**Proteasuria - the impact of active urinary proteases on sodium retention in nephrotic syndrome**

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The authors have declared that no conflict of interest exists.

Running head: proteasuria

Key words: proteasuria – proteinuria - nephrotic syndrome – ENaC – serine protease – proteolysis – aprotinin

Word count: 4074 words (excl. abstract and figure legends)

**Abstract**

Sodium retention and extracellular volume expansion are typical features of patients with nephrotic syndrome. In recent years, from *in vitro* data, endoluminal activation of the epithelial sodium channel (ENaC) by aberrantly filtered serine proteases has been proposed as an underlying mechanism. Recently, this concept was supported *in vivo* in nephrotic mice that were protected from proteolytic ENaC activation and sodium retention by the use of aprotinin for the pharmacological inhibition of urinary serine protease activity. These and other findings from studies in both rodents and humans highlight the impact of active proteases in the urine, or proteasuria, on ENaC-mediated sodium retention and edema formation in nephrotic syndrome. Targeting proteasuria could become a therapeutic approach to treat patients with nephrotic syndrome. However, pathophysiologically relevant proteases remain to be identified. In this review, we introduce the concept of proteasuria to explain tubular sodium avidity and conclude that proteasuria can be considered as a key mechanism of sodium retention in patients with nephrotic syndrome.

1. **Introduction**

Nephrotic syndrome is the most extreme manifestation of proteinuric kidney disease and leads to sodium retention with peripheral edema and fluid accumulation in the pleura, peritoneum and, rarely, the pericardium. Sodium retention in nephrotic syndrome has traditionally been explained by two opposing mechanisms, namely, the underfill and the overfill theories 1, 2. The underfill theory was formulated by Epstein about 100 years ago 3, stating that hypoalbuminemia due to urinary protein losses leads to intravascular volume depletion and to underfilling through the loss of oncotic pressure. As a consequence, fluid accumulates in the interstitial space, provoking secondary sodium retention by the kidneys. According to the underfill theory, this is aimed at restoring intravascular volume and is mediated primarily by a stimulated renin-angiotensin-aldosterone-system (RAAS) 4. This theory was widely adopted by medical textbooks and is taught to all medical students from early on. In contrast, the overfill theory, first formulated in 1979, states that edema formation could be a direct consequence of nephrotic syndrome, leading to primary sodium retention by the diseased kidney and to extracellular volume expansion, or overfill 5. This view was stimulated by studies in patients with nephrotic syndrome who had sodium retention in the absence of intravascular volume depletion or a stimulated RAAS 6-8.

Hitherto, the exact mechanisms leading to primary sodium retention by nephrotic kidneys have been ill-defined. Over the last few years, endoluminalactivation of the epithelial sodium channel (ENaC) by aberrantly filtered serine proteases has been suggested as a mechanism that could explain sodium retention, and this would be in keeping with the overfill theory. In this review, we introduce the term “proteasuria” to indicate the increased excretion of active plasma proteases in the urine of patients with nephrotic syndrome. There is strong evidence that proteasuria can be considered as a key mechanism of sodium retention in patients with nephrotic syndrome.

1. **Role of the epithelial sodium channel (ENaC) in sodium retention during nephrotic syndrome**

Much of the knowledge of the molecular mechanisms of sodium retention in nephrotic-range proteinuria has been derived from studies in rodents with experimental nephrotic syndrome induced by anthracyclines in mice 9-12 or by puromycin aminonucleoside in rats (PAN nephrosis) 13-16. Micropuncture studies have suggested that the distal tubule expressing the ENaC is the site of sodium retention in nephrotic syndrome 14, 16 while other sodium transporter such as the Na/H exchanger 3, NaK2Cl cotransporter and thiazide-sensitive NaCl cotransporter were found to be downregulated 17. This is supported by the finding that treatment with the ENaC blocker amiloride prevented volume retention in both nephrotic rats 14 and mice 11. However, a mineralocorticoid receptor blockade by canrenoate was not effective 11, and sodium retention was also observed in adrenalectomized rats 15 and in aldosterone-resistant mice lacking the serum-and-glucocorticoid-kinase 1 (SGK1) 9 pointing to mineralocorticoid-independent activation of ENaC in experimental nephrotic syndrome.

Among the complex and abundant regulatory mechanisms for ENaC including aldosterone 18, intra- and extracellular Na+ concentration, phosphorylation and ubiquitination status, lipid environment or palmitoylation (for review see 19), a special and unique feature of ENaC is its complex post-translational regulation by serine proteases. This leads to endoluminal channel activation by the cleavage of specific sites in the extracellular domains of the  and -subunits 20-22. Serine proteases involved in the physiological regulation of ENaC include furin, acting intracellularly, the membrane-anchored prostasin, and soluble tissue kallikreins 23-25. Proteolytic cleavage at three putative furin sites (two in α-ENaC, one in γ-ENaC) occurs before the channel reaches the plasma membrane. A second cleavage event in γ-ENaC is mediated by extracellular serine proteases distal to the furin site at specific cleavage sites, leading to the release of a peptide 43 amino acids in length (Fig. 1). Proteolytic activation of ENaC involves an increased open probability on a single channel level 26 and the recruitment of a population of near-silent channels without a change in membrane abundance, as shown with the prototypical serine protease trypsin 26, 27.

In 2009, Svenningsen et al. proposed that in nephrotic syndrome, ENaC might be proteolytically activated by the serine protease plasmin after the aberrant filtration of plasminogen from damaged glomeruli and its conversion to plasmin by the tubular urokinase-type plasminogen activator (uPA) 28. *In vitro*, plasmin is able to cleave the -subunit of ENaC at two distinct sites and induces a robust increase in amiloride-sensitive currents in oocytes expressing ENaC 29, 30. In Svenningsen et al.’s study, the activation of inward currents was observed after incubation with protein-rich urine samples of nephrotic rats and humans, which was accompanied by proteolysis of the -subunit of ENaC 28.

The concept of proteolytic ENaC activation by aberrantly filtered plasminogen/plasmin has been embraced as an attractive explanation for sodium retention in nephrotic syndrome 31, 32. It is compatible with the overfill theory, which postulates a primary sodium retention within the diseased kidney 1. However, the experimental data on plasminuria in nephrotic rats were not decisive in proving the essential role of plasmin, because ENaC activation was shown only *in vitro* but not *in vivo* 28. The latter could only have been shown by the inhibition of urinary plasmin activity or by a knockout model with plasminogen deficiency.

1. **Significant role of proteasuria in experimental nephrotic syndrome**

Recently, the significant role of urinary serine protease activity in ENaC activation and volume retention has been shown in wild-type mice with experimental nephrotic syndrome 11. In this study, we confirmed the finding of high serine protease activity in urine samples from nephrotic mice and humans, as shown by Svenningsen et al. 28. Urinary (or endoluminal) serine protease activity was completely inhibited by the addition of aprotinin *in vitro*. Treating nephrotic mice with sustained-release aprotinin pellets accomplished high urinary concentrations of the drug and resulted in a complete inhibition of urinary serine protease activity *in vivo.* This translated into the complete prevention of sodium retention and body weight gain despite an unaltered level of proteinuria. Although aprotinin is not a diuretic and has no direct inhibitory effect on ENaC, its effects were comparable to those of the ENaC blocker amiloride, which similarly prevented sodium retention in nephrotic mice as previously seen in nephrotic rats 14. This study has provided strong *in vivo* evidence that urinary serine protease activity is responsible for ENaC activation and sodium retention in experimental nephrotic syndrome. This explains the results of the study of Ichikawa et al., who found sodium retention only in the proteinuric kidney after unilateral puromycin injection and who postulated that intrarenal factors were responsible for tubular sodium retention 16.

However, the effect of aprotinin in experimental nephrotic syndrome is not yet proof of the essential role of urinary plasmin as the sole factor inducing sodium retention. In addition to plasmin, aprotinin inhibits a wide range of other serine proteases 33, and there is a possibility that other serine proteases might also be involved. Nevertheless, this study proves that the excretion of active, aprotinin-sensitive serine proteases in the urine - or proteasuria - is responsible for ENaC activation and sodium retention in experimental nephrotic syndrome (Fig. 1). In the context of proteasuria, different serine proteases may act in a cascade, as is known from the coagulation or complement system 20, 34. In nephrotic syndrome with proteasuria, plasma serine proteases continue to interact in the tubular lumen in a complex manner. However, little of pathophysiologic relevance is known about the exact identity, interactions of the serine proteases and also the role of protease inhibitors that are filtered at the same time and might counteract protease activity 35.

1. **Proteasuria in patients with nephrotic syndrome**

Proteasuria was also shown to occur in urine samples from patients with nephrotic syndrome of a different etiology. Proteasuria was quantified using a universal protease substrate library 36 and serine proteases were identified using proteomic profiling 37. In urine samples from nephrotic patients, total proteolytic activity was increased compared to that of healthy control persons 37-39. With class-specific inhibitors, serine protease activity accounted for more than 80% of the total protease activity, with the remainder being aspartate, cysteine, and metalloproteases. Using a proteomic approach, serine proteases seen in nephrotic syndrome were typically those that circulate in soluble form in the plasma and belong to the coagulation and complement cascade 40, 41. Therefore, proteasuria in nephrotic syndrome reflects the translocation and excretion of active proteases from the plasma compartment into the urine. The urinary excretion of proteases occurring in the healthy state, however, results mainly from proteases expressed in the kidney and shedded into the urine.

The molecular weight of the proteases excreted in nephrotic syndrome is well above that of albumin and prevents glomerular filtration in the healthy state. However, during glomerular disease, these proteases can aberrantly be filtered and can enter the tubular lumen, similar to albumin. It is therefore not surprising that urinary excretion of serine proteases parallels albuminuria, the gold standard marker of glomerular proteinuria. For the serine protease plasmin, a close correlation of urinary plasmin excretion with proteinuria has been shown in preeclampsia 42 and diabetic nephropathy 43, 44. In patients with CKD of a different origin, excretion of the serine proteases plasminogen and plasma kallikrein as zymogens has been closely correlated to albuminuria 45, 46. In that study, 44% and 30% of the patients had active plasmin and plasma kallikrein, respectively, in the urine as measured with chromogenic substrates. From these data, it is reasonable to assume the presence of significant proteasuria in every patient with gross proteinuria (e.g.>1-2 g per day). Given the similar data from nephrotic mice and humans there is no reason to assume that there are fundamental differences in the role of proteasuria in promoting proteolytic ENaC activation.

1. **Proteasuria as determinant of sodium homeostasis in patients with nephrotic syndrome**

In patients with nephrotic syndrome, proteasuria represented by excretion of active plasmin or plasma kallikrein or aprotinin-sensitive serine proteases was strongly associated with extracellular volume expansion (or overhydration), as assessed by bioimpedance spectroscopy 11, 45, 46. In children with nephrotic syndrome and edema, urinary plasminogen excretion was increased 25-fold compared to values obtained after the remission of the disease, and it coincided with the ability to stimulate inward cunts in collecting duct cells; this ability also disappeared during remission 47.

Currently, the evidence from human studies supporting a role for proteolytic activation of ENaC in nephrotic syndrome is rather weak and only associative. The most specific evidence supporting the proteolytic ENaC activation in humans comes from a study of proteinuric CKD patients who underwent nephrectomy for kidney cancer 48. Using antibodies specific for furin- or prostasin-cleaved γ-ENaC (Fig. 1), the authors demonstrated furin-mediated cleavage under physiologic conditions and second-hit processing of γ-ENaC at the prostasin site. At this point, it should be recalled that the predominant γ‑ENaC cleavage sites of prostasin (K181) and plasmin (K189) are distinct 30 and that the antibody specific to the prostasin cleavage was raised against the γ-ENaC sequence V182-A190. Thus, the binding of this antibody excluded a direct cleavage of γ-ENaC by plasmin at K189.

1. **Role of aldosterone and the question of underfill or overfill**

Aldosterone is the master stimulator of ENaC activity and increases ENaC-mediated sodium transport through a plethora of actions involving channel transcription, membrane abundance, channel activity, and also proteolysis 18, 19. In rodent models of nephrotic syndrome, aldosterone is not a prerequisite for edema formation, and sodium retention also occurs in aldosterone-suppressed or even aldosterone-blocked conditions 11 (Fig. 2) as well as in aldosterone-deficiency 49 or aldosterone-resistance 9. However, nephrotic syndrome in rodents is associated with hyperaldosteronism and hyperreninism, which indicates underfill and might contribute to volume retention by stimulating ENaC. With regard to the debate on under- vs. overfill, this discrepancy can be reconciled by confessing that both theories might hold true. Therefore, experimental nephrotic syndrome in rodents involves elements of both over- and underfill, as sodium retention is primarily caused by proteolytic ENaC activation and is followed by underfill, due to severe hypoalbuminemia. This can be compared to the clinical picture seen in some pediatric patients with nephrotic syndrome, who are hypotensive and have frank signs of underfill 50. An explanation for this might be the failure to compensate for urinary albumin losses 1. In adult patients with nephrotic syndrome, plasma albumin concentration and plasma renin activity have been negatively correlated with each other (r=-0.70, p<0.02), supporting this notion 8. Therefore, the two theories are not mutually exclusive and can coexist, and in Fig. 3 we present a reconciled scheme. Since both under- and overfill theories represent two ends of a continuous spectrum, it is conceivable that some patients may feature elements of both.

According to the concept of proteasuria, we believe that the quality and quantity of excreted serine proteases can make a difference with regard to ENaC activation and sodium retention. This can vary according to the underlying glomerular disease (diabetic nephropathy or immunological disease or others) and the extent of podocyte damage (podocytopathy). Although proteasuria correlates with overall proteinuria and albuminuria, variations in the excretion of serine proteases could also explain why there might be patients with high proteinuria but with little or no edema.

1. **Proteasuria confers salt sensitivity in nephrotic syndrome**

Salt sensitivity is a term used to describe an increase of blood pressure upon increased salt intake. Hypertension in CKD patients is typically salt-sensitive 51 and improves upon reduced salt intake 52. In the context of kidney disease, salt sensitivity can be defined as the inability to excrete sodium adequately or to maintain a neutral sodium balance upon increased salt intake. Salt sensitivity in CKD can be caused by two independent and additive mechanisms 53: first, by reduced glomerular filtration, and/or second, by increased tubular reabsorption, also referred to as tubular salt avidity and indicated by nearly sodium-free urine with a sodium concentration below 20 mM 54.

In murine experimental nephrotic syndrome, tubular salt avidity is caused by proteasuria (Fig. 2). When put on a high salt intake, healthy wild-type mice maintain their body weight by increasing their urinary sodium excretion and suppressing aldosterone secretion. After the induction of nephrotic syndrome, the onset of proteasuria is followed by a massive body weight gain, indicative of sodium retention and salt sensitivity. Treatment of nephrotic mice with aprotinin suppresses urinary serine protease activity and prevents body weight gain (Fig. 2). This longitudinal experiment proves that proteasuria confers salt sensitivity upon formerly salt-insensitive mice and relates to the urinary excretion of aprotinin-sensitive serine proteases.

There is evidence that proteasuria also confers salt sensitivity upon proteinuric CKD patients. In cross-sectional studies investigating variables associated with overhydration and sodium retention, proteasuria, represented by urinary plasmin or plasma kallikrein activity, was independently associated with overhydration 45. Notably, there was a continuous association between proteasuria and overhydration in CKD patients, which was also evident in those with low-range proteinuria, commonly referred to as being non-nephrotic. In agreement with the overfill theory, plasma renin activity and aldosterone concentration were negatively correlated with overhydration in this analysis 45. In the presence of renal salt-sensitivity, it is once more important to advise the patient to limit salt intake to prevent sodium retention and exacerbation of blood pressure control 55, 56.

1. **Proteasuria and promotion of kidney injury and disease progression**

Proteinuria is a sensitive marker of kidney damage and strong predictor of progression of kidney disease to end-stage renal disease (ESRD) 57, 58. Up to now, it is not clear if proteinuria is a risk marker that solely reflects kidney damage or if it is involved in the progression of kidney disease by exerting nephrotoxic effects on podocytes and tubuli 57. In this regard, it could be hypothesized that proteasuria might be involved in kidney damage and progression of kidney failure by direct and also indirect effects 59. Unlike the evidence of proteasuria influencing sodium handling *via* ENaC, the role of proteasuria in mediating kidney damage is more speculative and there are only few data supporting this. Hruby et al. demonstrated that treatment of rats with experimental necrotizing glomerulonephritis with aminocaproic acid (inhibiting plasminogen conversion) and the serine protease inhibitor aprotinin ameliorated kidney damage and reduced proteinuria 60. In a recent study these results were supported by the finding that plasminogen induced podocyte injury *in vitro* by mediating oxidative stress after binding to plasminogen receptors 61. It is remarkable that active plasmin is readily detectable in proteinuric CKD whereas in the plasma it can be barely detected 45. In a study using a sophisticated proteomic approach to analyze degradation products of cellular proteins at the N-terminus, Rinschen et al. found increased cleavage of extracellular domains of glomerular proteins after induction of PAN nephrosis in rats 62. Overall, these preliminary data on glomerular integrity suggest a possible role of proteasuria in mediating kidney injury and podocyte loss. This would herald a dual role of proteasuria in the progression of kidney disease with ENaC activation, sodium retention and hypertension on the one hand and direct effects on the glomerulus on the other.

1. **Targeting proteasuria to treat sodium retention in kidney disease**

The beneficial effects of the serine protease inhibitor aprotinin on sodium retention in murine experimental nephrotic syndrome are proof of the principle that targeting proteasuria could be a new therapeutic approach to treating sodium retention in nephrotic patients 11. Compared to a direct ENaC blockade with the diuretic amiloride, the inhibition of excessive urinary serine protease activity could protect from ENaC-mediated volume retention while minimally interfering with basal ENaC function. Therefore, it might confer protection from the development of life-threatening hyperkalemia, which limits amiloride treatment in clinical practice, particularly in patients with kidney failure 63-66. In a recent clinical trial with a double-blind randomized cross-over design, amiloride was compared to hydrochlorothiazide in nine patients with type 2 diabetes and gross proteinuria 67. Both diuretics were equally effective in lowering blood pressure. However, amiloride caused hyperkalemia and acute kidney injury in two out of nine patients. In a recent case report, addition of amiloride to a regimen containing RAAS blockade and a loop diuretic disrupted the refractory edematous state of the severely hypertensive patient 68. Currently, more controlled studies are needed to support the use of amiloride in the treatment of nephrotic edema.

Before the inhibition of proteasuria can be translated to clinical medicine, more research must be done to reveal the exact identity of those proteases that are pathophysiologically relevant for ENaC activation. This would allow a more targeted pharmacological inhibition and would avoid the use of broad-spectrum protease inhibitors, such as aprotinin or camostat, that have potential negative effects on other proteases of the plasma compartment that exert important physiological functions. Relevant proteases can be identified using affinity chromatography 28 or a proteomic approach 69, but the candidates derived from this approach need to be validated, using animal models, for pathophysiological relevance *in vivo*. So far, plasminogen has been identified as a highly abundant serine protease in the urine, and it has been proposed as involved in proteolytical ENaC activation 28. However, definitive proof from a knockout model is lacking. Another example is the aprotinin-sensitive serine protease plasma kallikrein, which we have hypothesized to be involved in proteolytic ENaC activation. It was detected in the urine of CKD patients and was found to stimulate amiloride-sensitive currents in ENaC expressing Xenopus laevis oocytes 46. However, mice with a genetic deletion of the plasma kallikrein gene (*klkb1-/-*) were not protected from ENaC activation and sodium retention in experimental nephrotic syndrome. This points to other serine proteases mediating proteolytic ENaC activation in this model or redundancy.

1. **Current therapeutic approaches in patients with proteasuria**

Protease inhibitors are a common class of pharmacological agents used in clinical medicine, particularly in nephrology, with the widespread use of angiotensin-converting enzyme or renin inhibitors. However, there are no specific pharmacological approaches to inhibit proteasuria. Although effective in mice, aprotinin is not an ideal drug for the treatment of proteasuric patients, given its side effects, in which kidney events have been described 70. In that study, aprotinin outcomes were analyzed retrospectively in patients undergoing cardiac surgery, and kidney events were defined by a rise in plasma creatinine or dialysis-dependent acute kidney injury. The negative effects of aprotinin might indicate interference with the serine proteases that are involved in the physiological regulation of kidney function or tubulotoxic effects, owing to the fact that aprotinin is heavily reabsorbed by the proximal tubule 71, 72. Aprotinin was removed from the market in 2008, but preparations are now underway for its reintroduction for the treatment of patients.

Camostat is an orally available serine protease inhibitor that was originally developed in Japan for the treatment of pancreatitis 73. Similar to aprotinin, camostat inhibits trypsin-like serine proteases and has shown beneficial effects in rat models of hypertension or CKD 74-77. Anecdotal reports from the 1990s suggest that camostat has beneficial effects in proteinuric patients, including the reduction of urinary protein excretion and edema 78, 79. However, these data are insufficient to warrant its use in nephrotic patients.

As of now, proteasuria can only be reduced as a whole in parallel with the reduction of the proteinuria level. Since proteasuria and proteinuria are closely correlate with each other, any reduction of proteinuria by RAAS blockers is expected to lower proteasuria. Therefore, any antiproteinuric therapy will be antiproteasuric at the same time. It is tempting to speculate that the benefits from lowering proteinuria are mediated, at least in part, by the reduction of urinary protease activity.

In nephrotic syndrome, ENaC inhibition using amiloride might be a good choice that seems to be underutilized by nephrologists. This might be due to the fact that there is a lack of controlled clinical studies showing an improved efficacy of amiloride over other diuretic regimens such as the most commonly used loop diuretics. In diabetic patients with nephropathy and proteinuria, a single oral amiloride dose failed to induce an enhanced natriuresis compared to diabetic patients without nephropathy 80. However, proteinuria was not in the nephrotic range (1.1 g vs. 0.1 g per day). It must be remembered that in the presence of underfill diuretic treatment including potent ENaC inhibition with amiloride can lead to acute prerenal failure as observed the above mentioned trial 67.

Besides its effect on ENaC, amiloride inhibits uPA, which converts plasminogen to plasmin in the tubule *in vivo* 28, 81. Therefore, it was speculated that amiloride might have a dual effect in nephrotic syndrome via direct ENaC inhibition and the prevention of plasmin formation. This has indeed been shown to occur in patients with diabetic nephropathy who were treated with amiloride and who showed reduced plasmin formation 43. The dominant role of plasmin formation by uPA was demonstrated in nephrotic mice with a genetic uPA deficiency (*uPA-/-*) that showed almost absent urinary plasmin formation 82, 83. However, this was not associated with protection from ENaC activation or sodium retention, which suggests once more that serine proteases other than plasmin might be involved. Notably, amiloride prevented sodium retention in nephrotic *uPA-/-* mice, clearly showing that the antiedematous effect of amiloride in nephrotic syndrome is related to the ENaC blockade 82.

ENaC expression and activity can also be modulated using the mineralocorticoid antagonist spironolactone, which prevents the upregulation of ENaC in high aldosterone or low salt intake conditions 84. This drug is more commonly used in addition to furosemide in pediatric patients with nephrotic syndrome 85. In a meta-analysis of adult patients with proteinuric CKD, low-dose spironolactone treatment (mostly 25 mg) has been found to reduce proteinuria by 30-40%, paralleled in many studies by lower blood pressure. It is remarkable that these effects occurred on top of a renin-angiotensin blockade. The incidence of hyperkalemia >6.0 mM was not significantly increased, but some patients developed reductions of GFR 86. The reduction of proteinuria by spironolactone can be explained by the lowering of blood pressure and sodium retention underscoring that the importance of ENaC for sodium homeostasis and blood pressure control.

1. **Conclusions**

The excretion of active plasma proteases in the urine of patients, called proteasuria, is a newly identified and important facet of nephrotic syndrome. Proteasuria is not only a disease marker but is also a risk factor with an impact on sodium balance. Urinary excretion of aprotinin-sensitive serine proteases is involved in proteolytic ENaC activation in experimental nephrotic syndrome and is associated with overhydration in patients. Proteasuria can be considered an important determinant of the renal salt sensitivity, and it provides a mechanism to explain edema formation, according to the overfill theory. Proteasuria is an amenable target for pharmacological treatment. However, it remains necessary to identify pathophysiologically relevant proteases.

**Acknowledgments**

This study was supported by a grant from the German Research Foundation (DFG, AR 1092/2-1).

We acknowledge the artwork by Marina Corral Spence.

**Conflicts of interest:**

None.

**Figure 1. Scheme of ENaC-mediated sodium retention in nephrotic syndrome**

In glomerular disease, increased permeability for proteins larger than albumin leads to excretion of aprotinin-sensitive serine proteases that, once active, may activate ENaC by cleavage at its -subunit, releasing an inhibitory peptide (red) and stimulating Na+ resorption.

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**Figure 2: Proteasuria confers salt sensitivity**

Healthy wild-type mice maintain body weight when put on a high salt intake involving suppression of aldosterone secretion (red and green line). After induction of nephrotic syndrome (red arrow) and onset of proteasuria (reflected by cleavage of the peptide substrate H-D-Val-Leu-Lys-pNA), there is a marked body weight gain indicative of sodium retention and salt sensitivity (blue line). This coincides with increased aldosterone secretion (blue line). High salt-treatment exacerbates body weight gain despite suppression of aldosterone (red line). Treatment of nephrotic mice with the serine protease inhibitor aprotinin prevents salt sensitivity, sodium retention and body weight gain (green line). This experiment shows that formerly salt-resistant healthy mice have become salt-sensitive after onset of proteasuria.

Modified from 11.



**Figure 3: Sodium retention in nephrotic syndrome**

Proteasuria as part of nephrotic proteinuria leads to sodium retention by direct endoluminal ENaC activation in agreement with the overfill theory. When proteinuria is sufficient to induce hypoalbuminemia and underfill, ENaC is additionally activated by the RAAS. There is a continuous relationship between both, and underfill can superimpose on any nephrotic patient with edema primarily due to proteasuria and overfill. Note that under- and overfill theories represent two ends of a continuous spectrum and some patients may be situated in between.

**overfill**

**underfill**

**ENaC activation**

**sodium retention**

**edema**

hypoalbuminemia

RAAS activation

**nephrotic proteinuria**

*proteasuria*

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