Hyponatremia with Persistent Elevated Urinary Fractional Uric Acid Excretion: Evidence for Proximal Tubular Injury?

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Key Words
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Abstract
Background/Aims: Hyponatremia associated with high urinary fractional excretion of uric acid which persists after serum sodium is corrected is the cardinal feature of salt losing nephropathy (SLN). We hypothesize that low grade proximal tubular injury is present in SLN because the proximal tubule is the main site of uric acid and sodium transport. Methods: Five subjects with SLN were compared to four subjects with recurrent hyponatremia and three healthy individuals. Urinary NGAL (neutrophil gelatinase associated lipocalin, a marker of tubular injury) and fasting urinary fructose levels (a marker of proximal tubular injury) were measured. Results: Subjects with SLN (n=5) showed elevated fractional uric acid excretion (22 ± 6 vs 4 ± 2 percent, p<0.0001), elevated urinary NGAL levels (62 ± 37 vs 9 ± 7 ng/mg creatinine, p=0.001) and fasting urinary fructose levels compared with the 7 controls (383 ± 465 vs 60 ± 34µmole/µg creatinine, p <0.001). A strong correlation between urinary NGAL levels and urinary fructose levels was observed (r =0.87, p<0.001). Conclusion: High urinary fractional excretion of uric acid in SLN is associated with elevated NGAL and fasting urinary fructose levels suggesting that SLN may involve tubular injury.

Introduction
Salt losing nephropathy (SLN) (also known as renal salt wasting) with hyponatremia and high urinary excretion of uric acid is a well described clinical entity [1]. The cardinal clinical features are extracellular volume depletion, hyponatremia and elevated fractional urinary...
excretion of uric acid (FEUA ≥ 10%) in the absence of adrenal and thyroid dysfunction. SLN can be confused with the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) which is also associated with hyponatremia and an elevated FEUA, but in the latter case the subject is clinically euvolemic. In addition, in SLN the elevation of FEUA persists after correction of hyponatremia whereas in patients with SIADH the elevated FEUA typically resolves after correction of hyponatremia [1].

The renal lesion and pathophysiological mechanism responsible for hyponatremia associated with elevated FEUA is unknown. It has been speculated that a proximal tubular defect is responsible for the urinary uric acid loss and sodium loss [2]. Indeed, some patients with renal salt wasting and hyponatremia show increased fractional excretion of phosphate (>20%) [1, 3], and rare cases of complete Fanconi syndrome have been reported [4]. Nevertheless, no studies have investigated whether biomarkers of renal tubular injury, such as urinary neutrophil gelatinase associated lipocalin (NGAL), are elevated in patients with this condition. In addition, we have recently identified fasting urinary fructose as a biomarker of proximal tubular injury (Lanaspa MA, submitted). Specifically, low levels of fructose are constantly being filtered at the glomerulus and then reabsorbed in the proximal tubule by specific transporters where the fructose is metabolized by the enzyme, fructokinase [5]. Proximal tubular injury might therefore be associated with reduced fructose reabsorption and increased urinary excretion. Moreover, accumulation of fructose in the renal cortex is also associated with renal damage, even in conditions not associated with a high fructose diet [6, 7].

We therefore hypothesized that proximal tubular injury is present in patients with hyponatremia and high urinary loss of uric acid. To test the hypothesis, we conducted an observational study on patients with hyponatremia who were referred to an outpatient nephrology practice from January 1st to December 31st, 2013 and measured their urinary excretion of NGAL and fructose levels.

**Subject and Methods**

Subjects referred to a nephrology practice in Beaumont, Texas with a diagnosis of recurrent hyponatremia (serum Na≤ 134 mEq/L) were investigated. The site of the first encounter was either in the office as an outpatient referral or in the hospital as an inpatient consult. The following screening laboratory tests were performed on first encounter:

- Serum (random): Na, K, BUN, Cr, Uric acid, Osmolality, Phosphorus
- Urine (random): Na, K, Osmolality, Uric acid, Phosphorus, Cr. FEUA, FENa, FEPO4
- Serum hormones: BNP, Cortisol, TSH.

FEUA is fractional urinary excretion of uric acid calculated as follows: \((U/P)UA ÷ (U/P)Cr \times 100\) where \((U/P)UA\) is the ratio of urinary uric acid in mg/dl to plasma level of uric acid in mg/dl. \((U/P)Cr\) is the ratio of urinary creatinine in mg/dl to plasma level of creatinine in mg/dl.

FENa is fractional urinary excretion of sodium calculated as follows: \((U/P)Na ÷ (U/P)Cr \times 100\) where \((U/P)Na\) is the ratio of urinary sodium in mEq/L to plasma level of sodium in mEq/L.

FEPO4 is fractional urinary excretion of phosphate calculated as follows: \((U/P)PO4 ÷ (U/P)Cr \times 100\) where \((U/P)PO4\) is the ratio of urinary phosphate in mg/dl to plasma level of phosphate in mg/dl.

A retrospective chart review was also performed on January 1st, 2013 to identify any subjects that had been referred to the practice either as an outpatient referral or inpatient hospital consult before January 1st, 2013 with a diagnosis of hyponatremia (serum Na≤ 134 mEq/L). The same screening laboratory tests were identified on chart review to ensure the same information is available to determine eligibility for inclusion in the study.

Subjects were enrolled in the study if they have serum Na≤ 134 mEq/L in the absence of hypothyroidism and adrenal insufficiency. The sources of the enrolled subjects were from (1) new referrals to the office
between January 1st and December 31st, 2013; (2) discharged patients from the hospital after being treated for hyponatremia between January 1st and December 31st, 2013; (3) established patients identified from chart review with hyponatremia seen in the office before January 1st, 2013; and (4) discharged patients with hyponatremia seen as hospital consults identified from chart review before December 31st, 2013.

Starting January 1st, 2013 all enrolled subjects were followed in the office every 3 months with serial testing of serum sodium and other indices. On December 31st, 2013, the study was censored. Subjects with hyponatremia were initially treated with water restriction with or without oral sodium tablets that varied from 1 gm (PO) TID or less as clinically indicated. During each office visit, blood pressure, pulse, volume status and medication list were evaluated by the attending nephrologist, SMKL.

On January 14, 2014 coded fasting serum and urine samples were collected, frozen at -70°C and shipped to the University of Colorado Denver. Urinary NGAL (Human neutrophil gelatinase-associated lipocalin) was measured by ELISA (Abcam, Cambridge, MA) and corrected for serum creatinine. Urinary NGAL levels in subjects with normal renal function presenting to emergency rooms is approximately 15 ng/mg creatinine, and levels greater than 130 ng/mg creatinine are considered diagnostic of acute kidney injury [8]. Urine fructose was measured by the Enzychrom Fructose calorimetric assay (Bioassays, Hayward CA). In the fasting state, urinary fructose is typically less than 100 umol/ug creatinine [9].

Subjects were assigned to Group I if they presented in first encounter with hyponatremia (serum Na⁺≤134 mEq/L) (in the hospital or clinic) with no evidence for adrenal or thyroid dysfunction (biochemically tested), an elevated fractional excretion of uric acid (FEUA> 10%) on January 14, 2014, and no clinical evidence of edema or hypervolemia but with clinical evidence of hypovolemia at first encounter. Controls (Group II) consisted of subjects presenting with recurrent hyponatremia regardless of volume status in first encounter and FEUA ≤ 10% on January 14, 2014. Three healthy staff members with no clinical history of comorbid conditions and on no medications served as healthy control subjects and was assigned to Group II. The study was approved by the Human Subjects Institutional Review Board of Christus Health System, St. Elizabeth Hospital, Beaumont, Texas. Informed consent was obtained from each study and control subject.

Statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium). The Variance ratio test (F-test) and Mann-Whitney test were used to test the difference in variances between two independent samples. The T-test was used to test the differences in the mean values between two independent samples. In order to quantify the association between two continuous variables, correlation analysis was performed.

Results

Five subjects with recurrent hyponatremia, increased fractional excretion of uric acid (≥ 10%), and evidence of clinical hypovolemia on first encounter were considered to have SLN (Group I). Four subjects with recurrent hyponatremia and normal urinary fractional excretion of uric acid (≤ 10 %) and three healthy subjects with no biochemical evidence of hyponatremia and comorbid conditions served as controls (Group II). All 5 subjects in Group I were identified on chart review with recurrent hyponatremia and were diagnosed as inpatients in first encounter before January 1st, 2013. They were managed with a combination of water restriction and/or oral salt tablets prior to entering into the study. Three subjects in Group II were referred to the clinic between January 1st and December 31st, 2013. Two were diagnosed in hospital and then referred to the clinic for follow up. One was directly referred to the outpatient clinic. One Group II subject was identified on chart review and was enrolled into the study starting January 1st, 2013.

Six subjects were established patients with the diagnosis of hyponatremia who had been followed in the clinic before January 1st, 2013. The average follow-up period for the 9 hyponatremic subjects was 40 months (range 2.5 months to 99 months).

Subjects with SLN and FEUA ≥10% were all female, average age of 72 (range 61-88 years), and had been diagnosed with SLN for 1 to 4 years, and frequently had comorbid conditions such as cardiovascular disease, cancer or inflammatory diseases (Table 1). Four subjects with hyponatremia and FEUAs≤10% had an average age of 72.5 years (range 68-84
years), consisted of both males and females (2 each), and also had been diagnosed within 1 to 7 years prior to presentation. Three healthy controls subjects (2 female and 1 male) with no known medical problems were recruited randomly from a pool of staff members of an outpatient dialysis clinic. The average age was 27 (range 21-35) (Table 2).

Subjects were tested while receiving salt tablets or water restriction. Mean serum sodium at time of testing (January 14, 2014) remained slightly hyponatremic in Group I subjects (133 ± 5 mEq/L, 95% CI, 126 to 139 mEq/L,) whereas it had largely corrected in the subjects with hyponatremia of other causes (139 ± 5 mEq/L, 95% CI, 135 to 144 mEq/L, p=0.04, two tailed T test) (Group II) (Tables 3 and 4).

Serum uric acid tended to be lower in the subjects considered to have SLN (4.7 ± 0.9 mg/dl) compared to control subjects (6.3 ± 2.1 mg/dl) but was not significant (p=0.12) (Tables 3 and 4). Fractional uric acid excretion averaged 22 ± 6 percent (95% CI, 14-30) in Group I versus 4 ± 2 percent (95% CI 2 to 7.3) in Group II (p<0.0001). Fractional excretion of phosphorus (FE PO4) in study subjects averaged 15±9.7% (95% CI 2.98 to 27.1) in Group I (n=5) versus 7.9 ±2.1 (95% CI 4.6 to 11.1) in Group II ( p=0.028) (Tables 3 and 4).

Evidence for renal injury was present in subjects in Group I, as noted by elevated urinary NGAL (62 ± 37 ng/mg creatinine, 95% CI for the mean 16 to 109) compared with controls (9 ± 7 ng/mg creatinine, 95% CI 3 to 15, p=0.001 by t test, p=0.0045 by Mann Whitney) (Figure 1). These changes appeared independent of eGFR measurements. Differences in NGAL remained significant when only Group I was compared with the 4 hyponatremia controls (62 ± 37 vs 11 ± 8 ng/mg creatinine, p<0.04) (Tables 3 and 4).
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All 5 study subjects (S1 to S5) and 4 controls (C1 to C4) were clinically assessed as having normal extracellular volume status at the time of their last office visit before January 14, 2014. None of the study subjects were taking medications that affect urinary excretion of uric acid.

The fasting urinary fructose level was also elevated in subjects from Group I (383 ± 465 µmol/µg creatinine, 95% CI 194 to 960) compared with controls (60 ± 34 µmol/µg creatinine, 95% CI 29 to 90) (p=0.001 by two tailed t test, p = 0.0284 by Mann Whitney) (Figure 2). A strong correlation was present between urinary NGAL levels and urinary fructose levels.
To examine the persistence of urinary uric acid excretion, serial laboratory values of the five study subjects (S1 to S5) in Group I and the 4 hyponatremic controls (C1 to C4) were reviewed by retrospective chart reviews (from January 1st, 2008 to December 31st, 2013, range of visits from 3 to 8 for each subject). From this review two subjects were identified (S1 and S2) that showed changing biochemical evidence suggestive of features of both SIADH and SLN. Subject S1 presented clinically as SLN but on subsequent visits appeared more like an SIADH as the FEUA reduced to <10% following correction of the serum sodium. Later she was started on chemotherapy for multiple myeloma and her FEUA increased to 20% despite a normal serum sodium of 137 meq/dl in July, 2012. In the subsequent 3 office visits she had an average serum sodium of 139 meq/dl with persistently elevated FEUA (24%). The second subject (S2) also presented with hyponatremia (serum Na 126 to 130 mEq/L) and was referred to SMKL’s office in January 2009 where initial laboratory values were compatible with SIADH. She was treated with fluid restriction until October 2012 when she was recognized to be hyponatremic with hypovolemia. Salt tablets 1 GM (PO)
TID was prescribed. In the subsequent 3 office visits her average serum sodium was 133 meq/dl with the corresponding mean urinary FEUA of 17%. In August, 2014, the patient was discovered to have invasive squamous cell carcinoma of the esophagus and was admitted to hospital for surgical resection.

Discussion

Our primary finding is that urinary NGAL is elevated in subjects with hyponatremia and persistent elevated FEUA≥10% compared to subjects with hyponatremia and FEUA≤10% or control subjects, with no overlap in values. While none of our subjects had levels considered diagnostic of acute kidney injury (>130 ng/mg creatinine) [8], the levels among control subjects were consistent with levels in normal healthy adults (10-15 ng/mg creatinine) [8] whereas the median level in our subjects with SLN and elevated FEUA was 60 ng/mg creatinine. While NGAL is primarily expressed in the collecting duct, increased urinary excretion also occurs with proximal tubular injury, in part because of reduced uptake by the proximal tubular cell [10, 11].

Hypouricemia and hyponatremia are frequently observed in patients with SLN and SIADH. Several groups have investigated the hypouricemia in SIADH [12-15]. The high uric acid excretion cannot be attributed to volume expansion, as the administration of saline does not increase FEUA to the levels observed in SIADH [16]. Furthermore, the administration of desmopressin (DDAVP) does not increase fractional urate excretion in humans [17], and while there is some suggestion that uricosuria may be mediated in part by the vasopressin receptor 1a [17], the administration of vasopressin to humans actually reduces urate excretion [18]. Furthermore, the observation that the high FEUA often resolves with correction of hyponatremia despite continued elevation of vasopressin levels in SIADH whereas elevation of FEUA is persistent in SLN despite correction of hyponatremia suggests that other unrecognized mechanisms may be involved in mediating uric acid excretion [3]. Since uric acid is reabsorbed and secreted almost entirely by the proximal tubule, an elevated FEUA could represent some type of proximal tubular dysfunction, and is consistent with our findings of elevated urinary NGAL in these subjects. The elevated urinary fructose levels might also be consistent with proximal tubular injury given that the proximal tubule is the site of fructose reabsorption in the kidney.

Clinically, separation of SIADH from SLN is important, as the latter may manifest with signs of extracellular volume depletion, and treatment of the former is best addressed by water restriction whereas the latter may also require the administration of salt tablets or saline infusion [3]. Nevertheless, changes in volume status can be subtle in SLN and the two conditions can be easily confused [1]. Furthermore, sodium excretion in subjects with renal sodium wasting and hyponatremia in SLN may not always be elevated as the level of sodium loss varies with dietary sodium intake [1, 3, 19]. Our study suggests the need to do further studies to determine if proximal tubular injury is present in both of these conditions, and if it is worse in subjects with SLN.

If proximal tubular injury is occurring in these subjects with hyponatremia and high FEUA, then what might be the mechanism? While speculative, two potential mechanisms seem possible. First, with chronic stimulation of vasopressin there is likely an increase in the medullary to cortical osmogradient, leading to increased extracellular tonicity in the outer medulla where it might induce the expression of aldose reductase in the S3 segment of the proximal tubule, as aldose reductase is induced by hyperosmolarity. In turn, increased expression of aldose reductase in the proximal tubule could lead to the generation of fructose and local tubular injury [20]. Second, it is also possible that there is an osmotic or other stimulus that might increase both vasopressin secretion by the posterior pituitary and the activation of aldose reductase-fructokinase. In this regard, fructose (but not glucose) is known to stimulate vasopressin levels in humans [21] and fructose also is metabolized in the
proximal tubule by fructokinase where it induces low grade injury and inflammation [6, 7, 20]. We have unpublished data that fructose induced stimulation of vasopressin is blocked in mice lacking fructokinase; and recently we have found that fructokinase is not only expressed in the proximal tubule, but also in the supraoptic nucleus (SON) of the brain (Song Z, Lanaspa MA and Roncal CA Fructose Driven Vasopressin Secretion May Contribute to Chronic Kidney Diseases under Chronic Dehydration, Experimental Biology Meetings, 2015). Thus, future studies should investigate whether some cases of SIADH and SLN may involve activation of the aldose reductase-fructokinase pathway especially since aldose reductase inhibitors are now available in some countries.

In recent years attention has focused on high serum uric acid as a potential risk factor for cardiorenal disease [22-25]. However, a low serum uric acid due to high fractional excretion may also be relevant to renal disease. In subjects with SLN, it remains unknown if the high FEUA reflects specific dysfunction of urate transporters, such as SLC2A9 or URAT1, or whether it represents subtle tubular injury as suggested by the relatively higher urinary NGAL values.

Limitations of this study include the small numbers and different ages of the subjects and healthy controls and the fact that subjects were evaluated sometime after their original presentation. Nevertheless, the absence of overlap with the urinary NGAL values suggests that a real difference exists between hyponatremic subjects with and without elevated FEUA.

**Conclusion**

Our data should be viewed as exploratory, and suggest tubular injury is present in some subjects with SLN as evidenced by elevated urinary NGAL and fructose levels. These studies suggest that SLN is more than just a vasopressin disorder, but may involve low grade proximal tubular injury. We hypothesize the proximal tubular injury may be due to underlying activation of the aldose reductase-fructokinase pathway. Finally, these studies suggest that SIADH may represent a spectrum with mild proximal tubular dysfunction identified solely by a high FEUA to more severe renal salt wasting reflecting more prolonged or severe injury. Consistent with this hypothesis, there is some evidence that the chronicity of hyponatremia is reflected by the duration of the elevated fractional excretion of uric acid [26]. We would suggest that future studies looking for proximal tubular injury and aldose reductase activation in SIADH and SLN may give clues for their underlying pathogenesis.

**Disclosure Statement**

Dr Johnson and Dr Lanaspa have patent applications related to blocking fructose metabolism with the University of Colorado. Dr Johnson, Lanaspa and Sanchez-Lozada are also part of a start-up company (Colorado Research Partners, LLC) to develop inhibitors of fructose metabolism. Dr Johnson is also on the Scientific Board of Amway. Dr. S-M Kurt Lee has no conflict of interest to declare.

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