Renal sodium handling in children with nephrotic relapse: relation to hypovolaemic symptoms

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Abstract We studied renal sodium handling during water diuresis in children in the early phase of relapse of minimal lesion nephrotic syndrome (MLNS). Findings were related to presence or absence of symptoms suggestive of hypovolaemia, and to neurohumoral factors, and were compared to results of similar studies in the same children in remission. Nine children (aged 7.8 ± 3.1 years) presented with hypovolaemic symptoms, and 10 (7.4 ± 4.3 years) without such symptoms. Both groups displayed severe proteinuria, hypoproteinaemia and oedema. Symptomatic patients showed tendency for a low glomerular filtration rate, and significantly impaired urine dilution, decreased fractional sodium and lithium excretions, and elevated diluting segment reabsorption \([C_{H_2O}/(C_{H_2O} + C_{Na})]\) and sodium/potassium exchange \([U_{K}/(U_{K} + U_{Na})]\). In the non-symptomatic patients these parameters were normal. Plasma renin and aldosterone were significantly elevated in the symptomatic children, and strongly correlated with all parameters of tubule sodium reabsorption. Weaker associations were found for plasma noradrenaline and atrial natriuretic peptide. Vasopressin was also relatively high in the symptomatic group, but showed no association with impaired urine dilution. The diffusely stimulated tubular sodium reabsorption in the symptomatic children, in association with stimulated neurohumoral factors, indicates that secondary sodium retention contributes to oedema formation in at least a subset of children developing a nephrotic relapse. This may be limited to the early stage, and be more pronounced in some patients than in others. The tubular defect responsible for maintenance of oedema in stabilized MLNS remains unclear.

Key words: nephrotic syndrome; renal sodium handling; children

Introduction

Volume retention is the major abnormality in the nephrotic syndrome, but the underlying mechanism is incompletely resolved. Primary renal excretion disturbance and secondary sodium retention due to hypovolaemia are two much debated mechanisms, and for both options convincing arguments are available. Indeed, rather than these two mechanisms being considered mutually exclusive, it is likely that both are involved, although to different extents in individual patients [1–3].

We have recently reported on a group of children presenting with early nephrotic syndrome [4], and suggested that a subset passes through a phase of clinically apparent hypovolaemia, whereas others enter directly into a steady state of hypoproteinaemia and oedema without experiencing hypovolaemic symptoms. A number of these children with nephrosis due to minimal lesions underwent sequential clearance studies during water diuresis, to define intrarenal sodium handling during the incipient nephrotic phase, and in remission. The results, which are reported here, permit distinction of children with clinical signs of hypovolaemia, stimulated vasoactive hormones, and avid tubular sodium reabsorption in various nephron segments, and children with stabilized oedema but apparently normal renal sodium handling.

Subjects and Methods

Patients

The data presented in this report concern 19 children (11 boys and eight girls, age range 2.2–13 years). They were largely selected out of participants in a previous study [4] on the basis of: (1) the presence of full-blown nephrosis during the relapse; and (2) the availability of measurements in relapse as well as in remission. All were known to have polyrelapsing steroid-sensitive nephrotic syndrome, due to biopsy-confirmed minimal lesions. No medication was taken at the time of relapse. During the measurements in remission,
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11 patients were still taking a low dosage of steroids. The protocol was approved by the Hospital Ethical Committee for Studies in Humans. Patients were included after obtaining their or their parents' informed consent.

Protocol

To obtain data in the early phase of relapse, participants were instructed to check urine for protein content at least twice weekly with dipsticks. They were urged to report to the outpatient ward when the dipsticks showed 3+ for three consecutive days, irrespective of the clinical condition. They were specifically instructed to continue the regular diet, without sodium restriction, until this admission. All had a plasma albumin of <25 g/l (selection criterion), and all had oedema. Volume excess was estimated from weight changes occurring during remission shortly after the relapse. The patients were divided according to presence or absence of hypovolaemic symptoms, the latter defined as (any combination of) tachycardia, peripheral vasoconstriction, oliguria, abdominal pain and diarrhoea. None were considered to have hypovolaemic symptoms, and 10 to have no such symptoms. Experiments were performed after hospital admission. Similar experiments were done in the same subjects during remission, i.e. ±4 months from the time of the relapse.

Urine voided after admission and before the tests was analysed for creatinine and albumin. Proteinuria was corrected for creatinine excretion (g/mmol creatinine). A level of 0.2 g/mmol creatinine corresponds with the accepted level for nephrotic proteinuria of 40 mg/h/m² [5]. The actual experiments were performed in the morning, within 24 h after admission. Lithium carbonate 300 mg/1.73 m² was administered orally on the previous evening. Between 09.00 and 10.00 h, after 1 h of recumbency, blood was sampled from an intravenous cannula, to measure concentrations of renin, aldosterone, noradrenaline, atrial natriuretic peptide, and albumin, and to measure colloid osmotic pressure. Next, erythrocyte and plasma volume were measured as, respectively, distribution volumes of 51Cr-labelled erythrocytes and 131I-albumin [6]. Blood volume was obtained by addition of erythrocyte and plasma volumes, and expressed per kg oedema-free weight.

Subsequently a water load was given (25 ml/kg) orally in about 30 min. Thirty min later a continuous infusion of insulin (10%), to measure glomerular filtration rate (GFR) and para-aminohippurate (PAH; 2.5%) to measure renal plasma flow (RPF) was started at a rate of 0.25 ml/min/m² after a priming dose of 0.5 ml/kg body weight. Following 45 min of equilibration, three 20-min urine collections were made. In this phase, maximal urine dilution was maintained by infusion of hypotonic saline (0.225% NaCl in 5% glucose) at a rate of 20 ml/min/1.73 m². Blood samples were taken halfway through each urine collection period for clearance measurements. Blood and urine samples were analysed for creatinine, PAH, osmolality, sodium, potassium and lithium. During the final urine collection period a blood sample was also taken for determination of plasma vasopressin.

The water diuresis protocol was adapted from the procedure described previously by others, in which the water load was followed by a 0.45% saline infusion [7]. We used a lower NaCl concentration (0.225%) to avoid NaCl expansion. The oral water load causes body fluid dilution of 4%. The hypothetical maximal further dilution obtained by the hypotonic infusion, i.e. if all fluid were retained, is ~2.25% in 1 h, which we considered a safe margin. In pilot studies, this protocol appeared superior to continued oral water loading, which often caused nausea and vomiting. Instead, our water diuresis procedure was tolerated well, and caused no nausea or unacceptable hypo-osmolality.

Analytical techniques

Sodium and potassium were measured by standard flame photometry, and lithium by means of a Perkin Elmer 3030 atomic absorption spectrophotometer. Osmolality was measured photometrically after hydrolysis to fructose [8], and PAH was determined photometrically by a chromoaldehyde reaction [9]. Radioimmuneassays were used to measure plasma concentrations of aldosterone, renin [10], atrial natriuretic peptide [11], and vasopressin [12]. Noradrenaline was estimated by high-performance liquid chromatography with fluorescence detection [13]. Colloid osmotic pressure was measured with a colloid osmometer built for 10 µl samples [14].

Calculations and statistics

Data are given as means±SD. Clearances and fractional excretions were calculated by standard formulae. To avoid inadequate urine dilution, averages of the final two urine samples were used occasionally. Statistical analysis of changes within groups was performed by paired Wilcoxon test. Differences between groups were assessed by means of the Mann–Whitney U-test. Correlations between plasma hormone concentrations and sodium handling parameters were assessed with Pearson's correlation matrix. For this assessment we used only the data obtained during relapse, since we were interested whether within this particular phase humoral factors play a role in the extreme renal sodium retention. By excluding the data obtained during remission from this evaluation, we avoided the effect that remission itself may have on this relationship, and the influence of the normal physiological relationship between these hormones and sodium excretion.

Results

Patients with hypovolaemic symptoms during relapse

Nine patients presented with hypovolaemic symptoms; eight of them were boys. Their mean age was 7.8 ± 3.1 years (range 3.5–12). Body weight during relapse was 25.8 ± 8.6 kg, and the estimated oedema averaged 9.8 ± 6.4% of body weight. All displayed heavy proteinuria, hypoalbuminaemia, and severely decreased plasma oncotic pressure. Average data are given in Table I. Blood volume, estimated from the addition of plasma and erythrocyte volumes, was not different from values obtained in the same subjects during remission.

Compared to data in remission, GFR and filtration fraction were low during relapse, but the differences just failed to reach significance (Table 2). The RPF was not altered. Sodium excretion and FEK, were significantly decreased. Indices for proximal tubule sodium reabsorption, maximal fractional water excretion and FE Li were decreased. Average plasma lithium
concentrations obtained in this group were also higher during nephrosis (0.220 ± 0.102 mmol/l) than in remission (0.150 ± 0.075 mmol/l, P < 0.05). Minimal urine osmolality was relatively high, as were the indices of distal sodium reabsorption [C H2O/(C H2O + C Na ) and UK/(U K + UNa)].

These patients displayed significantly elevated levels of plasma renin and aldosterone compared to the findings in remission (Table 3). Plasma potassium concentrations during nephrosis (4.8 ± 1.2 mmol/l) were somewhat but not significantly higher than during remission (4.4 ± 0.6 mmol/l). Plasma vasopressin and noradrenaline were also high, but not significantly elevated, whereas plasma ANP tended to be low.

Patients without hypovolaemic symptoms during relapse

Three of the 10 patients presenting without hypovolaemic symptoms were boys. The mean age of this group was 7.4 ± 4.3 years (range 2.2–13). Body weight during relapse was 27.4 ± 15.1 kg, and the estimated oedema averaged 8.7 ± 4.3% of body weight. All patients had heavy proteinuria and hypoalbuminaemia, and severely decreased plasma oncotnic pressure (Table 1 gives average values). Blood volume tended to be low, but the difference with blood volume in remission was not significant.

Compared to remission, GFR was unchanged, whereas RPF was significantly elevated (Table 2). Filtration fraction tended to be low (P = 0.066). Sodium excretion, FENa and FEH2O were not significantly decreased, nor were the indices for distal sodium reabsorption [C H2O/(C H2O + C Na ) and UK/(U K + UNa)] increased. Minimal urine osmolality was relatively high, and fractional water excretion relatively low, but this was not significantly different from the findings in remission. This group displayed similar plasma lithium concentrations during nephrosis and remission (respectively, 0.165 ± 0.089 and 0.144 ± 0.067 mmol/l, not significant). Although there were some numerical variations, these were not statistically significant.

Table 1. Urine and plasma protein and blood volume during remission and nephrosis in patients with and without hypovolaemic symptoms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinical symptoms of hypovolaemia</th>
<th>No clinical symptoms of hypovolaemia</th>
<th>Nephrosis without vs with symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remission</td>
<td>Nephrosis</td>
<td>P value</td>
</tr>
<tr>
<td>Proteinuria (g/mmol creat.)</td>
<td>0.08±0.11</td>
<td>1.85±1.12</td>
<td>0.008</td>
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<td>Plasmal albumin (g/l)</td>
<td>45±5</td>
<td>16±3</td>
<td>0.008</td>
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<tr>
<td>Plasma oncotic pressure (mmHg)</td>
<td>23.5±2.4</td>
<td>8.5±3.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Blood volume (ml/kg)</td>
<td>78±12</td>
<td>75±11</td>
<td>0.37</td>
</tr>
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</table>

Table 2. Renal sodium handling during remission and nephrosis in patients with and without hypovolaemic symptoms

<table>
<thead>
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<th>Variable</th>
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<th>No clinical symptoms of hypovolaemia</th>
<th>Nephrosis without vs with symptoms</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Remission</td>
<td>Nephrosis</td>
<td>P value</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>125±31</td>
<td>89±34</td>
<td>0.069</td>
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<tr>
<td>RPF (ml/min/1.73m²)</td>
<td>657±192</td>
<td>597±235</td>
<td>0.52</td>
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<tr>
<td>Filtration fraction (%)</td>
<td>19.8±3.3</td>
<td>15.4±3.4</td>
<td>0.083</td>
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<tr>
<td>Urine osmolality (moom/kg)</td>
<td>52±15</td>
<td>131±125</td>
<td>0.028</td>
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<tr>
<td>UNa/Ucreat (mmol/mmol)</td>
<td>44±31</td>
<td>6±5</td>
<td>0.005</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>1.1±0.6</td>
<td>0.3±0.3</td>
<td>0.011</td>
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<tr>
<td>FEH2O (%)</td>
<td>12.4±3.4</td>
<td>5.9±4.7</td>
<td>0.015</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>31.3±8.0</td>
<td>15.2±6.8</td>
<td>0.008</td>
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<tr>
<td>C H2O/(C H2O + C Na ) (%)</td>
<td>92.5±2.2</td>
<td>95.9±3.0</td>
<td>0.021</td>
</tr>
<tr>
<td>UK/(U K + U Na ) (%)</td>
<td>0.38±0.18</td>
<td>0.74±0.21</td>
<td>0.008</td>
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differences, no significant changes were found in hormone levels.

Patients with and without hypovolaemic symptoms: comparison and correlations

For unclear reasons, there was a predilection for boys to present with hypovolaemic symptoms ($P<0.05$). Age, severity of proteinuria, hyperalbuminaemia and oedema were not different (Table 1). Blood volume was comparable. However, clear distinctions could be made in behaviour of renal haemodynamics and sodium handling. In both groups filtration fraction was low, but in the symptomatic children this was related to a low GFR, whereas in the non-symptomatic children this was related to a high RPF (Table 2). Furthermore, in the symptomatic patients, clearances of sodium and lithium were severely reduced, and the markers of distal sodium reabsorption elevated compared to the non-symptomatic patients.

Stimulated values of plasma renin, aldosterone, noradrenaline and vasopressin were primarily found in the symptomatic compared to the non-symptomatic patients, whereas plasma ANP was high in the non-symptomatic compared to the symptomatic patients. In this group too, plasma potassium during the studies in nephrosis ($4.6 \pm 0.7$ mmol/l) were not significantly different from the values measured in remission ($4.4 \pm 0.5$ mmol/l).

We also calculated correlations between hormones and renal sodium handling in the nephrotic phase (as explained in the Methods, the measurements made during remission were excluded). Relevant correlations are presented in Table 4. It appeared that $FE_{Na}$ and $FE_{Li}$ showed strong negative correlations with renin and aldosterone, and a weak negative correlation with noradrenaline. The quotient $[U_{K}/(U_{K}+U_{Na})]$ showed strong positive correlations with plasma renin and aldosterone. Plasma ANP was positively correlated with $FE_{Na}$ and $FE_{Li}$. Neither of these hormones showed any correlation with minimal urine osmolality or fractional water excretion. Also, plasma vasopressin was not correlated with minimal urine osmolality. Figure 1 gives the correlations for aldosterone as an example. Clearly, there is some overlap between the two patient groups.

**Discussion**

Although sodium retention in the nephrotic syndrome is traditionally attributed to hypovolaemia, a number of observations reported in the past years point to a role for primary renal sodium retention. Probably both mechanisms operate simultaneously to a variable extent, but it is hard to discriminate between these two mechanisms within individuals. To elucidate this issue further, we studied renal sodium handling in children with an early relapse of minimal lesion nephrotic syndrome (MLNS), anticipating that in this stage a hypovolaemic component dictating renal sodium handling would be prevalent in at least some patients, but not in all.

Remarkable distinctions could be made between the children presenting with symptoms suggestive of hypovolaemia and children without such symptoms. Only the former group showed avid renal sodium retention, low GFR, and increased tubular sodium reabsorption, in association with high levels of renin and aldosterone. Importantly, these differences were largely confirmed by the paired comparison with data obtained in remission, which showed normalization in the symptomatic patients, but no change in the asymptomatic patients. In this respect, the present set of data in children with MLNS is unique.

A decreased GFR, normalizing in remission, was observed exclusively in the group with avid sodium retention. Bohlin and Berg [15] reported that children with MLNS and active sodium retention, defined as significant weight gain over the immediate past days, had lower GFR than children presenting with oedema in a steady state. Our data confirm this finding. In addition, we show that these patients have suppressed $FE_{Li}$ and maximal urine flow, both considered qualitative markers of proximal tubule sodium reabsorption [16]. The somewhat higher plasma lithium levels found in these patients also point to impaired lithium excretion between ingestion and clearance experiments. Relatively low values of $FE_{Li}$ and maximal urine flow were reported previously in children [17] and adults [18–20] with MLNS. Besides these changes, the present patients also showed stimulated diluting segment sodium reabsorption, indicated by the term $C_{H2O}/(C_{H2O}+C_{Na})$, and stimulated distal Na–K exchange, indicated by the term $U_{K}/(U_{K}+U_{Na})$ [21]. In aggregate, these changes suggest that the increased sodium reabsorption in the sodium-retaining patients was not limited to a single nephron segment, but rather a generalized tubular activity.

A question to be addressed is whether the increased tubular sodium reabsorption found exclusively in the patients presenting with hypovolaemic symptoms is

<table>
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<th>FE_{Na}</th>
<th>FE_{Li}</th>
<th>U_{K}/(U_{K}+U_{Na})</th>
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<tbody>
<tr>
<td>r coeff</td>
<td>P value</td>
<td>r coeff</td>
</tr>
<tr>
<td>Renin</td>
<td>0.56</td>
<td>0.012</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.60</td>
<td>0.006</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>0.55</td>
<td>0.015</td>
</tr>
<tr>
<td>ANP</td>
<td>0.33</td>
<td>0.019</td>
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</tbody>
</table>
indeed the consequence of hypovolaemia. We have reported that blood pressure is not low in these children [4]. In addition, blood volume and renal plasma flow appeared normal. Thus, we have no direct data to substantiate hypovolaemia. However, it should be realized that the homeostatic drive to maintain circulating volume is very strong [22] and, even though we measured plasma volume and erythrocyte volume separately, such techniques may not be sufficiently sensitive to detect subtle deviations. The notion of a decreased circulating volume is supported by findings of increased reabsorption at different nephron levels, and of elevated levels of plasma renin and aldosterone. In line with this idea, we found significant correlations between sodium reabsorption parameters and plasma renin, aldosterone and noradrenaline in all children during relapse. Note that the data obtained during remission were deliberately excluded from this correlation, to avoid the influence that remission itself may have on this relationship. In adults with the nephrotic syndrome significant correlations were reported between sodium excretion and aldosterone [1,20] but not with PRA [1]. In children the available data are sparse. Tulassay et al. [23] found that sodium excretion correlated weakly with PRA but not with aldosterone in nine children with nephrosis. Bohlin and Berg [15] found no correlation between sodium excretion and plasma aldosterone in 14 children with nephrosis, even although their study counted subjects with active sodium retention and subjects with oedema in steady state. Naturally, significant relationships as found by us do not necessarily indicate causal relations. For instance, despite the correlation between lithium reabsorption and plasma aldosterone, it is unlikely that aldosterone affects proximal tubule reabsorption or lithium clearance [24].

Plasma vasopressin, ANP and noradrenaline were less discriminative than renin and aldosterone, but some relevant tendencies were present. Compatible with a decreased circulating volume, noradrenaline tended to be stimulated and ANP to be suppressed in the patients with avid sodium retention. Rascher and Tulassay [25] also found a positive relation between plasma ANP and sodium excretion in children with nephrosis. Half the children of that study showed elevated plasma vasopressin. Plasma vasopressin was also high in the present symptomatic patients, but was not correlated with impaired urine dilution. However, the symptomatic children also tended to have a low GFR, which contributes independently to impaired urine dilution, as has been demonstrated in adults with nephrosis [26]. For these reasons, assessment of renal sodium handling from maximal free water clearance should be done with appropriate caution.

The children presenting without hypovolaemic signs had oedema without evidence of active sodium retention. In these children we found normal plasma hormone levels, not different from data in remission. A further remarkable finding was that renal plasma flow was elevated only in these patients. Similarly elevated renal plasma flow was found previously in adults with MLNS [1]. Others reported normal renal plasma flow in children with MLNS, however, no distinction was made between patients without and with active sodium retention [27]. Normal plasma hormones and elevated renal plasma flow make it likely that the effective circulation in these children was not impaired. This agrees with the finding by Kuster et al. [28] that systolic blood pressure in children with established MLNS is often higher than in remission. Altogether, these data suggest that this subset of children with MLNS restores sodium excretion by normal homeostatic mechanisms. However, the primary renal sodium excretion impairment remained, unequivocally shown by the persistence of oedema.

Despite the likelihood of a renal sodium excretion defect in these patients, we found no change in tubular sodium handling. The only apparent change was the filtration impairment, evident from the persistently low filtration fraction. This is different from what we had expected. It has been reported that rats with puromycin-induced nephrosis, a model of MLNS, display impaired glomerular filtration, in association with decreased sodium reabsorption in the proximal tubule.
and loop of Henle [29]. The resulting sodium delivery to the collecting tubules is probably normal, and sodium retention was therefore understandably attributed to increased reabsorption in the collecting tubules [29]. Compatible with this idea, recent studies in similar models showed impaired natriuretic response to ANP [30–33], which acts in the inner medullary collecting duct. If the tubular defect in children with MLNS was also concentrated to this part of the nephron, one would expect to see a shift of tubular sodium reabsorption, in particular in this situation of stable oedema. However, this was not the case. The presently used clearance techniques may be too insensitive to detect subtle shifts in tubular reabsorption. On the other hand, one has to consider that the tubular defect responsible for sodium retention in experimental nephrosis and human MLNS are not the same. In this respect, it may be relevant that various studies have demonstrated normal natriuretic response to ANP infusions in adults with nephrotic syndrome [34–36]. Similar studies in children have not been reported.

Finally, the finding of both normovolaemic and hypoatraemic presentation in early juvenile nephrosis deserves discussion. Clearly, absence of hypoatraemic symptoms could not be attributed to less depletion of albumin, since plasma albumin and COP were equally low in symptomatic and asymptomatic patients. Following the earlier suggestion by Cameron [37], we have explained this difference in hypoatraemic and normovolaemic presentation of early MLNS to a hyperacute development of relapse in the former case, resulting in a temporary disequilibrium in intravascular and interstitial albumin pools [4,38]. However, since protein excretion was not different in the two groups, we have no direct evidence for this idea. Circumstantial evidence was provided by the observation in a single patient that hypoatraemic symptoms and stimulated renin–aldosterone axis found at first presentation subsequently disappeared despite further decrease in plasma albumin concentration [4]. Whether this is a general feature of hypoatraemic MLNS in children still has to be substantiated.

In sum, children with a full-blown relapse of MLNS and symptoms suggesting hypoatraemia display diffuse increase in tubular sodium reabsorption. Generalized increase in sodium reabsorption is compatible with a decreased effective circulation, in agreement with other tendencies found in these patients, such as neurohumoral activation and low GFR. Children without symptoms suggesting hypoatraemia tend to have normal neurohumoral activity, GFR and renal sodium handling, the only sign of impaired sodium excretion being the presence of oedema. These data indicate that secondary sodium retention contributes to oedema formation in at least a subset of children developing a nephrotic relapse. This may be limited to the early stage, and be more pronounced in some patients than in others. It remains unclear why such children can generally maintain blood pressure. Also unclear remains the nature of the tubular defect responsible for maintenance of oedema in stabilized MLNS.

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