Leptinaemia in patients dialysed with different buffers and dialysis membranes

Sir,

Leptin is a 16 kDa protein, produced by OB gene, which is expressed and secreted by adipocytes [1]. It appears that the kidney is a major site of leptin clearance. It has been shown that both dialysed patients and kidney transplant recipients have elevated leptin levels [2,3]. Measurement of pre- and post-haemodialysis leptin levels failed to demonstrate a significant clearance of leptin during haemodialysis with a conventional cuprophone membrane [4], whereas Nakazono et al. [5] found that the polysulfone membrane dialysers removed serum leptin, while cellulose membrane dialysers did not. On the other hand, Coyne et al. [6] reported that high-flux dialysis lowered plasma leptin concentrations an average of 30%, but biocompatibility did not influence leptinaemia. So far there have been no data on the influence of dialysate buffer on leptinaemia in haemodialysed patients. Therefore, the aim of this study was to assess leptin in patients dialysed...
on acetate and bicarbonate buffers, as well as the effects of two kinds of membranes (polysulfone and cuprophane) on leptinemia in haemodialysed patients.

The study was performed on 53 clinically stable haemodialysed patients (mean age 55 ± 15, age range 24–84 years). The causes of renal failure in these patients were chronic glomerulonephritis (n = 21), chronic interstitial nephritis (n = 10), diabetic nephropathy (n = 8), polycystic kidney disease (n = 6) and other or unknown causes (n = 8). All the diabetics were treated with subcutaneous insulin.

Blood was drawn from patients in the morning between 8.00 and 9.00 am to avoid circadian variations [7] before the start of one dialysis session (and heparin administration) and after haemodialysis from the arterial line of haemodialysis system immediately before discontinuation of the extracorporeal circulation. Ultrafiltrate samples were also taken. All the patients had required regular haemodialysis for 4–5 h a day three times a week (on average 31 ± 31 months). Blood flow was usually 150–200 ml/min with a dialysate flow rate of 500 ml/min. Ultrafiltration was varied according to patient’s actual weight. Among all patients, 41 subjects were dialysed on polysulfone membranes (F resenius, Bad Homburg, Germany) and 12 on cuprophane membranes (Gambro n = 6, Nipro n = 4, Terumo n = 1 or Braun n = 1 dialysers). Ten patients were dialysed with acetate dialysates and 43 with bicarbonate dialysates. The patients’ height and weight were recorded (mean BMI 24.0 ± 3.8 kg/m²). All the patients were informed about the aim of the study and gave their consent.

The following parameters were assessed: haemoglobin, red blood cell count, total protein, albumin, cholesterol, triglycerides, urea (before and after HD), pH (before and after HD), bicarbonate levels (before and after HD), calcium, phosphorus, alkaline phosphatase, C-reactive protein by means of standard laboratory methods. Serum leptin concentrations, leptin levels in ultrafiltrate obtained from haemodialysed patients were measured with a commercially available radioimmunoassay (Linco Research, St Charles, MO, USA). This method detects 0.5 ng/ml leptin. TNFα and IL-1 concentrations were estimated by ELISA using kits from Endogen, USA.

Data are expressed as means ± SD and were compared using the Student’s t-test or the Mann–Whitney test as appropriate. P < 0.05 was considered to be statistically significant.

The major new finding of this study was that leptin levels (Figure 1) as well as TNFα concentrations (6.87 ± 3.19 pg/ml vs 9.08 ± 4.09 pg/ml, P < 0.05) are lower in patients dialysed with acetate buffer than with bicarbonate buffer, despite the fact that bicarbonate buffer is considered to be more physiological than acetate. Interleukin 1 levels were similar (27.59 ± 5.99 pg/ml acetate buffer, 28.38 ± 9.93 pg/ml bicarbonate buffer). In patients dialysed with acetate buffer, pH before and after HD was significantly lower than in patients dialysed with bicarbonate buffer (7.29 ± 0.05 vs 7.37 ± 0.05 before HD, P<0.01, 7.41 ± 0.03 vs 7.46 ± 0.03 after HD, P<0.001). Bicarbonate levels before and after HD were significantly lower in patients dialysed with acetate buffer when compared with patients dialysed with bicarbonate buffer (18.09 ± 2.13 mmol/l vs 22.79 ± 2.53 mmol/l before HD, P<0.001 and 21.24 ± 1.70 mmol/l vs 28.11 ± 2.05 mmol/l after HD, P<0.001). The reduction rate of serum leptin defined as serum concentrations of leptin before and after haemodialysis was, however, lower in patients dialysed with bicarbonate buffer than with acetate buffer (6.65 ± 18.45 ng/ml vs 2.09 ± 6.71 ng/ml, P<0.05). In all cases studied, leptin levels in ultrafiltrate were below the detection limit of 0.5 ng/ml. Both groups (with regard to both dialysate buffer and dialysers membrane) did not differ significantly regarding BMI, sex, duration of renal replacement therapy, erythropoietin requirements and serum erythropoietin levels, haemoglobin concentration, erythrocyte count, total protein, albumin, cholesterol, triglycerides, C-reactive protein, urea before and after haemodialysis. In patients dialysed with acetate buffer, calcium concentration was significantly lower (1.83 ± 0.17 mmol/l vs 2.04 ± 0.30 mmol/l, P<0.05), phosphorus level was slightly, but non-significantly higher (7.61 ± 2.27 mg/dl vs 6.33 ± 2.14 mg/dl, P=0.51). We could not find any significant differences between leptin levels in patients dialysed on cuprophane or polysulfone dialysers (Figure 1). When we compared the rate of reduction in serum leptin levels, we found that polysulfone membranes caused a significant reduction in leptin levels relative to cuprophane membranes. The area of dialysers have no effect on leptinemia and the rate of reduction in serum leptin levels.

Our finding that bicarbonate dialysis is associated with elevated leptin levels in haemodialysed patients may be of potential interest as patients treated by acetate dialysis have significantly lower serum concentrations of leptin before and after HD, pH elevated leptin levels in haemodialysed patients may be of potential interest as patients treated by acetate dialysis have significantly lower serum concentrations of leptin before and after HD, and after haemodialysis from the arterial line of haemodialysis system immediately before discontinuation of the extracorporeal circulation. Ultrafiltrate samples were also taken. All the patients had required regular haemodialysis for 4–5 h a day three times a week (on average 31 ± 31 months). Blood flow was usually 150–200 ml/min with a dialysate flow rate of 500 ml/min. Ultrafiltration was varied according to patient’s actual weight. Among all patients, 41 subjects were dialysed on polysulfone membranes (F resenius, Bad Homburg, Germany) and 12 on cuprophane membranes (Gambro n = 6, Nipro n = 4, Terumo n = 1 or Braun n = 1 dialysers). Ten patients were dialysed with acetate dialysates and 43 with bicarbonate dialysates. The patients’ height and weight were recorded (mean BMI 24.0 ± 3.8 kg/m²). All the patients were informed about the aim of the study and gave their consent.

The following parameters were assessed: haemoglobin, red blood cell count, total protein, albumin, cholesterol, triglycerides, urea (before and after HD), pH (before and after HD), bicarbonate levels (before and after HD), calcium, phosphorus, alkaline phosphatase, C-reactive protein by means of standard laboratory methods. Serum leptin concentrations, leptin levels in ultrafiltrate obtained from haemodialysed patients were measured with a commercially available radioimmunoassay (Linco Research, St Charles, MO, USA). This method detects 0.5 ng/ml leptin. TNFα and IL-1 concentrations were estimated by ELISA using kits from Endogen, USA.

Data are expressed as means ± SD and were compared using the Student’s t-test or the Mann–Whitney test as appropriate. P < 0.05 was considered to be statistically significant.

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Our finding that bicarbonate dialysis is associated with elevated leptin levels in haemodialysed patients may be of potential interest as patients treated by acetate dialysis have significantly lower pH levels and lower bicarbonate levels than those treated by bicarbonate dialysis both before and after a haemodialysis session. Recently, Teta et al. [8] reported that adipocytes (differentiated from confluent mouse 3T3-L1 cells) exposed to acid show a decrease in leptin secretion. In addition, acidotic remnant rats seem to exhibit lower leptin levels than their bicarbonate-treated counterpart. It suggests that, in vivo, a low pH may decrease leptin synthesis as we found in patients treated by acetate dialysis. Teta et al. [8] also conclude that correction of acidosis may exacerbate hyperleptinaemia [8]. Sharma et al. [4] reported that serum leptin was not removed by cellulose membranes. Nakazono et al. [6] reported that polysulfone membrane
dialysers (Fresenius) removed serum leptin, while the cellulose membrane dialysers (Asahi) did not. They did not measure leptin concentrations in ultrafilters. Wright et al. [9] showed a significantly lower leptin and CRP in patients dialysed with bioincompatible membranes (cuprophane, Gambro GFE) than on polyethylene glycol grafted cellulose membranes (Asahi Biowet). On the other hand, Hillon et al. [10] found a significant decrease in leptin during haemofiltration or haemodialfiltration with high-flux polyanamide membranes in spite of lack of leptin in the ultrafiltrate. This study is in general agreement with the report of Coyne et al. [6] that high-flux dialysers lowered leptin levels an average of 30%. We could not observe, in contrast to Coyne et al. [6], any significant effects of polysulfone dialysers on TNFα concentrations (polysulfone dialysers 8.51 ± 4.08 pg/ml, cuprophane dialysers 10.34 ± 4.47 pg/ml, \( P = 0.12 \)). Level of TNFα is known to be increased in haemodialysis patients exposed to cellulose dialysis membranes [11]. In our study we did not find any significant differences in TNFα concentrations upon the type of dialysers used (polysulfone or cuprophane). C-reactive protein was unrelated to the type of dialysers used. Our results suggest that not only the type of dialyzer but also a kind of dialysate buffer may affect leptinaemia in haemodialysed patients. However, further investigations, to clarify whether there are changes in synthesis or clearance of leptin by these more biocompatible membranes and more physiological dialysate buffers are required.

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