated with BMI for controls ($r = 0·80$, $P < 0·001$) and HD patients (Kendal's $T_{partial} = 0·31$, $P < 0·05$) and that $leptin_{22–56}$ (adjusted for leptin) correlated with BMI for HD patients ($r = 0·61$, $P < 0·003$) but not for controls ($T_{partial} = 0·1$, $P > 0·2$).

Based on the above data, it is reasonable to propose that leptin fragments are related to the BMI of HD patients and that their levels may be elevated due to renal failure. To test this hypothesis, we assessed whether HD has an effect on leptin and leptin

Table 2 Leptin, leptin$_{22–56}$ and leptin$_{57–92}$ levels for the haemodialysis patients before and after two successive dialyses and on the intermediate day

	Leptin (ng/ml) Mean ± SD (Median)	Leptin$_{22–56}$ (ng/ml) Mean ± SD (Median)	Leptin$_{57–92}$ (ng/ml) Mean ± SD (Median)
Pre first session	65·0 ± 66·8* (33·1)	6·59 ± 6·02* (3·68)	5·27 ± 5·98* (3·17)
Post first session	41·3 ± 49·6† (17·2)	3·14 ± 2·56† (2·46)	2·57 ± 3·44† (0·74)
Dialysis-free day	67·7 ± 84·9*,† (31·4)	5·48 ± 6·46* (2·55)	5·13 ± 5·70*,† (3·32)
Pre second session	65·6 ± 70·7* (32·9)	5·18 ± 6·44* (3·61)	3·55 ± 4·07* (1·82)
Post second session	40·0 ± 43·4† (24·6)	4·29 ± 3·43* (3·61)	2·99 ± 3·31 (0·73)
Controls	30·4 ± 32·7 (19·5)	1·87 ± 1·80 (1·56)	1·50 ± 1·92 (0·68)

*$P < 0·05$ *vs.* controls, †$P < 0·05$ *vs.* preceding value.

fragments by measuring these levels before and after two consecutive HD treatments and on the intermediate dialysis-free day. It is evident that HD temporarily reduces leptin levels of HD patients to levels that are not different from those of the controls and that are significantly different from the pre-HD value (Table 2). Leptin values were reduced in 31/36 pre/postevaluations (15/18 after the first and 16/18 after the second treatment). Similar to total leptin levels, both leptin fragments are reduced to levels not different from those of controls after the first session of HD (and significantly different from the pre-HD value) and are increased back to levels higher than those of controls on the next morning (Table 2). Leptin$_{22–56}$ remained elevated during the dialysis-free day and did not change after the second dialysis session. Leptin$_{57–92}$ increased significantly from postdialysis to the dialysis-free day, remained elevated during this day, and after the second dialysis session, was again decreased to levels not different from controls. For both leptin and leptin fragment levels, the intradialysis changes were not correlated with the membrane surface area, ultrafiltration coefficient, volume of ultrafiltrate and urea reduction ratio.

Table 3 shows the relative leptin and leptin fragment changes by membrane type. Reduction of leptin with polysulphone (PSF) was highest, while the leptin levels remained essentially unchanged with cellulose acetate. No statistically significant differences were found between pairs of membranes probably due to the grossly unequal number of values (ANOVA: $0·05 < P < 0·08$). When we compared the grouped results of the cellulosic membranes *vs.* those of the grouped synthetics (PSF and PMMA), the difference was significant (cellulosic: 18·1 ± 21·8% *vs.* synthetic: 37·7 ± 23·5%, $P < 0·03$). The relative changes of leptin levels during HD treatment were not correlated with the volume of ultrafiltrate, membrane surface area, or ultrafiltration coefficient of the dialyser used during HD. For the leptin fragments, there were no statistically significant differences between pairs of membranes or between the grouped cellulosic *vs.* synthetic membrane types (leptin$_{22–56}$: −7·6 ± 97·2 *vs.* −22·9 ± 65·6, $P = 0·68$; leptin$_{57–92}$: 12·0 ± 99·4 *vs.* 12·0 ± 117·8, $P = 0·997$).

To investigate the possibility of an effect of leptin or its fractions on erythropoiesis, we used the ratio: erythropoietin (EPO) dose (/kg of BW/week)/haematocrit (i.e. EPO dose normalized by haematocrit). This index compensates for the fact that, in clinical practice, EPO dose is not independent of the haematocrit (or haemolgobin). The ratio increases during worsening of the erythropoiesis due to decrease of the haematocrit or increase of the weekly EPO dose or (usually) both. On the other hand, the ratio decreases on improvement of erythropoiesis because of increasing haematocrit and concomitant decrease of the exogenously required dose of EPO administration. Total leptin was found to have a weak, but not statistically significant, correlation with the EPO dose/haematocrit ratio ($T = −0·20$, $P = 0·14$). Of the two leptin fragments, leptin$_{22–56}$ was significantly negatively correlated to the above index ($T = −0·42$, $P < 0·01$; Fig. 2). When the analysis was performed to evaluate the correlation of leptin fragments with total weekly EPO dose (not adjusted for body weight or haematocrit), similar results were obtained with only leptin$_{22–56}$ showing a significant correlation with weekly EPO dose ($T = −0·46$, $P < 0·01$).

Table 3 Relative (%) changes in leptin, leptin$_{22–56}$, and leptin$_{57–92}$ levels by membrane type during haemodialysis

Membrane	Number of dialyses	%Leptin change (Mean ± SD)	%Leptin$_{22–56}$ change (Mean ± SD)	%Leptin$_{57–92}$ change (Mean ± SD)
Hemophane	22	−17·9 ± 22·7	+0·6 ± 107·7	+24·2 ± 108·0
Polysulphone	4	−50·8 ± 14·3	−23·2 ± 89·7	−67·7 ± 23·3
PMMA	4	−24·5 ± 25·1	−22·6 ± 44·6	+91·6 ± 122·2
Cuprophane	2	−22·1 ± 1·70	−39·2 ± 36·6	−39·7 ± 52·9
Polyethylen-glycol	2	−35·8 ± 17·4	−55·5 ± 27·2	−48·5 ± 37·2
Cellulose acetate	2	+1·3 ± 18·5	−18·1 ± 32·1	−12·0 ± 23·4
All membranes	36	−22·5 ± 23·4	−11·0 ± 90·5	−11·9 ± 102·0

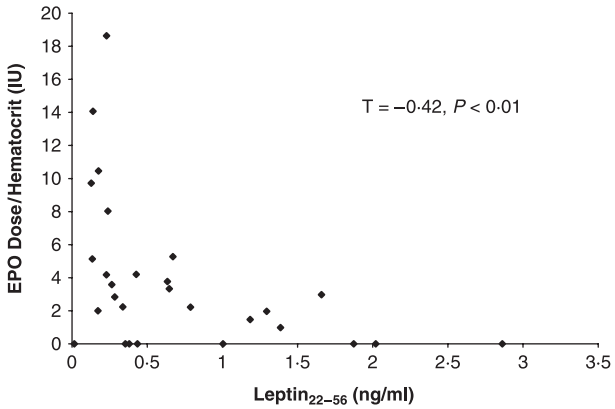

Fig. 2 Correlation of EPO dose/haematocrit index with $leptin_{22–56}$ levels (ng/ml).

Conclusions

Although previous studies have shown that leptin levels of HD patients are significantly elevated (Heimburger *et al*., 1997; Howard *et al*., 1997; Merabet *et al*., 1997; Sharma *et al*., 1997; Young *et al*., 1997; Nordfors *et al*., 1998; Nishikawa *et al*., 1999; Widjaja *et al*., 2000), no prior studies have investigated whether leptin fragments are detectable in human serum and what the effect of renal failure and haemodialysis on leptin fragment levels is. Thus, a novel finding in our study is that in addition to total leptin levels and similar to other hormones such as parathyroid hormone, leptin fragments in the N-terminal and mid-region of the leptin molecule ($leptin_{22–56}$ and $leptin_{57–92}$, respectively) are elevated in renal failure patients and exhibit a similar decline after haemodialysis. The C-terminal fragment has been studied *in vitro* by others as discussed below, but whether this fragment is elevated in renal failure and affected by haemodialysis could not be determined due to the lack of a specific antibody.

Elevated leptin levels in patients with renal failure may be related to suboptimal clearance of leptin by the kidneys, as suggested by impaired renal extraction of leptin with renal insufficiency (Sharma *et al*., 1997) and the evidence that renal leptin uptake could account for ~80% of all leptin removed from plasma (Meyer *et al*., 1997). Similarly, if renal leptin uptake and degradation results in complete digestion of the protein, renal failure would impair significantly the clearance of leptin fragments. Because it has been previously proposed that leptin is not only cleared but also degraded in the kidneys (Sharma *et al*., 1997), however, and because we have shown that leptin is not detectable in the urine of normal subjects in the fed or fasted state, i.e. a state of increased leptin clearance (data not shown), it is reasonable to hypothesize that leptin degradation in the kidneys would result in increased levels of leptin fragments, as shown herein. Leptin levels correlate significantly with the degree of residual renal function as assessed by serum creatinine, urea nitrogen, or creatinine clearance in some (Howard *et al*., 1997; Shoji *et al*., 1997) but not all previous studies (Heimburger *et al*., 1997; Merabet *et al*., 1997; Nakazono *et al*., 1998), and renal transplantation performed in renal failure patients led to a significant decrease in leptin levels (Landt *et al*., 1998). Other factors such as insulin and cytokine levels may also play an important role in the elevated leptin levels present in renal failure (Mantzoros, 1999; references therein).

Another important factor in determining leptin clearance appears to be the type of dialyser and dialysis membrane used, with the pore size being of particular importance. As expected from the molecular weight of leptin (16 kDa), no clearance of leptin occurred across modified cellulose membranes or low-flux membranes (Sharma *et al*., 1997; Nakazono *et al*., 1998; Wiesholzer *et al*., 1998). Synthetic PSF membranes seem to remove leptin more efficiently, with higher clearance rates seen in high-flux dialysers and membranes with larger pore size (Coyne *et al*., 1998; Nakazono *et al*., 1998; Wiesholzer *et al*., 1998). Our findings of the greatest clearance of leptin with synthetic membranes and minimal clearance with cellulose membranes are in agreement with these prior studies. However, clearance of leptin fragments does not appear to be affected by the membrane type, which could reflect different physicochemical properties of the fragments *vs.* the intact molecule.

Because leptin levels are elevated in patients with renal failure and leptin has been shown to have an anorectic effect, it is reasonable to hypothesize that leptin may contribute to the anorexia, malnutrition and weight loss commonly seen in HD patients. Patients with chronic renal failure have higher leptin to fat mass ratios, which correlates inversely with dietary protein intake and lean tissue mass (Young *et al*., 1997). Moreover, the leptin to fat mass ratio in HD patients showed a significant inverse correlation with weight change over a 17-month period, raising the possibility that higher leptin levels had induced weight loss (Odamaki *et al*., 1999). While the physiological significance of elevated leptin fragments in renal failure is not known, it is possible that they may play a role in modulating food intake, given their previously demonstrated biologic activity in rodents. The N-terminal fragment $leptin_{22–56}$ inhibited food intake in a significant and dose-related manner when administered into the lateral ventricles of rats, whereas the C-terminal fragment $leptin_{116–167}$ demonstrated only minimal inhibition of feeding (Samson *et al*., 1996).

Leptin fragments have also shown distinct effects on neuroendocrine function. *In vitro*, the C-terminal fragment $leptin_{116–130}$ inhibited gonadotropin release from rat pituitaries (Gonzalez *et al*., 2000; Tena-Sempere *et al*., 2000) in contrast to the stimulatory or neutral effect of native leptin (Yu *et al*., 1997; Tena-Sempere *et al*., 2000). However, similar to native leptin (Nagatani *et al*., 1998; Tena-Sempere *et al*., 1999), $leptin_{116–130}$ reversed the

suppression of prolactin and LH release that typically occurs during fasting in adult male rats (Gonzalez *et al*., 1999) and inhibited testosterone secretion from rat testis (Tena-Sempere *et al*., 2000). Sexual dysfunction is highly prevalent among male HD patients (Rodger *et al*., 1984), and potential contributing factors include elevated prolactin, LH and PTH levels and decreased testosterone levels (Bogicevic & Stefanovic, 1988). Thus, it is reasonable to speculate that elevated leptin and/or leptin fragments may interfere with the proper regulation of these neuroendocrine axes and thus contribute to the hypogonadism seen in HD patients, but this requires further study in future interventional studies.

Leptin's role in haematopoiesis was first proposed based on the structural similarity between the leptin receptor and cytokine receptors (Bennet *et al*., 1996; Cioffi *et al*., 1996). Bone marrow adipocytes express the ob gene, and leptin has a proliferative effect on haematopoietic stem cell populations, acting synergistically with EPO to increase erythropoiesis (Bennet *et al*., 1996). A significant negative correlation between the weekly EPO dose and leptin levels has been observed in a cross-sectional study of patients with chronic renal failure (Stenvinkel *et al*., 1998). In our study, leptin has a weak (but not significant) negative correlation with the EPO dose adjusted for haematocrit but leptin_{22-56} had a stronger and significant negative correlation with this index, suggesting a differential effect of this active leptin fragment. Moreover, the demonstration that EPO treatment has a positive effect on nutritional parameters in HD patients (Barany *et al*., 1991) raises the possibility that this effect may be related to compensatory leptin changes. In fact, treatment of HD patients with EPO led to a significant decline in serum leptin levels without a significant change in BMI compared to no change in leptin levels in patients not treated with EPO (Kokot *et al*., 1998).

In conclusion, this study demonstrates that not only leptin but also leptin fragments (leptin_{22-56} and leptin_{57-92}) are elevated in HD patients and correlate with BMI independently of leptin and that haemodialysis leads to an acute and significant decrease in leptin and leptin fragment levels. While observational data do not definitively prove whether the elevated levels of leptin fragments contribute to the anorexia, malnutrition and hypogonadism commonly seen in renal failure patients, these hypothesis-generating data are clearly suggestive given the previously demonstrated biological activity of leptin fragments on feeding and neuroendocrine function and may provide the basis for future interventional trials. Finally, the significant negative correlation between leptin_{22-56} and EPO index suggests that this leptin fragment may favour erythropoiesis, which could have significant clinical implications and deserves further study.

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