Glycyrrhetinic acid food supplementation lowers serum potassium concentration in chronic hemodialysis patients

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Hyperkalemia is a common life-threatening problem in hemodialysis patients. Because glycyrrhetinic acid (GA) inhibits the enzyme 11\(\beta\)-hydroxy-steroid dehydrogenase II and thereby increases cortisol availability to the colonic mineralocorticoid receptor, it has the potential to lower serum potassium concentrations. To test this, 10 patients in a 6 month prospective, double-blind, placebo-controlled crossover study were given cookies or bread rolls supplemented with glycyrrhetinic acid or placebo. Twenty-four-hour blood pressure measurements were performed at baseline and week 6 and 12 of each treatment period. The ratio of plasma cortisol/cortisone was significantly increased in all patients on GA as compared to baseline or placebo, indicating appropriate enzyme inhibition. Nine of the 10 patients had a persistent decrease in predialysis serum potassium concentration. On GA, mean predialysis serum potassium was significantly lower than at baseline or on placebo. On placebo, serum potassium was significantly elevated above the upper limit of normal in 76% compared to 30% of measurements during GA treatment. Furthermore, on this treatment the frequency of severe hyperkalemia significantly decreased from 9% to 0.6%. No differences were found in parameters reflecting sodium retention. Although these studies show that prolonged GA supplementation persistently lowers serum potassium in dialysis patients, a long-term toxicity study will be mandatory before we recommend the routine use of this treatment.

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Hyperkalemia is a common and sometimes life-threatening problem in patients with end-stage renal disease and is a frequent reason for emergency dialysis in patients undergoing chronic renal replacement therapy.\(^4\) Severe hyperkalemia (serum potassium > 6.0 mmol/l) was reported to occur in up to 12%\(^2\)-\(^4\) of hemodialysis patients. In these patients, three mechanisms account for hyperkalemia: an increase in potassium intake, a decreased potassium excretion and a shift of intracellular potassium to the extracellular fluid. Metabolic acidosis, the main mechanism for intra– extracellular potassium shift, is not a relevant cause of hyperkalemia at steady state in adequately dialysed patients. The efficacy of a dietary restriction of potassium intake as a measure to avoid hyperkalemia is often limited for nutritional and compliance reasons.\(^1\) In patients without renal function, the gastrointestinal excretion of potassium has a pivotal role.\(^3,5\) Thus, pharmacological interventions enhancing the gastrointestinal excretion of potassium are of potential relevance. The gastrointestinal mode of disposal of potassium is enhanced by cation exchange resins, together with a cathartic, usually sorbitol.\(^6\) The efficacy of the unpleasant resin therapy has been questioned\(^7\) and gastrointestinal side effects, including colonic necrosis, have been observed.\(^8,9\) Thus, alternative agents to enhance gastrointestinal potassium removal are warranted.

Potassium secretion is an established mechanism of rectal and colonic mucosa.\(^10,11\) This loss is regulated, at least in part, by the mineralocorticoid receptor (MR).\(^12\) The activation of the MR by fludrocortisone enhances the rectal electrical potential difference, an effect that is mimicked by inhibiting the enzyme 11\(\beta\)-hydroxysteroid dehydrogenase (11\(\beta\)-HSD2) in segments of normal rectal colon obtained from humans.\(^13\)

The 11\(\beta\)-HSD2 enzyme converts endogenous cortisol into cortisone in mineralocorticoid target tissues including epithelial cells of the colon.\(^14,15\) This mechanism protects the MR from promiscuous activation by cortisol (Figure 1).\(^16–18\) Therefore, we hypothesize that inhibition of 11\(\beta\)-HSD2 might enhance intestinal potassium loss. In a 2-week preliminary proof of principle study, we showed that inhibition of 11\(\beta\)-HSD2 by glycyrrhetinic acid (GA), the active ingredient

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of licorice, decreased serum potassium concentrations in patients on dialysis, an observation suggesting that this xenobiotic has the potential to be a potassium-lowering agent in dialysis patients. To determine the efficacy and safety of GA, we designed a trial with a 3-month treatment period and a 3-month placebo period.

RESULTS

Effect of GA on potassium concentrations in blood, urine, and dialysate and on the serum ratios of cortisol/cortisone and aldosterone/renin

Predialysis serum potassium concentrations were significantly lower during periods with GA ingestion. In Figure 2 the values obtained after 6 and 12 weeks on GA or placebo were combined and compared with baseline measurements. Mean observed serum potassium values were 5.5 ± 0.6 mmol/l during baseline, 4.4 ± 0.6 mmol/l (week 6) and 4.6 ± 0.6 mmol/l (week 12), whereas on GA and 5.4 ± 0.7 mmol/l (week 6) and 5.3 ± 0.7 mmol/l (week 12) during the placebo period. Predialysis potassium concentrations during the GA period were significantly lower when compared with the concentrations at baseline or during the placebo periods (P<0.01 for all). A combined linear model relating absolute individual differences to drug exposure and time revealed a significant difference in serum potassium concentrations between placebo and GA (−0.15 ± 0.6 mmol/l and −0.95 ± 0.45 mmol/l, respectively, P<0.001) as compared with baseline values.

Of all the serum potassium measurements obtained routinely before every dialysis session, 70% of the measurements were in the defined normal range of 3.5–4.7 mmol/l while patients were on GA, whereas only 24% were within these limits in the placebo period (P<0.001, Table 1). GA treatment induced a rapid (within days) and sustained decline in predialysis serum potassium concentrations (Figure 3). For the whole study period, mean dialysate potassium concentrations were higher on GA than on placebo (3.1 ± 0.4 mmol/l vs 2.8 ± 0.4 mmol/l, P<0.05, Figure 3).

From 4 out of the 10 patients who dropped out during the study at least one blood sample during the GA period was obtained. When the predialysis serum potassium concentrations of these samples were compared with those obtained at
baseline or during the placebo period, a uniform decline was observed in all patients while on GA (5.7 ± 0.4 mmol/l vs 4.5 ± 0.3 mmol/l, P < 0.01). Furthermore, no significant changes in blood pressure or weight gain occurred during the observation time (data shown below).

During the GA period, the ratio of plasma cortisol/cortisone increased in all patients and was significantly higher than at baseline or during the placebo phase of the trial (Table 2). When these values were plotted against the predialysis potassium concentrations given in Figure 2, a significant inverse correlation (P < 0.002) was found (Figure 4). The increase in the cortisol/cortisone ratio resulted from a uniform decrease in plasma cortisone in the GA period (Table 2). Plasma aldosterone concentrations and the aldosterone/renin ratios decreased significantly during GA phases when compared with baseline and placebo measurements (Table 2).

In an attempt to determine whether GA enhances urinary potassium excretion, 24 h urine samples were obtained from the day before dialysis at baseline and after 6 and 12 weeks on each treatment modality. As expected, these values showed a high variability among patients with some residual renal function (baseline 46 ± 39 mmol/24 h; GA 40 ± 27 mmol/24 h; placebo 43 ± 30 mmol/24 h). The variability of urinary sodium excretion was even more pronounced (baseline 97 ± 113 mmol/24 h; GA 158 ± 184 mmol/24 h; placebo 157 ± 133 mmol/24 h).

Table 2 | Laboratory values at baseline, during glycyrrhetinic acid (GA) and placebo treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline mean (± s.d.)</th>
<th>GA mean (± s.d.)</th>
<th>Placebo mean (± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/ml)</td>
<td>101 ± 25</td>
<td>116 ± 38</td>
<td>102 ± 56</td>
</tr>
<tr>
<td>Cortisone (ng/ml)</td>
<td>12.7 ± 3</td>
<td>8.0 ± 1.7**</td>
<td>11.2 ± 3.2</td>
</tr>
<tr>
<td>Ratio cortisol/cortisone</td>
<td>8.1 ± 2.1</td>
<td>14.3 ± 3.3***</td>
<td>9.7 ± 3.3**</td>
</tr>
<tr>
<td>Renin (ng/l)</td>
<td>18 ± 18</td>
<td>23 ± 14</td>
<td>27 ± 22</td>
</tr>
<tr>
<td>Aldosterone (pmol/l)</td>
<td>1030 ± 1539</td>
<td>356 ± 301**</td>
<td>689 ± 709*</td>
</tr>
<tr>
<td>Ratio aldosterone/renin</td>
<td>88.7 ± 67</td>
<td>18 ± 13**</td>
<td>37 ± 36*</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>136 ± 2.9</td>
<td>136 ± 3.8</td>
<td>137 ± 3.9</td>
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<tr>
<td>Bicarbonate (mmol/l)</td>
<td>23 ± 2.6</td>
<td>23 ± 1.4</td>
<td>23 ± 2.6</td>
</tr>
<tr>
<td>Urea reduction rate (%)</td>
<td>74.7 ± 4.3</td>
<td>75.8 ± 5.5</td>
<td>77.5 ± 4.3</td>
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<tr>
<td>Parathyroid hormone (pg/ml)</td>
<td>234 ± 177</td>
<td>219 ± 122</td>
<td>217 ± 174</td>
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<tr>
<td>1.25-OH-Vitamin D (pmol/l)</td>
<td>41 ± 15</td>
<td>36 ± 17</td>
<td>41 ± 20</td>
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<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.9 ± 1.4</td>
<td>5.5 ± 1.2</td>
<td>6.1 ± 1.8</td>
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<tr>
<td>Insulin (mmol/l)</td>
<td>16 ± 23</td>
<td>12 ± 16</td>
<td>18 ± 21</td>
</tr>
<tr>
<td>Homeostatic Model Assessment Index</td>
<td>4.6 ± 8</td>
<td>3.2 ± 5</td>
<td>5.2 ± 8</td>
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<tr>
<td>HbA1c (%)</td>
<td>4.8 ± 0.6</td>
<td>6.1 ± 0.9**</td>
<td>6.0 ± 0.8*</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.2 ± 0.9</td>
<td>3.8 ± 0.7*</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.6 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1</td>
<td>1.7 ± 1.1</td>
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<tr>
<td>Hemoglobin (g/l)</td>
<td>115 ± 5.7</td>
<td>116 ± 8.8</td>
<td>115 ± 11.3</td>
</tr>
<tr>
<td>White cells (G/l)</td>
<td>6.1 ± 1</td>
<td>6.9 ± 3</td>
<td>6.4 ± 1.4</td>
</tr>
<tr>
<td>Platelets (G/l)</td>
<td>221 ± 84</td>
<td>239 ± 89</td>
<td>220 ± 70</td>
</tr>
</tbody>
</table>

Values presented in the GA and placebo columns represent the mean of the two measurements at week 6 and 12 on each modality.

*P < 0.05 as compared with baseline measurements.

**P < 0.01 as compared with baseline measurements.

***P < 0.001 as compared with baseline measurements.

*P < 0.05 as compared with GA period.

**P < 0.01 as compared with GA period.
Side effects of GA

Mean pre- and post dialysis systolic/diastolic blood pressure values were comparable on GA and placebo (142/66 ± 14/11 mm Hg vs 142/65 ± 13/10 mm Hg and 137/66 ± 12/8 mm Hg vs 139/66 ± 14/9 mm Hg, respectively). Similarly, no differences were observed in the 24 h ambulatory systolic and diastolic blood pressure measurements (baseline: 132/80 ± 15/11 mm Hg; GA: 130/80 ± 14/13 mmHg; placebo: 128/77 ± 10/10 mm Hg). Mean 24 h ambulatory heart rate was also unaffected by the treatment modality (70 ± 13 vs 72 ± 14 vs 71 ± 14 b.p.m.).

Pre- and post dialysis weight as well as the interdialytic weight gain, were assessed at every treatment session. No significant differences were found in these parameters when the GA period was compared with the placebo period (predialysis weight: 76 ± 15 kg vs 76 ± 14 kg; interdialytic weight gain: 2.02 ± 0.63 kg vs 2.07 ± 0.55 kg, respectively).

Orally administered GA was well tolerated in all patients who concluded the trial. Reasons for dropout during the study were mostly GA-unrelated and are given in the Materials and Methods section. It is noteworthy that three patients (two on GA, one on placebo) terminated the study prematurely because of diarrhea (up to five bowel movements/day in one patient on GA).

Although the HOMA index for insulin sensitivity tended to be lower during GA treatment, HbA1c concentrations increased during the GA and placebo periods (Table 2). Total serum cholesterol concentrations were significantly reduced during GA administration (Table 2). A moderate increase in blood liver enzyme concentrations of ALAT and γ-GT was observed during the GA period (Table 2). Other serum and hematology parameters were not influenced by GA (Table 2).

DISCUSSION

We show here that the potassium-lowering effect of GA in patients with chronic renal failure persists for 3 months without inducing hypertension or other relevant side effects. In addition, GA appears to diminish the concentrations of aldosterone, a steroid considered to be relevant for myocardial fibrosis.

The mechanism underlying the uniform effect on serum potassium concentrations with a clinically significant reduction of hyperkalemic episodes deserves some pathophysiological considerations. Although a shift of potassium from the extracellular to the intracellular compartment might be a potential mechanism accounting for lowering serum potassium concentrations during a short-term intervention, as in our previous study, for mass balance reasons this mechanism cannot explain the present diminished plasma potassium levels lasting 3 months under steady-state conditions. Furthermore, the main driving forces of such a shift, that is, increased insulin concentrations or metabolic acidosis, were not observed when patients were on GA. The decline in serum potassium concentrations on GA is also not explained by changes in dietary potassium intake because: (i) predialytic and postdialytic weight did not change during the study and dialysis prescriptions remained constant, and (ii) predialytic parameters, including urea, phosphate, bicarbonate, and creatinine concentrations, were not affected by intake of GA. Thus, the most likely mechanism for the diminished plasma potassium concentration is enhanced extrarenal disposal. With respect to the anatomic site of potassium loss, we can only speculate. It is possible that GA enhanced rectal and colonic loss of potassium, most likely by activation of the MR by hydrocortisone as a consequence of the inhibition of 11β-HSD2 by GA. Alternatively, GA might have modulated potassium loss by sweat. The effect of GA on 11β-HSD2 was reflected by an increased ratio of cortisol/cortisone in all patients. Such an inhibition of the gate-keeper enzyme of the MR allows cortisol to bind to the MR and deploy a mineralocorticoid action, which causes renal sodium retention, volume overload and hypertension in humans with functioning kidneys. Therefore, in this study, special attention was paid to interdialytic weight gain and blood pressure assessed by 24 h ambulatory and in-clinic pre- and post dialysis recordings. Both body weight and blood pressure were unaffected by GA. The observed absence of hypertension is in line with previous studies where patients were treated with the MR agonist fludrocortisone. It is conceivable that an escape mechanism for sodium retention is operative in the colonic epithelial cells, similar to that observed in the kidney following prolonged administration and/or secretion of aldosterone.

Aldosterone and the serum aldosterone/renin ratio decreased on administration of GA (Table 2), an effect best explained by a diminished kalemia-related release of aldosterone. A moderate release of aldosterone is of potential clinical benefit as both the Randomized Aldactone Evaluation Study and the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study indicate that inhibition of aldosterone action by spironolactone or eplerenone is beneficial in humans with heart failure.
failure. Although the mechanism of this beneficial effect of aldosterone blockade in the Randomized Aldactone Evaluation Study and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study trials is still debated, whether it is due to some genomic or non-genomic effects of aldosterone, the concept that high aldosterone concentrations induce cardiac hypertrophy and fibrosis appears to be accepted knowledge. Thus, the present observation of a decline of aldosterone concentrations after GA dosing can be interpreted as a potentially beneficial effect. However, cardiac fibrosis and hypertrophy are observed when the MR is activated by aldosterone, and therefore cardiac fibrosis is of concern when the MR is activated by the glucocorticoid cortisol as a consequence of 11β-HSD2 inhibition (Figure 1). Because GA appears to show antioxidative properties in various tissues including the heart, and oxidative stress is a relevant mechanism in MR activation-induced fibrosis, a GA effect blunting the MR-induced fibrosis might exist. Although in our trial no cardiac side effects have been observed, the issue of structural heart damage by GA deserves further investigations.

Recent accumulating evidence from studies in animals and humans suggest a potential beneficial effect of 11β-HSD1 inhibition on the metabolic syndrome. Because GA not only inhibits 11β-HSD2, but also 11β-HSD1, the enzyme catalyzing the transformation from inactive cortisone into active cortisol, we investigated the effect of GA on glucose, HbA1c, insulin-sensitivity and blood lipids. Surprisingly, HbA1c increased slightly on both GA and placebo. The reason for this increase is unknown. The number of cookies (≈ 50 g/d) or bread rolls (≈ 30 g/d) consumed during both study periods appears to be insufficient to explain this increase. In line with previous investigations using a specific 11β-HSD1 inhibitor, insulin sensitivity (HOMA index) tended to improve while the patients were taking GA. This is probably best explained by a diminished intracellular ratio of biologically active 11β-hydroxylglucocorticoids to inactive 11-keto-steroids.

Previously, the mineralocorticoid hormone fludrocortisone has been given to lower plasma potassium concentrations in patients on hemodialysis. These studies were unblinded and comprised no placebo group. Kaisar compared prospectively patients with and without fludrocortisone for 3 months in an open-label trial and did not find a significantly lower midweek predialysis serum potassium concentration in patients on fludrocortisone. The dose of fludrocortisone given in the latter study was lower than that in the previous reports. Thus, the dose of fludrocortisone showing a potential clinically relevant effect appears to be unknown. If relatively high doses of fludrocortisone are required in the future, side effects might be anticipated. Fludrocortisone shows a 10 times higher glucocorticoid potency than cortisol. Thus, systemic glucocorticoid effects might appear especially in patients on dialysis because 80% of fludrocortisone is recovered in urine following an oral or intravenous dose in healthy normal volunteers. GA on the other hand deploys its mineralocorticoid effects specifically in MR-expressing target tissues, such as in the cortical collecting duct or in the colonic epithelial cells, because the MR-protecting enzyme 11β-HSD2 is selectively expressed in cells with MR. The rigorous control of the 11β-HSD2 gene has recently been elucidated by our group and is at least in part attributable to the presence of defined positive regulatory components and differential methylation of CpG islands in the promoter.

The moderate increase of the serum liver enzymes ASAT and γ-GT when on GA indicates liver toxicity. Interestingly, GA has been used in humans with hepatitis C infection and animals with different liver toxicities as a hepatoprotective agent. In this study, the increased liver enzymes returned to baseline values during the placebo period. Thus, a severe hepatotoxic effect of GA appears to be unlikely. Nevertheless, the effect of GA on liver enzymes in dialysis patients deserves further consideration in future studies.

In conclusion, the present investigation indicates that the long-term administration of GA to dialysis patients lowers predialysis potassium concentrations and reduces the frequency of dangerous hyperkalemic episodes without inducing weight gain or hypertension. Before GA can be recommended as a potassium-lowering agent, the pharmacokinetics and the chronic toxicity have to be evaluated formally in dialysis patients.

**MATERIALS AND METHODS**

**Study design and patients**

The study was approved by the Ethics Committee of the University of Berne, Switzerland and registered at www.clinicaltrial.gov (NCT 00384384). Based on an expected serum potassium concentration decrease of 0.6 ± 0.6 mmol/l during treatment with GA, a minimum number of 10 patients was estimated to provide a statistical power of 80%.

Twenty maintenance hemodialysis patients were randomly allocated by a concealed randomization procedure to start either with GA (n = 10) or placebo (n = 10) and enrolled in a prospective, placebo-controlled 6 months crossover study (Figure 5). All patients gave written informed consent. No patients with nephrotic syndrome or cholestasis were included. Exclusion criteria were age <18 years, dialysis vintage of less than 3 months, therapy with glucocorticoids, insulin-treated diabetes mellitus and changes in antihypertensive medication in the 2 weeks before inclusion. All patients were asked to keep their fluid and dietary potassium intake as constant as possible during the study period. Intake of licorice and flavonoids were not allowed. Dropout reasons during the study were: Death (one patient on placebo), kidney transplantation (one patient on placebo), diarrhea (two patients on GA, one on placebo), improvement of renal function with no further need of dialysis (one patient on GA), hospitalization for low back pain (one patient on placebo), withdrawal of consent (one patient on GA, one on placebo), relocation (one patient on placebo). Ten patients completed the study and were available for analysis. Patient characteristics are shown in Table 3.

Blood samples were obtained at baseline and at week 6 and 12 of the GA and the placebo period. All patients were dialyzed three times per week.
times weekly. Blood samples were drawn before dialysis after the long hemodialysis interval of 3 days and after 10 min supine rest. Standard laboratory methods were used to determine sodium, potassium, bicarbonate, creatinine, urea, liver enzymes, lipids, parathyroid hormone (PTH), renin (substrate), insulin, glycosylated hemoglobin (HbA1c), aldosterone, cortisol and cortisone at the time points mentioned above. Moreover, serum potassium concentrations were measured before each dialysis session with a ‘point of care’ AVL 988-4 device (AVL Medical Instruments, Schaffhausen, Switzerland).

Pre- and post dialysis body weight and blood pressure were recorded at every treatment session and oscillometric 24 h ambulatory BP measurements (Profilomat II, Disetronic, Burgdorf, Switzerland) were performed at baseline and week 6, 12, 18 and 24.

**Administration of GA and placebo**
Pharmaceutical quality 18-β-glycyrrhetinic acid dried powder (Vital-Chem, Zhuhai, PR China, GMP Certificate number F0012, Certificate of analysis/batch number JV050708) was administered orally with permission from the Swiss authorities (Swissmedic). According to the patients’ preference, either cookies or bread rolls were supplemented with 500 mg GA or placebo (500 mg dextrose) and given twice per day. Selection of this dose was based on the results of our previously published pilot study.19 The cookies and bread rolls were manufactured by our own hospital kitchen and baked at a temperature of 80 °C (GA remains stable up to 160 °C).

**Prescription of dialysis and antihypertensives**
Intraindividual dialysis prescription parameters, including duration of dialysis, blood flow, dialysate flow, conductivity and filter were kept constant during the whole study period. Changes in ultrafiltration rate and, if necessary, dialysate potassium concentrations were allowed by protocol and registered for analysis. All patients followed a conventional three times weekly dialysis schedule. Dialysis dose was measured as equilibrated Kt/V (Kt/V_e) and urea reduction rate (Table 2 and Table 3).

Prescription and dose alterations of ‘potassium-relevant’ drugs (antihypertensives and exchange resins) are shown in Table 4. Dosage of calcium channel blockers (given in 4 out of 10 patients) was not changed, with the exception of one patient (No. 2) who stopped 5 mg of amlodipine during the placebo period. The doses of furosemide, a drug known to inhibit 11β-HSD in high doses, was kept constant.52,53

**Analysis of plasma cortisol and cortisone by gas chromatography-mass spectrometry**
2.5 μg of medroxyprogesterone (Steraloids, Newport, RI, USA) was added to 0.75 ml of plasma as a recovery standard and the samples were extracted with 10 ml of methylene chloride (Merck KGaA,
Darmstadt, Germany) on a rotator for 20 min. After centrifugation at 3000 r.p.m. for 5 min, the two layers were separated and the organic layer was transferred into a new tube. Methylene chloride was evaporated under a stream of nitrogen at 45 °C. Stigmasterol (Steroids) was added as a standard for derivatization and chromatography (2.5 μg in methanol). The solvent was evaporated under nitrogen at 60 °C and the samples were derivatized to form methyloxime-trimethylsilyl ethers. 100 μl of methoxy amine hydrochloride 2% in pyridine (Pierce Biotechnology Rockford, IL, USA) was added and the extracts were heated for 1 h at 60 °C for the formation of methyloximes. Pyridine was evaporated under a stream of nitrogen at 60 °C, 100 μl of trimethylsilyl imidazole (Pierce Biotechnology) was added and the extracts were heated at 60 °C for 16 h to form the trimethylsilyl ethers. The derivatized steroids were purified by gel filtration on Lipidex 5000 (Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA) columns. The samples were analyzed by gas chromatography-mass spectrometry using a gas chromatograph 6890N (Agilent, Santa Clara, CA, USA) equipped with a mass selective detector 5973N (Agilent) by selected ion monitoring during a temperature-programmed run over 35 min. One characteristic ion was chosen for each compound analyzed (m/z 605 for cortisol, m/z 531 for cortisone, m/z 443 for medroxyprogesterone and m/z 394 for stigmasterol). Calibration lines in the range of 5 to 500 ng were established (correlation coefficient for cortisol was 0.9973, for cortisone 0.9992).

Statistical analysis
All results are given as means ± s.d., unless otherwise mentioned. Statistical analyses were performed with the software packages GraphPad 5 (GraphPad Software, La Jolla, CA, USA) and SAS 9.2 for Windows (The SAS Institute, Cary, NC, USA). Combined general linear models were used to relate absolute differences from baseline values of all the studied parameters to the time of observation (6, 12, 18 or 24 weeks), to the drug exposure, and to the patient studied. Student-Newman-Keuls tests were used for comparison of means. The same modeling approach was used to study changes in body weight, ultrafiltration, blood pressure and point of care measurements of potassium available from each dialysis session during the study. The interdialytic weight gain was calculated by subtracting the post dialysis patient weight of the previous dialysis from the actual predialysis weight. Additional statistical methods included Kruskal-Wallis, the two-sided Mann–Whitney U-test for nonparametric analyses and Spearman’s correlation.

DISCLOSURE
All the authors declared no competing interests.

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