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Abstract:
Despite the incompletely understood multiple etiologies and underlying mechanisms, cardiorenal syndrome is characterized by decreased glomerular filtration and sodium avidity. The underlying level of renal sodium avidity is of primary importance in driving a congested heart failure phenotype and ultimately determining the response to diuretic therapy. Historically, mechanisms of kidney sodium avidity and resultant diuretic resistance were primarily extrapolated to cardiorenal syndrome from non-heart failure populations, yet the mechanisms appear to differ between these populations. Recent literature in acute decompensated heart failure has refuted several classically accepted diuretic resistance mechanisms and reshaped how we conceptualize diuretic resistance mechanisms in cardiorenal syndrome. Herein, we propose an anatomically based categorization of diuretic resistance mechanisms, to establish the relative importance of specific transporters and translate findings toward therapeutic strategies. Within this categorical structure, we discuss classical and novel mechanisms of diuretic resistance.

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Classic and Novel Mechanisms of Diuretic Resistance in Cardiorenal Syndrome

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Abstract

Despite the incompletely understood multiple etiologies and underlying mechanisms, cardiorenal syndrome is characterized by decreased glomerular filtration and sodium avidity. The underlying level of renal sodium avidity is of primary importance in driving a congested heart failure phenotype and ultimately determining the response to diuretic therapy. Historically, mechanisms of kidney sodium avidity and resultant diuretic resistance were primarily extrapolated to cardiorenal syndrome from non-heart failure populations, yet the mechanisms appear to differ between these populations. Recent literature in acute decompensated heart failure has refuted several classically accepted diuretic resistance mechanisms and reshaped how we conceptualize diuretic resistance mechanisms in cardiorenal syndrome. Herein, we propose an anatomically based categorization of diuretic resistance mechanisms, to establish the relative importance of specific transporters and translate findings toward therapeutic strategies. Within this categorical structure, we discuss classical and novel mechanisms of diuretic resistance.
Cardiorenal syndrome (CRS) comprises a spectrum of heart-kidney disorders arising from variable etiologies and precipitated by factors such as hemodynamic, neurohormonal, and inflammatory disorders. Despite CRS’ multifaceted and incompletely understood pathophysiologies, the resultant clinical profile is characterized by decreased glomerular filtration, sodium avidity, and diuretic resistance (DR). CRS may occur in conjunction with acute kidney injury, occur in the absence of tubular injury, and/or acute kidney injury may occur as a direct result of progressive severe CRS. Although this distinction is likely important, current biomarkers of tubular injury are unable to provide clear prognostic information or guide clinical therapies in CRS, and on a population level, tubular injury biomarkers are not generally associated with the small to moderate sized changes in creatinine commonly seen in heart failure (HF) populations. Despite this limitation, recent investigations provide new insights into the mechanisms classically considered to cause DR in CRS and have discovered novel resistance mechanisms. Understanding the mechanisms of DR can better inform clinicians on therapeutic strategies to treat this complex syndrome.

Defining Diuretic Resistance

Qualitatively, DR can be defined as an unsatisfactory rate of diuresis/natriuresis despite an adequate diuretic regimen. These defining aspects of DR are subjective, interdependent, and problematic to measure. Importantly, a state of hypervolemia is the first aspect in differentiating pathologic DR from adaptive maneuvers by the kidney to defend body fluid homeostasis, as diuretic response will be poor in the setting of hypovolemia even in a diuretic naive subject. The assessment of hypervolemia is outside the scope of this review but has been extensively reviewed. Secondly, judging the adequacy of the diuretic regimen is highly subjective and variable between physicians and the clinical scenario. For example, a diuretic-
induced natriuresis of 75mmol may be ideal to maintain euvolemia in a patient with chronic HF adhering to a low sodium diet but represent severe resistance in hypervolemic acute decompensated heart failure (ADHF). Lastly, clinically available measurements of diuresis are severely limited. Commonly used metrics such as weight changes and net input-output measurements are notoriously imprecise even in rigorous clinical trials where measurement inaccuracies should be minimized.(12, 13) Furthermore, urine and weight-based metrics only capture changes in total body water, yet the primary inciting pathophysiology of extracellular volume expansion is sodium accumulation.(14) This is of critical importance since patients with ADHF have a wide interpatient variability in sodium content of diuretic-induced urine and a positive sodium balance is still associated with more than a 2 fold increase risk of death even in patients with a negative fluid balance.(15)

Despite these ambiguities, qualitative assessments of DR can provide prognostic information. Diuretic efficiency integrates the diuretic response in context of the loop diuretic dose, dividing either fluid output, weight change, or sodium output by the loop diuretic dose administered (i.e. mL/40mg IV furosemide).(16) Diuretic efficiency has been validated in multiple populations and is a valuable prognostic marker.(17-20) However, diuretic efficiency lacks clinical utility since a modest response to a low dose diuretic can result in good diuretic efficiency that is clinically irrelevant if inadequate to decongest the patient. Additional limitations include the absence of an established threshold to define resistance across populations and the aforementioned limitations if urine output or weight change are utilized.

Natriuresis-based quantitative definitions of DR overcome many of the limitations from urine volume and weight-based definitions and are accumulating evidence for use in clinical practice. Early studies found lower spot urine sodium concentrations following the first IV loop
diuretic dose were associated with worse prognoses, including worsening kidney function, worsening HF, and mortality. (21-27) Based on the urine sodium concentrations in the observational reports, experts have recommended measuring the urine sodium concentration 1-2 hours after the initial diuretic dose and titrating the diuretic dose to achieve a spot urine sodium concentration > 50-70 mmol/L. (8, 28) Yet this recommended urine sodium concentration threshold neither indicates DR without considering the diuretic dose nor ensures a net negative sodium balance if achieved. Importantly, urine sodium concentration alone does not account for urine flow rate and many hospitalized ADHF patients have a urine sodium concentration > 50-70 mmol/L in the absence of diuretic administration. (29, 30) An alternative strategy is to estimate the cumulative sodium excretion over the diuretic period using a natriuretic response prediction equation (NRPE) based upon a spot urine sodium and creatinine collected 2 hours after an IV loop diuretic dose. (30, 31) The NRPE estimates the instantaneous rate of urine production, which is the product of the estimated glomerular filtration rate (eGFR) and the ratio of serum to urine creatinine, converted into sodium excretion with a constant to calculate 6-hour sodium excretion. (30)

\[
\text{Na output (mmol)} = \text{eGFR} \times (\text{BSA}/1.73) \times (\text{Cr})_{\text{serum}} / (\text{Cr})_{\text{urine}} \times 60 \text{ minutes} \times 3.25 \text{ hours} \times (\text{Na}_{\text{urine}} / 1000\text{ml})
\]

\[Na = \text{sodium}; \text{eGFR} = \text{estimated glomerular filtration rate}, \text{BSA} = \text{body surface area}, \]
\[\text{Cr}_{\text{serum}} = \text{serum creatinine}; \text{Cr}_{\text{urine}} = \text{urine creatinine}; \text{Na}_{\text{urine}} = \text{urinary sodium concentration}\]

The NRPE has been validated against measured 6-hour cumulative sodium excretion, demonstrating excellent discrimination across the range of natriuretic responses (area under the curve \(\geq 0.90\)). (30, 31) An online calculator for the NRPE is available at cardiorenalresearch.net.
Future work is required to integrate the natriuretic response with the diuretic dose and associated outcomes to create a complete definition of DR.

**Mechanisms of Loop Diuretic Resistance**

Multiple studies, old and new, have illustrated that the underlying level of renal sodium avidity is of primary importance in determining diuretic response. Some of the seminal work in diuretic response research demonstrated that healthy volunteers provided a very low salt diet had dramatically reduced response to even the first dose of loop diuretic. This pre-diuretic or basal sodium avidity describes the proclivity of the kidney to excessively retain sodium in the absence of diuretics. Collectively these works have demonstrated that basal sodium avidity: has wide interpatient variability despite similar disease severity characteristics and eGFR, fluctuates significantly over time within an individual, is a strong driver of diuretic response, and in hospitalized ADHF patients is not primarily driven by the previous IV diuretic response in the post-diuretic period.

Our current knowledgebase of hypothetical mechanisms underlying kidney sodium avidity and resultant DR relies primarily on animal models or human studies performed in euvoletic healthy controls and patients with hypertension or chronic kidney disease. Although chronic kidney disease is a co-morbid condition in up to one-half of patients with HF, diminished GFR and the systemic aberrations appear to have less of a prominent role on natriuresis and diuretic response in CRS. There is a paucity of studies in patients with CRS on contemporary medical therapy, and DR mechanisms appear to differ based on the clinical context and population. Recent literature in patients with ADHF has refuted several classically accepted mechanisms and reshaped how we extrapolate resistance mechanisms from these
historical populations to CRS. Classical mechanisms of DR that appear to not be primary drivers on a population level are listed in Table 1.

Historically, DR mechanisms have been combined under the umbrella term *diuretic braking*. Importantly, diuretic braking is not a mechanism, but rather a catchall descriptive term for the observation that response to the same dose of diuretic will decrease with subsequent doses. Diuretic braking as originally described in healthy volunteers is adaptive and actually allows the wide therapeutic window and safe use of loop diuretics. A patient with a GFR of 120 mL/min filters approximately 1,400 g of sodium/day. If breaking did not occur and ~20% of filtered sodium were excreted on a continuous loop diuretic infusion, the patient would excrete a lethal ~280 g of sodium and ~50 L of urine over a 24-hour period. The kidney adaptations encompassed by diuretic braking are a double-edged sword, allowing safe use of loop diuretics but if triggered inappropriately also impairing decongestion.

To encourage a mechanism-based system distinguishing the specific kidney pathophysiology driving resistance with the potential to guide therapeutic decisions, classic and novel mechanisms of DR with known or potential significance are presented in Figure 2 using an anatomical categorization. DR can broadly be dichotomized into *Extra-tubular* and *Tubular* DR, with the latter further divided based upon the anatomical nephron segments to establish the relative importance of specific transporters and translate findings toward therapeutic strategies.

**Extra-tubular diuretic resistance**

In this classification system, *Extra-tubular* includes all potential DR mechanisms not involving tubular cells or the tubular lumen. Classic mechanisms of *Extra-tubular* DR (and pseudo-DR) include poor kidney perfusion, venous congestion, intra-abdominal hypertension,
obstructive nephropathy, impaired loop diuretic delivery to the nephron from hypoalbuminemia, high dietary sodium intake, increased organic anions or medications reducing organic acid transport of diuretics into the nephron, and reduced GFR. Although there are minor intra-tubular manifestations in several of the above mechanisms, the *Extra-tubular* mechanisms largely can be distilled down into either 1) reduced filtration/delivery of sodium to diuretic targets or 2) reduced delivery of diuretic to the tubules. We evaluated the impact of collective *Extra-tubular* DR mechanisms to *Tubular* mechanisms in patients with ADHF.(44) Diuretic response, expressed as diuretic efficiency (natriuresis per doubling of bumetanide dose administered ), represented the cumulative effects of both *Extra-tubular* and *Tubular* DR, while diuretic response relative to the amount of drug delivered to the site of action (natriuresis per doubling of renally excreted bumetanide) represented *Tubular* DR alone. *Tubular* mechanisms explained >70% of the variability in diuretic response, indicating natriuresis is determined primarily by *Tubular* mechanisms. *Extra-tubular* mechanisms minimally contribute to DR at the population level in the mild to moderate aberrations found in most patients with CRS. This contemporary report confirms the findings of Brater et al four decades ago, concluding the majority of furosemide resistance in patients with HF is not from impaired drug delivery but from a change in the nephron’s response.(45)

Reductions in renal blood flow via low cardiac output or venous congestive nephropathy have been explored as *Extra-tubular* DR mechanisms. Low cardiac output has been shown in multiple recent analyses to be of limited importance to kidney function or diuretic response at the general ADHF population level. (16, 46-48) Likewise, hemodynamic measures of venous congestion were unrelated to diuretic efficiency and weakly correlated with GFR in contemporary ADHF cohorts.(16, 47) Vasodilators, dopamine, and milrinone failed to augment
diuresis or weight loss in relatively unselected patients with ADHF. In animal HF models, negative renal pressure applied to the urinary collection system improved natriuresis without improving renal plasma flow, indicating mechanical therapies targeted to the kidney may have future utility unlike nonselective vasodilation. Outside of patients in cardiogenic shock or equivalent low perfusion states, hemodynamic mechanisms appear to play a secondary role in DR. Put another way, inotropes and venodilators should be used for cardiac rather than renal indications.

As loop diuretics are > 90% bound to albumin, hypoalbuminemia is classically considered an important mechanism of DR through reduced drug delivery to the tubular lumen. This hypothesis is founded in patients with nephrotic syndrome or cirrhosis receiving low diuretic doses. Although potentially relevant in profound hypoalbuminemia, differences in serum albumin do not contribute meaningfully to diuretic delivery or resistance in the spectrum of albumin levels observed in ADHF populations. No association between baseline serum albumin concentrations and weight loss (p=0.43), diuretic efficiency (p=0.53), or freedom from congestion (p=0.30) existed in large trials of ADHF, validating mechanistic studies.

Classic paradigms assert high sodium intake as a cause of Extra-tubular DR. We consider this a cause of “pseudo- diuretic resistance” since high dietary sodium intake does not directly reduce diuretic response, and if anything increases it. The relationship between sodium intake and DR is complex, with insufficient evidence to define a specific sodium intake goal for patients with ADHF. A randomized trial of intense sodium restriction (<0.8g/day) did not result in better weight loss, decongestion, or duration of IV diuretic therapy compared to liberal sodium intake (3-5g/day) in patients with ADHF. Higher sodium intake might be
beneficial in ADHF populations provided a net negative sodium balance is achieved with diuretic therapy. (65) Concomitant hypertonic saline and high-dose loop diuretics produced greater natriuresis and urine volume than high-dose loop diuretics alone among ADHF patients with DR. (67-70)

Unlike in chronic kidney disease, GFR and the associated increase in organic anions appear to have less of a prominent role on diuretic response in ADHF. (41, 44) Only sodium and water filtered by the kidney are available for diuretic manipulation, with the GFR setting the maximal “diuresable” sodium and filtrate volume. Yet, eGFR poorly correlates with net fluid output ($r^2=0.0; p=0.35$) and diuretic efficiency ($r^2=0.02; p<0.001$) in patients with ADHF across a wide range of eGFRs. (16) Although GFR influences natriuresis through a reduction in the total number of nephrons and thus filtered sodium load, it does not impact the individual nephron’s diuretic response. While urea clearance ($r=0.75; p=0.001$) and low eGFR ($r=0.58; p=0.001$) strongly correlated with decreased diuretic delivery to the kidney, the relevance of diminished diuretic delivery and lower GFR is minimized by a substantially higher fractional excretion of sodium (FENa) with lower GFRs, helping to explain the lack of overall association between eGFR and diuretic response in heart failure. (44) Notably, if only looking at diuretic response (FENa) based on the diuretic delivered by the tubule, eGFR is significantly inversely associated with diuretic response (i.e., the lower the eGFR the more the FENa increases). (44) (Figure 3)

Therefore, defects in glomerular filtration are less important mediators of DR in CRS than Tubular mechanisms.

Tubular diuretic resistance

Recent literature has challenged the notion that many of the classic Tubular mechanisms observed in other populations (chronic kidney disease, hypertension, healthy volunteers) remain
relevant in a CRS population. Post-diuretic sodium reabsorption occurs through yet-to-be determined Tubular mechanisms in healthy volunteers and is widely cited as a significant mechanism of Tubular DR in CRS. (8, 10, 28, 42, 71) Post-diuretic sodium reabsorption describes a reflexive process where the kidney significantly increases sodium reabsorption following diuretic-induced natriuresis, offsetting the diuretic response in the setting of intermittent diuretic bolus dosing and high dietary sodium intake. (42) While post-diuretic sodium reabsorption is significant in healthy, euvolemic volunteers, it is not a significant driver of DR in hypervolemic ADHF at the population level. (10, 29) \textbf{(Figure 4)} To the contrary, in a recent study we observed the opposite; increasing diuretic-induced natriuresis was followed by greater spontaneous sodium excretion in the post-diuretic periods. (29) Importantly, intensification of diuretic therapies in poor diuretic responders was shown to increase diuretic-induced natriuresis, but the post-diuretic sodium excretion remained unchanged. Thus, the strongest driver of both diuretic-induced and post-diuretic sodium excretion was basal kidney sodium avidity. These results may inform why studies of continuous infusion diuretic administration and intense sodium restriction have failed to improve outcomes in unselected ADHF populations. (66, 72)

Tubular mechanisms involving the tubular cells or lumen are categorized below by the anatomical location as \textit{Pre-Loop of Henle}, \textit{Loop of Henle}, and \textit{Post-loop of Henle}.

\textit{Pre-loop of Henle}

The proximal convoluted tubule (PCT) has the greatest capacity (~70%) for filtered sodium reabsorption and expresses several druggable targets, including carbonic anhydrase, \textit{Na$^+$/H$^+$} exchanger 3 (NHE3) and sodium-glucose co-transporter 2 (SGLT2). (73) SGLT2
inhibitors strongly decreases PCT sodium reabsorption in animal models and healthy volunteers via the direct effects on SGLT2 and likely also via suppression of transport through NHE3. (74, 75) Despite this, acute addition of neither SGLT2 inhibitors nor acetazolamide to loop diuretics in patients with HF produce a dramatic augmentation of diuresis. (76, 77) Although counterintuitive that the nephron segment that reabsorbs the most salt has the least importance in diuretic response and resistance, it becomes more intuitive when considering that fine tuning of sodium excretion is predominantly a function of the distal nephron. We used fractional excretion of lithium (FELi) to assess sodium exiting the PCT and loop of Henle and FENa to assess net sodium excretion into the urine. (78) In the proximal tubules and loop of Henle, sodium and lithium are reabsorbed in parallel, but in the distal nephron only sodium is reabsorbed. By measuring the differences in FELi and FENa in the urine, the relative contributions of the proximal and distal nephron’s sodium reabsorption can be assessed. FELi did not differ between patients with HF and controls and did not differ across quartiles of DR. (Figure 5) Following administration of IV diuretic, FELi increased by 12.6% ± 10.8% (P<0.001), but FENa increased by only 4.8% ± 3.3%. These results suggest that on a population level IV loop diuretic therapy yields meaningful inhibition of sodium reabsorption in the loop of Henle, but the nephron segments distal to the loop reabsorb most of that sodium.

**Loop of Henle**

Loop diuretic’s dose-response curve exhibits a sigmoidal pattern along a logarithmic scale, which is shifted rightward and downward in HF. (Figure 6A) This rightward shift is a significant mechanism of DR and can be extreme in many patients. Administration of 40mg IV furosemide to a healthy volunteer saturates the sodium-potassium-chloride transporter (NKCC2), increasing sodium exit from loop of Henle (FELi) by 20-25% and also producing a natriuresis of ~20% of filtered load (FENa). (79, 80) Yet a median 160mg (IQR 40 – 270mg) of IV furosemide
only increased FELi by 12.6% ± 10.8% in patients with HF with what historically would be considered saturating urinary loop diuretic concentrations.(78) Importantly, this resistance can be partially mitigated by administering higher loop diuretic doses than commonly prescribed. In a recent study of diuretic resistant patients, we found sequential diuretic titration up to 500mg of IV furosemide equivalents linearly increased peak post-diuretic FENa (p<0.001).(30) (Figure 6B)

Post-loop of Henle
On a population level, the ultimate natriuretic response to a high dose loop diuretic appears to be determined primary by distal nephron compensatory reabsorption. Continuous loop diuretic exposure in animals causes rapid distal nephron hypertrophy and hyperfunction.(81-83) Contemporary studies in patients with HF have confirmed the distal nephron has the greatest contribution to DR.(44, 78) However, the specific tubular sodium transport pathways responsible for this compensatory reabsorption in human CRS patients have yet to be characterized.

Classically, distal nephron sodium reabsorption has focused on a thiazide sensitive electroneutral and an amiloride sensitive electrogenic NaCl reabsorptive pathways, with transport ascribed primarily to the sodium-chloride cotransporter (NCC) and the epithelial sodium channel (ENaC) respectively.(84, 85) The assumption has been that the majority of distal nephron sodium reabsorption was attributable to NCC based upon the increase in number of NCC on the membrane with chronic loop diuretic therapy.(85, 86) Furthermore, adjuvant thiazide and thiazide-like diuretics show clear clinical efficacy when added to loop diuretics.(87) However, the majority of thiazides interact with multiple sodium transport pathways such as carbonic anhydrase, pendrin, and the sodium-dependent chloride/bicarbonate exchanger (NDCBE), thus making the clinical efficacy of thiazide-loop combination a non-specific finding
in support of NCC as the major distal compensatory reabsorptive pathway. ENaC is the primary channel for sodium reabsorption in the principal cells of the connecting tubules and collecting ducts.\(^{(88)}\) Aldosterone activates ENaC, increasing sodium reabsorption.\(^{(88)}\) Yet, the limited studies performed with spironolactone have not led to a detectable change in diuresis or decongestion. The addition of spironolactone 200mg daily for 3 days to IV furosemide with and without chlorothiazide did not increase diuresis in healthy volunteers receiving chronic furosemide.\(^{(38)}\) In the ATHENA-HF trial, patients with ADHF randomized to spironolactone 100mg daily plus IV loop diuretic therapy for 4 days did not increase diuresis or decongestion.\(^{(89)}\) Although the null effect noted in these studies is likely multifactorial, we would argue that the summative results suggest that ENaC is not a dominant pathway driving DR in HF.\(^{(89-92)}\)

Despite the classic focus on NCC and ENaC, extensive work in animals involving pharmacologic inhibitor and knockout studies has established an additional stoichiometrically important distal sodium reabsorptive pathway likely involving pendrin.\(^{(93-96)}\) Pendrin is chloride-bicarbonate anion exchanger located on the apical membrane of intercalated cells in the collecting ducts, resulting in chloride reabsorption.\(^{(97)}\) Although some debate exists as to the stoichiometry and collaborating channels and transporters, NDBCE and pendrin may work in concert to allow electroneutral sodium reabsorption independent of NCC or ENaC.\(^{(98, 99)}\) Many parameters make pendrin based NaCl reabsorption an excellent candidate for the primary distal tubular NaCl reabsorption pathway in humans receiving loop diuretics. Unlike ENaC and NCC, which have important clinical phenotypes associated with either gain or loss of function genetic disorders in humans, there is no kidney phenotype associated with genetic disorders of pendrin unless a second hit such as acute illness or diuretic therapy is superimposed.\(^{(100-102)}\)
Furthermore, administration of a selective pendrin inhibitor as monotherapy to rodents results in no change in electrolyte handling or urine output, whereas when administered to rats treated with chronic furosemide it increases urine output by 60%.(103) As such, pendrin is an excellent candidate for human DR in that it offers the clear plasticity to act as a salvage pathway when distal delivery of sodium is increased (i.e., after loop diuretic administration) but not when salt delivery is normal or reduced (i.e., in the inter-dose period). Prospective mechanistic research is needed in patients with CRS and DR to ascertain the relative importance of the distal nephron’s sodium reabsorption pathways and inform how findings from healthy controls and animal models can be extrapolated to this distinct patient population.

The aquaporin channel has been explored as a DR mechanism via the addition of tolvaptan, a vasopressin-2 receptor antagonist, to IV loop diuretics in multiple AHF studies. AHF trials without DR found tolvaptan increased urine output and weight loss when added to modest IV loop diuretic doses.(104, 105) Patients with AHF and DR on high-dose IV loop diuretics randomized to either thiazides or tolvaptan had similar weight loss, urine output, and diuretic efficiency; however, tolvaptan produced approximately 50% less sodium excretion than thiazides (p<0.001).(106) While definitive conclusions cannot be formed, the vasopressin system appears not to have a dominant contribution to diminished sodium excretion in diuretic resistant CRS.

Hypochloremic alkalosis has drawn renewed attention as a DR mechanism. Although historically conceptualized as Extra-tubular, recent findings indicate the effect is likely mediated by Tubular mechanisms. The kidney appears to sense salt primarily through chloride, with a dominant effect of chloride over sodium on renin secretion, tubuloglomerular feedback, and regulation of multiple ion transporters via WNK (With-No-Lysine) serine–threonine kinases.(107-111) Hypochloremia is independently associated with worse diuretic response and
less decongestion in ADHF trials even after correction for serum sodium. (112-114) Mechanistic studies using FELi to quantify sodium exit from the proximal tubule and loop of Henle found no difference in FELi between hypochloremia and normochloremia, suggesting hypochloremia may impact distal tubular sodium handling. (114) While a pilot study of chloride repletion with lysine-chloride has been conducted, (114) no data currently exists to support sodium free chloride supplementation or diuretic therapy modification on the basis of serum chloride. However, one of hypertonic saline’s primary theorized mechanisms of benefit in DR is based on the notion that it is repleting a chloride depleted state.

**Mechanism-based therapies to restore Diuretic Response in Cardiorenal Syndrome**

The mechanism for DR should be investigated and should be specifically targeted if identifiable. Commonly the specific mechanism is unknown, and thus therapy should target the most prevalent DR mechanisms at the ADHF population level while considering the therapy’s safety profile. The subsequent discussion assumes a hemodynamically stable, intravascularly hypervolemic patient free from urinary tract obstruction.

Initial therapies should assume a Tubular DR mechanism as this class of mechanisms appear to have the greatest importance in HF. Increasing, often doubling, the IV loop diuretic dose should be the initial strategy, and doses up to IV 500mg furosemide equivalents (80mg oral furosemide = 40mg IV furosemide = 20mg oral/IV torsemide = 1mg oral/IV bumetanide) have demonstrated progressive improvement in peak natriuresis. (30) In addition to increasing peak natriuresis, bolus dose escalation also increases the time therapeutic drug levels exceed the diuretic threshold. (42) Peak diuretic response should be assessed 2 hours after the increased dose (e.g., assess urine output or spot urine chemistry parameters), and if suboptimal the dose should be increased, usually doubled, and the post-dose assessment repeated. (8, 28) While these
strategies are based on expert opinion currently, a randomized prospective trial comparing guideline-based usual care diuretic dosing to a natriuretic prediction equation-guided dosing based on urine sodium and creatinine measurements is underway and will provide evidence to guide diuretic titration strategies. (NCT04481919)

Once a therapeutic loop diuretic dose is identified, it is important to understand that patients with DR have not only poor diuretic-induced natriuresis but also limited sodium excretion during off-diuretic periods. (29) Although diuretic dose titration can increase “on-diuretic” natriuresis, post-diuretic sodium excretion remains low from high basal sodium avidity and this is an important source of sodium excretion in most hospitalized HF patients. (29) As such, diuretic resistant patients will require more frequent bolus diuretic dosing (e.g., every 6 or 8 hours) or high-dose IV continuous loop diuretic infusion to achieve a net negative daily sodium balance since natriuresis primarily occurs when therapeutic drug levels are present in these patients.

If diuretic response is not adequate after maximizing loop diuretic therapy, post-loop of Henle DR mechanisms should be targeted next. (8, 28) While the relative importance of prioritizing specific distal nephron resistance mechanisms is not yet known, thiazides and thiazide-like diuretics have been historically utilized more and experience is greater with this class. Although a strategy of early addition of a thiazide to a loop diuretic when response is inadequate may have superior efficacy, the safety profile of combined loop/thiazide is unfavorable compared to high dose loop diuretic monotherapy. Combination diuretic therapy was independently associated with increased risk of hypokalemia, hyponatremia, worsening creatinine and mortality. (115) Additionally, the multicenter, randomized, controlled DOSE-AHF trial found minimal increased risk associated with high dose loop diuretics aside from a transient
and minimal rise in serum creatinine.\textsuperscript{(72)} Based on the limited evidence, expert opinion suggests loop diuretic dose escalation to moderate or preferably high dose (i.e., bolus dose $\geq 240\text{mg$ furosemide equivalents) should be prioritized over early introduction of combination thiazide diuretic use until randomized trials can better inform practice.\textsuperscript{(8, 28)} All thiazides appear to have equal efficacy at equipotent doses without evidence of superiority for one agent, even in patients with low eGFR.\textsuperscript{(87, 106)}

Although sodium reabsorption through pendrin-based mechanisms is hypothesized to be a significant DR mechanism, no specific inhibitor to pendrin is currently available for human use. Theoretically, chronic use of carbonic anhydrase inhibitors (i.e., acetazolamide and most thiazide or thiazide like diuretics) can downregulate pendrin, but pendrin-directed therapies cannot be recommended presently.

In contrast to loop-thiazide combinations, the addition of ENaC inhibitors or mineralocorticoid receptor antagonists (MRA) to loop diuretics does not worsen hypokalemia. In the absence of clinical trial data in CRS and DR populations, medication choice and dosing are guided by extrapolations from other populations. If spironolactone is chosen, a loading doses of \textit{300-400mg/day} may be initially needed to rapidly achieve therapeutic levels of the active metabolites with long half-lives.\textsuperscript{(92)} Amiloride could provide better inhibition of ENaC by avoiding spironolactone’s pharmacokinetic issues and inhibiting aldosterone independent ENaC activation. Theoretically, this may be of particular importance in DR with significant proteinuria where a significant quantity of filtered proteases is more likely to be present. Conversely, aldosterone had broad sweeping effects on sodium homeostasis and these non-ENaC effects could be important in improving diuretic response. Although there is limited data to support the
practice, addition of ENaC inhibitors and/or high dose MRA to loop-thiazide diuretics is reasonable in refractory DR.(116)

The remaining therapies discussed below have less evidence to support their use and/or are targeting less dominant DR mechanisms. Therefore, each may be considered based upon individual variables.

Salt supplementation

Most diuretics activate neurohormonal systems and further aggravate sodium avidity, the underlying DR physiology. Increasing salt intake may suppress some sodium avidity mechanisms, improving diuretic response. Although initial reports of hypertonic saline supplementation were clouded by concerns of data integrity, single-center experiences in the United States have also reported increasing diuretic response when combined with aggressive diuretic therapies.(70, 117) Common algorithms utilize 150mL bolus doses of 3% saline with concomitant diuretics and frequent serum sodium monitoring.(70) Patients with DR and hypochloremia and/or hyponatremia may be ideal candidates.

Proximal tubular diuretics

Both acetazolamide and SGLT2i have a historical record supporting their safety for other indications, yet the PCT’s limited role in DR suggests their efficacy to be marginal in this population.(118) Prospective trials of acetazolamide and dapagliflozin early in ADHF are ongoing that will better inform this question.(118, 119) While SGLT2i and acetazolamide will increase diuretic response in patients with CRS, available data indicates the magnitude of diuretic response is substantially less than strategies that target the loop of Henle and distal nephron. One advantage of SGLT2i as a diuretic adjuvant is that it has a dual benefit as a chronic medication to improve outcomes such as death and rehospitalization.(120, 121)
**Inotropes and Vasodilators**

Randomized trials of novel and traditional vasoactive therapies have not increased diuretic response and are associated with increased adverse events. (49-52, 122) Adding positive inotropes or vasodilators with the sole purpose of improving diuresis in the absence of a definable hemodynamic target has not been shown to be effective and is not recommended. Although dopamine did not increase diuresis in patients with CRS, there has never been a study of dopamine in truly diuretic resistant CRS patients. (51) Given the theoretical advantages, low dose dopamine should only be considered as salvage therapy in severally diuretic resistant patients.

**Ultrafiltration**

Ultrafiltration (UF) has not proven to afford superior decongestion and is associated with more adverse events than diuretic therapies in CRS. (123) The overwhelming majority of patients with CRS and DR can achieve significant natriuresis with multi-segment diuretic therapies. (116, 124) UF should be reserved for salvage therapy in CRS refractory to aggressive pharmacologic therapies. Anecdotally, UF has particular benefit in a scenario of extreme congestion where the pathophysiology of DR may be related to “congestive nephropathy” and the overall congested state (i.e., worsened hemodynamics from cardiac congestion, vasodilation due to translocation of bacteria due to gut congestion, etc). In this setting, UF may break a congestion-induced vicious cycle, restoring diuretic response and allowing durable benefit to the patient.

In summary, cardiorenal syndrome is characterized by a high sodium avid state and DR. Despite many classic and novel potential resistance mechanisms, a rightward shift in the loop diuretic dose-response curve and distal nephron sodium reabsorption are the dominant drivers on
a population level. Future research into the specific kidney transporters causing cardiorenal syndrome is crucial to guiding therapeutic treatment strategies.

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Dr. Testani reports grants and personal grants and personal fees from 3ive labs, personal fees from Bayer, grants and personal fees from Boehringer Ingelheim, grants and personal fees from bristol myers squibb, personal fees from Astra Zeneca, personal fees from Novartis, personal fees from Cardionomic, personal fees from MagentaMed, grants and personal fees from Reprieve medical, grants and personal fees from FIRE1, personal fees from W.L. Gore, grants and personal fees from Sanofi, grants and personal fees from Sequana Medical, grants from Otsuka, grants from Abbott, grants and personal fees from Merck, personal fees from Windtree Therapeutics, personal fees from Lexicon pharmaceuticals, personal fees from Regeneron, outside the submitted work; In addition, Dr. Testani has a patent Treatment of diuretic resistance US20200079846A1 issued to Yale and Corvidia Therapeutics Inc, a patent Methods for measuring renalase WO2019133665A2 issued to Yale, and a patent Treatment of diuretic resistance pending to Reprieve Medical. Dr. Rao has a patent Treatment of diuretic resistance US20200079846A1 issued to Yale and Corvidia Therapeutics Inc with royalties paid to Yale University, Dr. Rao and Dr. Testani have a patent Methods for measuring renalase WO2019133665A2 issued to Yale. Dr. Rao reports personal fees from Translational Catalyst. Dr. Cox reports grants from AstraZeneca and Cumberland Emerging Technologies.

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**Author Contributions:**

Zachary Cox: Conceptualization; Writing - original draft; Writing - review and editing. Veena Rao: Writing - review and editing. Jeffrey Testani: Conceptualization; Writing - original draft; Writing - review and editing.
References:


80. Beutler JJ, Boer WH, Koomans HA, Dorhout Mees EJ. Comparative study of the effects of furosemide, ethacrynic acid and bumetanide on the lithium clearance and diluting segment


Table 1: Insignificant classical mechanisms of diuretic resistance

<table>
<thead>
<tr>
<th>Insignificant mechanisms of diuretic resistance in cardiorenal syndrome*</th>
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<tbody>
<tr>
<td>Reduced cardiac output</td>
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<tr>
<td>High sodium intake</td>
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<tr>
<td>Reduced glomerular filtration rate</td>
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<tr>
<td>Post-diuretic sodium reabsorption</td>
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<tr>
<td>Increased proximal tubule sodium reabsorption</td>
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<tr>
<td>Reduced diuretic delivery to site of action via:</td>
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<tr>
<td>• Hypoalbuminemia</td>
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<tr>
<td>• Albuminuria</td>
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<td>• Increased organic ions</td>
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*These mechanisms may be relevant in selected patients but, on average, do not appear to be major drivers of diuretic resistance on a population level in hospitalized patients with acute decompensated heart failure.
**Figure 1: Utilization of the Natriuretic Response Prediction Equation**

Figure 1 Caption:
A urine sample is collected approximately 2 hours after the IV loop diuretic bolus dose is administered. The urine sodium and urine creatinine values from this sample are input into the online Natriuretic Response Prediction Equation, available at [www.cardiorenalresearch.net](http://www.cardiorenalresearch.net). The calculated natriuretic response is available in approximately 3 hours and is highly accurate in predicting the diuretic response.

**Figure 2: Classic and Novel Significant Mechanisms of Diuretic Resistance**

Figure 2 Caption:
Diuretic resistance mechanisms are categorized by their anatomic location and by their significance. Diuretic resistance can arise from multiple mechanisms and the mechanisms differ across individuals in a population. Mechanisms with evidence to support their relevance at a population level in humans with heart failure and/or cardiorenal syndrome are bolded in red text. Mechanisms that are hypothesized to be significant but currently lack adequate evidence in humans with heart failure and/or cardiorenal syndrome are listed in black text.

Adapted with permission.(8) ENaC: epithelial sodium channel; NCC: sodium-chloride co-transporter; NDCBE: sodium-dependent chloride/bicarbonate exchanger

**Figure 3: Fractional excretion of sodium over 6 hours by estimated glomerular filtration rate**

Figure 3 caption:
Fractional excretion of sodium (FENa) over the 6 hours from a measured 6-hour urine collection following a dose of intravenous loop diuretic in patients with acute decompensated heart failure is dichotomized by the estimated glomerular filtration rate (eGFR). Patients with a lower eGFR compensated by increasing the FENa to minimize the impact of reduced eGFR on diuretic response. Adapted with permission.(44)

**Figure 4: Compensatory post-diuretic sodium reabsorption is not significant in acute heart failure**

Figure 4 Caption:
**Healthy Volunteers:** Compensatory post-diuretic sodium reabsorption is illustrated by graphing hypothetical urinary sodium excretion over a 48-hour period divided into 6-hour blocks. A 24-hour pre-diuretic period (blue bars) is followed by a 6-hour natriuretic period from an IV loop diuretic dose in the red bar and subsequent 18-hour post diuretic period (blue bars). The horizontal dotted black line denotes the average rate of urinary sodium excretion needed every 6 hours (31 mmol/6 hours) to achieve a net even 24-hour sodium balance on a 3g (130mmol) sodium restricted diet, considering insensible sodium losses (6mmol). In the Pre-Diuretic Period, the urinary sodium excretion rate equals the sodium intake. The diagonally hashed portion of the red bar above the dotted line represents the quantity of natriuresis exceeding the expected rate from dietary intake. In the Post-Diuretic Period, the diagonally hashed blue space indicates the amount of post-diuretic sodium reabsorption, where urinary sodium excretion is depressed following a diuretic period and results in a net even sodium balance. **Hypervolemic Acute Heart Failure:** In contrast to healthy volunteers, patients with hypervolemic acute heart failure do not have compensatory post-diuretic sodium reabsorption. Adapted with permission.(29)

**Figure 5: Proximal convoluted tubule’s marginal involvement in diuretic resistance**

*Previously unpublished figure from published data.(78)
Figure 6: Diuretic dose-response relationship in cardiorenal syndrome

Figure 6 caption:

**Panel A:** The loop diuretic dose-response curve is shown for healthy normal controls (blue line) and patients with acute decompensated heart failure (ADHF) (red line). Sodium \((\text{Na}^+)\) excretion is plotted against the plasma diuretic concentration on a logarithmic scale. Compared to normal, patients with ADHF have rightward (R) shift and downward (D) shift in the dose-response curve. Patients with ADHF require a significantly higher loop diuretic dose and have a diminished ceiling response. Adapted with permission.\(^8\)

**Panel B:** Patients with ADHF and diuretic resistance were given ascending doses of intravenous furosemide (blue bars). The fractional excretion of sodium (FENa) increased with ascending furosemide doses up to approximately 500mg. The incremental improvements in natriuresis occurred with doses commonly considered to be above the diuretic “ceiling” and illustrate the rightward shift of the dose-response curve in ADHF. Adapted with permission.\(^30\)

*IV furosemide equivalents where 1mg IV bumetanide = 40mg IV furosemide*
Figure 1

Diuretic and Natriuretic response predicted in ~3 hours with excellent discrimination across a range of natriuretic responses (area under the curve ≥ 0.90)
### Importance of specific cause/mechanism on diuretic resistance

<table>
<thead>
<tr>
<th>Extra-Tubular</th>
<th>Tubular</th>
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<tr>
<td><strong>Significant</strong></td>
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<td>Venous congestion</td>
<td>Loop of Henle</td>
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<td>Increased intra-abdominal pressure</td>
<td>Inadequate loop diuretic dose</td>
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<td>Kidney vasoconstriction and hypoperfusion</td>
<td>Rightward shift in loop diuretic dose-response curve</td>
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<tr>
<td>Unknown but hypothesized to be significant</td>
<td>Post-Loop of Henle</td>
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<td>Compensatory distal tubular sodium reabsorption</td>
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<td>Pendrin</td>
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Figure 3

Box plot showing the FENa (%) distribution for eGFR<60 mL/min/1.73m² and eGFR≥60 mL/min/1.73m². The p-value is 0.009.

- FENa (%)
- eGFR<60 mL/min/1.73m²
- eGFR≥60 mL/min/1.73m²
**Healthy Volunteers:** Compensatory Post-Diuretic Sodium Reabsorption in the hours following diuretic-induced natriuresis negates the diuretic effect.

**Hypervolemic Acute Heart Failure:** Compensatory Post-Diuretic Sodium Reabsorption is not a significant mechanism of diuretic resistance on a population level.
Figure 5

(A) Distal Tubules: Na⁺ but No Li⁺ Reabsorption

Proximal Tubule and Loop: Na⁺ & Li⁺ Reabsorption in parallel

FELi > FENa = Distal tubule reabsorption

(B) Pre-Diuretic FELi (%)
- Control
- HF Patients

(B) p=0.82

(C) Pre-Diuretic FELi (%)
- Q1
- Q2
- Q3
- Q4

Diuretic resistant → Diuretic responsive

(C) p=0.41