Why do SGLT2 inhibitors reduce heart failure hospitalization? A differential volume regulation hypothesis.

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ABSTRACT

The effect of a sodium glucose cotransporter 2 inhibitor (SGLT2i) in reducing heart failure hospitalization in the EMPA-REG OUTCOMES trial has raised the possibility of using these agents to treat established heart failure. We hypothesize that osmotic diuresis

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induced by SGLT2 inhibition, a distinctly different diuretic mechanism than other diuretic classes, results in greater electrolyte-free water clearance, and ultimately in greater fluid clearance from the interstitial fluid (IF) space than from the circulation, potentially resulting in congestion relief with minimal impact on blood volume, arterial filling, and organ perfusion. We utilize a mathematical model to illustrate that electrolyte-free water clearance results in a greater reduction in IF volume compared to blood volume, and that this difference may be mediated by peripheral sequestration of osmotically inactive sodium. By coupling the model with data on plasma and urinary sodium and water in healthy subjects administered either the SGLT2i dapagliflozin or loop diuretic bumetanide, we predict that dapagliflozin produces a 2-fold greater reduction in IF volume compared to blood volume, while the reduction in IF volume with bumetanide is only 78% of the reduction in blood volume. Heart failure is characterized by excess fluid accumulation, in both the vascular compartment and interstitial space, yet many heart failure patients have arterial underfilling due to low cardiac output, which may be aggravated by conventional diuretic treatment. Thus, we hypothesize that by reducing IF volume to a greater extent than blood volume, SGLT2 inhibitors might provide better control of congestion without reducing arterial filling and perfusion.

INTRODUCTION

The EMPA-REG OUTCOME trial, which evaluated the SGLT2 inhibitor (SGLT2i) empagliflozin in patients with increased cardiovascular risk, demonstrated a large and unexpected reduction in cardiovascular mortality, heart failure hospitalization, and all-cause mortality[1, 2]. More recently, the Canagliflozin Cardiovascular Assessment program (CANVAS) showed a similar decrease in heart failure hospitalization although the rate of death from cardiovascular causes was not reduced significantly [3]. Differences in heart failure hospitalization rates were apparent early, within the first few months in both trials, suggesting treatment may have "rescued" individuals on the verge of heart failure. This finding was unexpected, and although hypotheses have been proposed [2, 4], there is no consensus understanding of the mechanism for this protective effect.

SGLT2 is predominantly located in the proximal tubule (PT) of the kidney, and reabsorbs sodium (Na^+) and glucose in a 1:1 molar ratio. Thus, SGLT2 inhibition reduces not only glucose reabsorption, but PT Na^+ reabsorption as well. In addition, SGLT2 inhibition likely stimulates an osmotic diuresis effect, particularly in the distal tubule. This is because, except in cases of excessive plasma glucose (greater than around 250 mg/dl), nearly all filtered glucose is normally reabsorbed in the PT and very little is delivered distally. However, with SGLT2i, unreabsorbed glucose flows into the distal nephron, and as water is successively reabsorbed along the nephron, glucose concentration and tubular fluid osmolality is increased, reducing the osmotic gradient between the tubular fluid and interstitium. This reduces passive reabsorption of water, particularly in the collecting duct, resulting in osmotic diuresis. Na^+ reabsorption may also be reduced through solvent drag.

The osmotic diuresis effect of SGLT2i is distinctly different from that of other types of diuretics. Most diuretics (thiazide, loop, potassium-sparing diuretics) act by directly reducing Na^+ reabsorption, and water follows passively. In contrast, the osmotic diuresis effect of SGLT2i (in addition to its direct effect on proximal glucose and sodium reabsorption) acts to reduce water reabsorption, and Na^+ reabsorption is reduced indirectly. Thus, SGLT2i may be expected to produce greater electrolyte-free water clearance than other Na^+-driven diuretics [5, 6].

While our understanding of body fluid and sodium has historically focused on the blood and interstitial fluid (IFIF) compartments, there has been growing appreciation over the last few years for the role of peripheral tissues, in particular the skin, muscle, and the skeleton, in dynamically storing and regulating body sodium [7-9]. Titze and colleagues have shown that sodium can be stored non-osmotically (without commensurate water retention) in the skin and other tissues, likely by binding with glycosaminoglycans. They have proposed that skin and other tissues act as negatively charged capacitors, trapping and releasing cations[9], effectively mitigating excess sodium and water accumulation or loss. For interventions that affect body water and sodium levels, this mechanism may play an important role [8, 10].

Many of the signs and symptoms of heart failure are the result of fluid accumulation in the interstitium, leading to peripheral and pulmonary edema, and effective treatment aims to reduce this congestion. However, while blood volume and IF volume are both increased in heart failure, much of the excess blood is accumulated in the venous system, and relative arterial underfilling due to low cardiac output maybe a problem in many patients with heart failure [11, 12]. Treatment with diuretics and vasodilators can reduce the

effective arterial circulating volume further, limiting the effectiveness of these treatments and setting in motion maladaptive compensatory responses [13-16].

We hypothesize that, as a result of osmotic diuresis and greater electrolyte-free water clearance, SGLT2i produces an unexpectedly greater clearance of fluid from the IF space, relative to clearance from the circulation, compared with traditional diuretics. In this study, we use a mathematical model of blood, IF, and peripheral Na^+ and water content, coupled with data from healthy volunteers treated with the SGLT2i dapagliflozin or with the loop diuretic bumetanide, to demonstrate this hypothesis. We further hypothesize that greater reductions in IF volume, relative to blood volume, might allow better control of congestion without reducing arterial filling and perfusion in heart failure patients. This may explain the early reduction in heart failure events observed in EMPA-REG and in CANVAS.

METHODS

Dapagliflozin/bumetanide pharmacodynamic drug-drug interaction study: Healthy subjects (n=42, 10 females and 32 males, aged 19 to 45 years [mean: 30 years], 28 Caucasian and 14 Black, with BMI values of 20.0 to 31.2 kg/m^2 [mean: 26.5 kg/m^2]) gave written, informed consent to participate in the Institutional Review Board-approved study. The subjects were randomized in this open label study to a loop diuretic bumetanide (1 mg/day), dapagliflozin (10 mg/day), or both for 7 days followed, in all groups, by 7 days of bumetanide plus dapagliflozin. Subjects were confined in a clinical trial unit for the study duration and received a fixed diet with ~110 mmol/day sodium and 60 mmol/day potassium for 2 days prior to and throughout dosing. Pooled 24h urine samples were collected on day –1 (baseline) and throughout the study and analyzed for volume, total

amounts of sodium, potassium, chloride, calcium, magnesium, phosphorus, glucose, creatinine, uric acid and osmolality. Fasting serum samples were obtained at baseline and throughout the study for determination of sodium, potassium, chloride, calcium, magnesium, phosphorus, bicarbonate, glucose, creatinine, uric acid, blood urea nitrogen and osmolality and plasma renin activity (PRA). The urine and plasma samples were assayed at an accredited Central Laboratory (PPD Central Laboratory, Austin, Texas, USA). The study did not assess plasma or IF volumes. For the purposes of the analyses described here, data from bumetanide or dapagliflozin given alone for the first 7 days of the study were used. Of note, the salt tablets provided in this study to precisely control sodium intake were poorly tolerated and associated with frequent adverse events including nausea, vomiting and dizziness.

Electrolyte-free water clearance (cH_2Oe) was calculated from urine volume (V_{urine}), urinary sodium (U_{Na}), urinary potassium (U_K), and serum sodium (S_{Na}), according to Equation 1.

$$cH_2Oe = V_{urine}\left(1 - \frac{U_{Na}+U_k}{S_{Na}}\right) \quad \text{Eq. 1}$$

Total freee water clearance (FWC) was calculated from urine volume, urine osmolality (U_{osm}), and plasma osmolality (P_{osm}), according to Equation 2:

$$FWC = V_{urine}\left(1 - \frac{U_{osm}}{P_{osm}}\right) \quad \text{Eq, 2}$$

Model:

We utilized a mathematical model, as illustrated in Figure 1, to theoretically evaluate the impact of free water clearance and osmotically inactive sodium storage on blood and IF volume, and to simulate the volume response to dapagliflozin and bumetanide. The model consists of three compartments: blood (plasma and red blood cells), interstitial fluid, and a peripheral Na^+ storage compartment. The peripheral compartment is a non-fluid compartment representing the Na^+ stored non-osmotically in the skin, muscle, and skeletal tissue [7-9].

The model makes the following assumptions:

1. Ingested water and Na^+ enter the blood compartment, and excreted water and Na^+ exit from the blood compartment.
2. Na^+ and water move freely between the IF and blood compartments across Na^+ concentration gradients.
3. Blood and IF compartments equilibrate quickly.
4. Na^+ intake is constant.
5. When blood Na^+ concentration exceeds the normal equilibrium level, there is a feedback to enhance water intake representing the effects of vasopressin-stimulated thirst. In response to increased diuresis, the increase in water intake is constrained to be less than 50% of the increase in water excretion[17].
6. When IF Na^+ concentration exceeds the normal equilibrium level, Na^+ moves out of the IF and is sequestered in the peripheral Na^+ compartment, where it is osmotically inactive. This process happens very fast, on the same order as Na^+ and water equilibration between the blood and IF.

The final two assumptions are required to be consistent with the observed constant plasma Na^+ concentrations in the healthy volunteer study, as well as prior knowledge of the response to loop diuretics: that increased water intake accounts for up to 50% of the water deficit due to excretion[17]. See supplement for model equations, parameters, and additional explanation.

The model inputs are the time courses of water and Na^+ intake and excretion. Once excretion and intake are specified, the amount of Na^+ and water in each compartment, the concentration gradient between compartments, and the Na^+ and water moving between each compartment can be calculated over time.

RESULTS

Data for urine excretion and serum concentrations of sodium and glucose, as well as urine volume and serum potassium excretion, are summarized in Figure 2. As shown in Figure 3A, while total FWC tended to decrease over time with both treatments, dapagliflozin increased cH_2Oe on day one, and it then returned to baseline. By comparison, cH_2Oe with bumetanide was not different from total FWC. The difference between FWC and cH_2Oe with dapagliflozin is likely the result of increased glucose excretion (Figure 2) and the associated osmotic diuresis effect. In other words, dapagliflozin produced a greater relative increase water excretion than in Na^+ excretion, compared to bumetanide. Plasma renin activity increased in the bumetanide arm but was

unchanged in the dapagliflozin arm, although the difference did not reach statistical significance (Figure 3B).

Theoretical effect of water and Na^+ excretion on blood and IF volume

Before turning to modeling the specific effects of dapagliflozin and bumetanide-generated Na^+ and water excretion on blood and IF volume, we first considered the following hypothetical test cases, in order to better understand the effects of water and Na^+ excretion separately.

Case 1: Response to an increase in water excretion without an increase in Na^+ excretion. Figure 4A shows the simulated response to an increase in urinary water excretion with no change in Na^+ excretion. Two subcases are shown – with and without osmotically inactive peripheral Na^+ storage. When Na^+ is not allowed to be stored peripherally, free water excretion increases blood Na^+ concentration and decreases blood and IF volume acutely, but ultimately has no sustained effect on any of these variables. Essentially all water removed is eventually replaced, and the system ends up at the same steady state where it began. However, when peripheral Na^+ storage is included in the model, an increase in free water clearance then results in a sustained reduction in IF volume. The acute changes in blood volume and blood Na^+ concentration are much smaller and both eventually return to normal. This aligns with the proposed role of peripheral Na^+ storage as a buffer to prevent large changes in blood volume and blood pressure [18].

In both cases, increased water excretion (panel 1) initially causes blood Na^+ concentration to begin to rise (panel 3). Quickly, water moves from the IF to the blood

(panel 4), and Na^+ moves from the blood to the IF (panel 5), across the concentration gradient. Thus, IF Na^+ concentration rises too and remains in near equilibrium with the blood. When peripheral Na^+ storage is included in the model, as IF concentration increases above normal, the higher concentration causes excess Na^+ to move out of the IF into the periphery (Panel 6). This sequestering of Na^+ in the periphery essentially removes Na^+ from the IF, allowing the concentration to remain low, and reducing the water volume needed to maintain the equilibrium Na^+ concentration.

In summary, essentially, removal of free water from the blood causes water to move out of the IF into the blood, which would tend to raise IF sodium concentration, but as IF concentration begins to increase, excess Na from the IF is stored non-osmotically in the periphery, allowing IF to maintain the equilibrium concentration at a lower volume.

Case 2: Response to an ***increase in Na^+ excretion without an increase in water excretion.*** Figure 4B shows the opposite case – the simulated response to an increase in urinary Na^+ excretion with no change in water excretion. This is a physiologically unlikely scenario, but is useful for understanding the system behavior. In this case, blood volume is decreased over time, while IF volume is predicted to increase. This occurs because as Na^+ is excreted from the blood, blood Na^+ concentration falls, and water moves out of the blood into the IF, while Na^+ moves from the IF into the blood stream. IF Na^+ concentration begins to fall, but this causes Na^+ to be pulled from the osmotically inactive sodium stores in the periphery back into the IF, preventing it from falling further. Thus, ultimately, Na^+ excreted from the blood stream is replaced by Na^+ returned from peripheral storage. This extra Na^+ in the IF allows water that moved from the blood to the IF to be held there, so that blood volume is reduced while IF is increased.

Case 3: Increases in both Na^+ and water excretion. The first two cases represented the extremes. Figure 4C shows the response to an increase in urinary Na^+ excretion with three different levels of increased water excretion. The orange, blue, and green lines correspond to a +1, 0, and -1 L change in 24-hour cH_2Oe. In all cases, blood volume is predicted to be reduced to a similar degree. The change in IF volume, on the other hand, is dependent on the change in water excretion relative to Na^+ excretion. When water excretion is large relative to Na^+ (cH_2Oe is increased), IF volume is greatly reduced, but when the ratio is smaller, IF volume decreases less or even increases. Thus, the model predicts that Na^+ excretion determines the degree of blood volume depletion, while free water clearance (water excretion in excess of Na^+) determines the degree of IF volume reduction.

Comparison of blood and IF volume response to dapagliflozin and bumetanide

We next sought to simulate the response to dapagliflozin and bumetanide, using the clinically observed water and Na^+ excretion time profiles as model inputs. Water and Na^+ excretion following treatment with dapagliflozin or bumetanide treatment were modeled as a decaying exponential, the shape of which was determined by least-squared fitting to the observed 24hr urine Na^+ and urine volume (Figure 5A, first column). It was also necessary to account for two additional features of the excretion data. First, in both groups, sodium excretion on day 0 was less than expected, given that study subjects were given daily 110mEq sodium tablets beginning 2 days before the study began. By day 7, sodium excretion was consistent with this sodium intake. The initial lower sodium excretion is likely due to tolerability issues and reduced absorption of the salt tablet, since

diarrhea, nausea, and vomiting were more common in the first 3 days of the study. To account for this, we modeled an increase in sodium intake from day 0 to day 1, which was then reflected in sodium excretion. We repeated the simulations assuming constant sodium intake and matching either the slope or absolute of the rise in sodium and water excretion on day 1 (Suppl. Figures 1 and 2). Changing these assumptions had small quantitative effects on the predicted blood and IF volume changes, but did not alter the conclusions of the simulations.

Secondly, in the bumetanide group, water excretion declined below baseline after an initial peak. It is unlikely that bumetanide directly reduces water excretion over time, but this may reflect a physiologic overcompensation in water retention. In Figure 5A, we modeled this as a gradual linear decrease in water intake after day 1. We repeated these simulations without accounting for this affect (Supplement Figure 3). Again, altering these assumptions produced small quantitative differences in volume changes, but did not affect the conclusions of the simulations.

We then simulated the time courses of serum Na^+ concentration, IF volume, blood volume, peripheral Na^+ storage, and water intake in response to the empirically fitted changes in Na^+ and water excretion. As shown in Figure 5A, the model predicted that dapagliflozin reduces IF volume three times as much as blood volume (480 ml vs 150 ml). In comparison, the predicted reduction in IF volume with bumetanide was only 80% of blood volume reduction (510 ml vs 780 ml) (Figure 5B). Thus, while dapagliflozin produced an overall weaker natriuresis and diuresis than bumetanide, it is predicted to have a relatively greater effect on IF volume than on blood volume, compared to

bumetanide. In other words, SGLT2i may be able to effectively reduce interstitial congestion without causing arterial underfilling due to a large blood volume reduction.

DISCUSSION

In this study, we coupled observed plasma and urinary water and electrolyte data with a mathematical model to simulate and compare effects of dapagliflozin and bumetanide on blood volume and IF volume. In healthy volunteers, dapagliflozin produced similar changes in total free water clearance but a larger increase and smaller subsequent decrease in electrolyte-free water clearance than bumetanide. Although a role for urea cannot be ruled out since it was not measured, dapagliflozin-induced reductions in proximal tubule glucose reabsorption likely account for this different. Glucose remaining in the tubules draws water across the osmotic gradient, leaving sodium and other electrolytes behind.

Our simulations suggest that the differential effect on electrolyte-free water clearance produces a relatively greater reduction in IF volume than in blood volume with dapagliflozin, compared to bumetanide. This differential effect on fluid compartments has potential therapeutic implications, particularly in heart failure. Heart failure is of course characterized by fluid accumulation, leading to peripheral and pulmonary edema. Managing congestion is a major goal in treating heart failure, and is achieved primarily

through use of loop diuretics. It also is a major challenge, because excessive blood volume reduction can have deleterious effects, including reduced arterial filling and organ perfusion, hypotension, and neurohormonal activation [13-16]. Neurohormonal activation, in turn, can cause greater sodium and water retention and reduced peripheral and renal perfusion due to vasoconstriction. Reduced renal blood flow may lead to renal impairment and further impairment in the ability to eliminate sodium and water. It has even been postulated that the higher mortality associated with high-dose loop diuretic use might be related to these effects (although cause and effect has not been established and these observations could also reflect confounding due to more severely ill patients requiring larger doses of diuretics) [19]. Although changes in PRA in the current healthy volunteer study were small, the fact that PRA increased with bumetanide but was unchanged with dapagliflozin is supportive of less neurohormonal activation with SGLT2i compared to loop diuretics. Few other data exist on the neurohumoral effects of SGLT2i and these have been collected in uncontrolled studies; however, the available data do not show activation of the renin-angiotensin system with SGLT2i which, if correct, is distinct from observations with conventional diuretics[20, 21].

If our model is correct and SGLT2i indeed produces larger reductions in IF volume relative to blood volume, they may more effectively relieve signs and symptoms of interstitial congestion, and provide some relief of elevated cardiac filling pressures, without deleterious effects of excessive blood volume depletion, including neurohumoral activation. These effects should occur soon after initiating treatment. It is tempting to speculate that the diuretic and natriuretic action of SGLT2i were related to the heart failure hospitalization prevention noted in EMPAREG-OUTCOME (empagliflozin) and CANVAS

(canagliflozin), although other hypotheses have also been proposed to explain this benefit[4]. Treatment with empagliflozin and canagliflozin also significantly reduced the rate of renal function decline, an effect that may also contribute to the protective effect of these drugs in heart failure. However, treatment with SGLT2i initially *decreases* in GFR, making it unlikely that renoprotection accounts for the rapid reduction in heart failure hospitalization seen with SGLT2i (although their renoprotective actions may contribute to the long-term cardiovascular benefits).

A critical component of the hypothesis of differential volume regulation proposed here is the ability of peripheral tissue to sequester Na^+ when excess water relative to Na^+ is removed and IF concentrations rise, and release Na^+ when the opposite occurs. The importance of peripheral Na^+ storage is increasingly recognized. In separate studies by Titze et al, reductions in peripheral Na+ (measured by Na-MRI) were greater with furosemide [8] than with dapagliflozin [10]. However, these studies are not directly comparable, since the first evaluated heart failure and patients and the second was in diabetic patients, and baseline peripheral sodium was quite different between the two studies. In addition, the dynamics of peripheral Na^+ storage are incompletely understood, and there may be differences in peripheral Na^+ storage between health and disease. Skin sodium content has been shown to be correlated with LV hypertrophy in chronic kidney disease[22]. Research is needed to fully understand the role of peripheral Na^+ storage in regulating IF volume in health and disease.

The hypothesis proposed here arises from predictions of IF and volume changes based on sodium and water excretion. Unfortunately, few studies have directly measured IF volume following treatment with SGLT2 inhibitors or loop diuretics, and no study has

directly compared the two. Hirose and colleagues did find that after 8 weeks of treatment with SGLT2i tofogliflozin, IF volume in patients with type 2 diabetes was significantly reduced by 400ml – similar to the reduction predicted by our simulations[23]. These investigators also reported significant reductions in body weight and free fat mass. Hematocrit increased, while mean arterial pressure was reduced slightly. Studies that more direct measure changes in IF and blood volume with these treatments are needed to validate the hypothesis.

This study utilized data from healthy volunteers. Based on knowledge of the mechanism of action of each drug, it may be reasonable to assume that the relative difference in cH_2Oe, and thus in relative IF volume reduction, will maintained in diseased states like heart failure. However, alterations in fluid status, renal function, and neurohumoral activation in diabetes and/or heart failure could impact the magnitude of the response. Studies in diabetic and heart failure patients should be conducted to further evaluate the hypothesis presented here.

Diuretics like bumetanide and dapagliflozin stimulate thirst, and thus increased water intake may compensate for some of the electrolyte-free water loss. Unfortunately, changes in water intake were not measured in this study, and therefore, we cannot rule out that any excess in water excretion was compensated by increased intake. However, this seems unlikely. Rats treated with furosemide have been shown to replace less than half of the deficit in fluid volume by increasing intake[17].

The unexpectedly low sodium excretion at baseline in both study arms, as well as the decline over time in water excretion in the bumetanide arm, introduced some uncertainty into the simulations. We attempted to address this by testing alternative hypotheses to explain these data (see supplement). These alternative simulations did not alter the conclusions regarding the greater relative effect of SGLT2i on IF volume, compared to bumetanide.

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References

1. Zinman, B., et al., *Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes.* N Engl J Med, 2015. **373**(22): p. 2117-28.
2. Fitchett, D., et al., *Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME(R) trial.* Eur Heart J, 2016. **37**(19): p. 1526-34.
3. Neal, B., et al., *Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes.* N Engl J Med, 2017.
4. Ferrannini, E., M. Mark, and E. Mayoux, *CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis.* Diabetes Care, 2016. **39**(7): p. 1108-14.
5. Shimizu, K., et al., *Solute-free versus electrolyte-free water clearance in the analysis of osmoregulation.* Nephron, 2002. **91**(1): p. 51-7.
6. Lindner, G., C. Schwarz, and G.C. Funk, *Osmotic diuresis due to urea as the cause of hypernatraemia in critically ill patients.* Nephrol Dial Transplant, 2012. **27**(3): p. 962-7.
7. Titze, J., *Water-free sodium accumulation.* Semin Dial, 2009. **22**(3): p. 253-5.
8. Hammon, M., et al., *23Na Magnetic Resonance Imaging of the Lower Leg of Acute Heart Failure Patients during Diuretic Treatment.* PLoS One, 2015. **10**(10): p. e0141336.
9. Titze, J., *Sodium balance is not just a renal affair.* Curr Opin Nephrol Hypertens, 2014. **23**(2): p. 101-5.
10. Schmieder, R., et al., *Os 12-03 Sglt-2-Inhibition with Dapagliflozin Reduces Tissue Sodium Content.* J Hypertens, 2016. **34 Suppl 1 - ISH 2016 Abstract Book**: p. e76.
11. Schrier, R.W., *Body fluid volume regulation in health and disease: a unifying hypothesis.* Ann Intern Med, 1990. **113**(2): p. 155-9.
12. Schrier, R.W., *Decreased effective blood volume in edematous disorders: what does this mean?* J Am Soc Nephrol, 2007. **18**(7): p. 2028-31.
13. De Vecchis, R., et al., *In right or biventricular chronic heart failure addition of thiazides to loop diuretics to achieve a sequential blockade of the nephron is associated with increased risk of dilutional hyponatremia: results of a case-control study.* Minerva Cardioangiol, 2012. **60**(5): p. 517-29.
14. Francis, G.S., et al., *Acute vasoconstrictor response to intravenous furosemide in patients with chronic congestive heart failure. Activation of the neurohumoral axis.* Ann Intern Med, 1985. **103**(1): p. 1-6.
15. Weinfeld, M.S., G.M. Chertow, and L.W. Stevenson, *Aggravated renal dysfunction during intensive therapy for advanced chronic heart failure.* Am Heart J, 1999. **138**(2 Pt 1): p. 285-90.
16. Greenberg, A., *Diuretic complications.* Am J Med Sci, 2000. **319**(1): p. 10-24.
17. Rabe, E.F., *Relationship between absolute body-fluid deficits and fluid intake in the rat.* J Comp Physiol Psychol, 1975. **89**(5): p. 468-77.
18. Titze, J., et al., *Extrarenal Na+ balance, volume, and blood pressure homeostasis in intact and ovariectomized deoxycorticosterone-acetate salt rats.* Hypertension, 2006. **47**(6): p. 1101-7.
19. Peacock, W.F., et al., *Impact of intravenous loop diuretics on outcomes of patients hospitalized with acute decompensated heart failure: insights from the ADHERE registry.* Cardiology, 2009. **113**(1): p. 12-9.
20. Heise, T., et al., *Pharmacodynamic Effects of Single and Multiple Doses of Empagliflozin in Patients With Type 2 Diabetes.* Clin Ther, 2016. **38**(10): p. 2265-2276.
21. Heise, T., et al., *Acute Pharmacodynamic Effects of Empagliflozin With and Without Diuretic Agents in Patients With Type 2 Diabetes Mellitus.* Clin Ther, 2016. **38**(10): p. 2248-2264 e5.

22. Schneider, M.P., et al., *Skin Sodium Concentration Correlates with Left Ventricular Hypertrophy in CKD.* J Am Soc Nephrol, 2017. **28**(6): p. 1867-1876.
23. Hirose, S., et al., *Impact of the 8-week Administration of Tofogliflozin for Glycemic Control and Body Composition in Japanese Patients with Type 2 Diabetes Mellitus.* Intern Med, 2016. **55**(22): p. 3239-3245.

Figure 1. Schematic of mathematical model of volume homeostasis.

Figure 2. Observe urinary sodium excretion (top left), serum sodium concentration(top right), urinary glucose excretion (middle left), serum glucose (middle right), urine volume (bottom left), and urinary potassium excretion (bottom right) during daily dosing of dapagliflozin or bumetanide. Data are mean (solid lines) and 25th and 75th quartiles.

Figure 3. Observed mean change in electrolyte-free water clearance clearance (cH20e) and total free water clearance (FWC) (A) and plasma renin activity (PRA) (B) following initiation of treatment with dapagliflozin or bumetanide. FWC and cH20e were not different in the bumetanide arm. In the dapagliflozin arm, cH20e was higher than FWC, which decreased over time.Dapagliflozin tended to produce a larger initial increase and smaller subsequent decrease in electrolyte-free water clearance than bumetanide. Although the differences were not statistically significant, plasma renin activity tended to increase with bumetanide but not dapagliflozin.

Figure 4. Hypothetical response to imposed changes in urinary volume excretion (first column) and urinary Na^+ excretion (second column). A) Response to increase in water excretion without an increase in Na^+ excretion, when peripheral Na^+ storage is excluded (orange) or included (green) in the model. Without peripheral storage, water excretion has no sustained effect. With peripheral storage, water excretion causes a sustained decrease in IF volume, with no change in blood volume. B) An increase in Na^+ excretion with no change in water excretion is predicted to produce a sustained blood volume reduction with no change in IF volume. C) The change in IF volume is dependent on the relative increase in water excretion compared to Na^+ excretion.

Figure 5. (A-B) Simulated volume responses to the increases in Na^+ and water excretion observed with dapagliflozin (A) and bumetanide (B). The first column in each panel shows the urinary Na^+ and water excretion profiles used as input to the model. The next three panels show the simulated electrolyte free water clearance, plasma Na^+ concentration, blood volume, and IF volume. Solid lines – simulation. Thick dashed lines (first column only) – simulated intake. Circles – mean observed data. Dashed gray lines – observed 25^{th} and 75^{th} quantiles. C-D) Dapagliflozin is predicted to produce a 3-fold decrease in IF volume relative to blood volume, while the reduction in IF with bumetanide is only 66% of the reduction in blood volume.