Circulation

ORIGINAL RESEARCH ARTICLE

Empagliflozin in Heart Failure

Diuretic and Cardiorenal Effects

Editorial, see p 1055

BACKGROUND: Sodium-glucose cotransporter-2 inhibitors improve heart failure–related outcomes. The mechanisms underlying these benefits are not well understood, but diuretic properties may contribute. Traditional diuretics such as furosemide induce substantial neurohormonal activation, contributing to the limited improvement in intravascular volume often seen with these agents. However, the proximal tubular site of action of the sodium-glucose cotransporter-2 inhibitors may help circumvent these limitations.

METHODS: Twenty patients with type 2 diabetes mellitus and chronic, stable heart failure completed a randomized, placebo-controlled crossover study of empagliflozin 10 mg daily versus placebo. Patients underwent an intensive 6-hour biospecimen collection and cardiorenal phenotyping at baseline and again after 14 days of study drug. After a 2-week washout, patients crossed over to the alternate therapy with the above protocol repeated.

RESULTS: Oral empagliflozin was rapidly absorbed as evidenced by a 27-fold increase in urinary glucose excretion by 3 hours ($P<0.0001$). Fractional excretion of sodium increased significantly with empagliflozin monotherapy versus placebo (fractional excretion of sodium, 1.2±0.7% versus 0.7±0.4%; $P=0.001$), and there was a synergistic effect in combination with bumetanide (fractional excretion of sodium, 5.8±2.5% versus 3.9±1.9%; $P=0.001$). At 14 days, the natriuretic effect of empagliflozin persisted, resulting in a reduction in blood volume (–208 mL [interquartile range, –536 to 153 mL] versus –14 mL [interquartile range, –282 to 335 mL]; $P=0.035$) and plasma volume (–138 mL, interquartile range, –379 to 154±453 mL; $P=0.04$). This natriuresis was not, however, associated with evidence of neurohormonal activation because the change in norepinephrine was superior ($P=0.02$) and all other neurohormones were similar ($P<0.34$) during the empagliflozin versus placebo period. Furthermore, there was no evidence of potassium wasting ($P=0.20$) or renal dysfunction ($P>0.11$ for all biomarkers), whereas both serum magnesium ($P<0.001$) and uric acid levels ($P=0.008$) improved.

CONCLUSIONS: Empagliflozin causes significant natriuresis, particularly when combined with loop diuretics, resulting in an improvement in blood volume. However, off-target electrolyte wasting, renal dysfunction, and neurohormonal activation were not observed. This favorable diuretic profile may offer significant advantage in the management of volume status in patients with heart failure and may represent a mechanism contributing to the superior long-term heart failure outcomes observed with these agents.

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Clinical Perspective

What Is New?

- Among patients with heart failure and diabetes mellitus, empagliflozin therapy resulted in increased natriuresis as monotherapy and demonstrated a clinically meaningful synergistic effect when combined with a loop diuretic.
- This enhanced natriuresis persisted over the 14-day study period, resulting in a reduction in plasma volume. However, it did not occur at the expense of off-target electrolyte wasting, renal dysfunction, or neurohormonal activation.

What Are the Clinical Implications?

- This favorable diuretic profile may offer significant advantage in the management of volume status in patients with heart failure and may be a contributory mechanism to the superior long-term heart failure outcomes observed with these agents.
- Additional study of the clinical utility of sodium-glucose cotransporter-2 inhibitors as natriuretic agents is warranted.

Central to the pathophysiology of heart failure (HF) is dysregulation of sodium and fluid homeostasis.[1–3] Specifically, there is maladaptive activation of sodium-conserving pathways despite appropriate or even excess intravascular volume. The resulting congestion is a primary driver of symptoms and hospitalizations and is strongly associated with worsened survival.[4–7] Currently, loop diuretics are the mainstay of therapy to counteract this sodium avidity.[8] However, loop diuretics augment sodium excretion at the expense of neurohormonal activation, which occurs in part as a result of direct antagonism of sodium chloride entry into the salt sensor of the kidney, the macula densa.[9–11] In addition to the well-established contribution of neurohormonal activation to HF progression and mortality, these pathways evolved to defend intravascular volume by increasing renal tubular sodium reabsorption.[12] Therefore, it is not surprising that loop diuretic resistance and persistent volume overload are common with these agents.[13,14]

Sodium-glucose cotransporter-2 inhibitors (SGLT-2is) are glucose-lowering drugs that have been shown to reduce HF hospitalizations in patients with type 2 diabetes mellitus and, more recently, to improve clinical HF outcomes in patients with HF with reduced ejection fraction, including those without diabetes mellitus.[15–18] Although the mechanisms behind these benefits are unknown, a candidate contributor is the diuretic effect of these agents. Several studies in presumably euvolemic individuals with diabetes mellitus have demonstrated a reduction in measured blood volume with these agents, and evidence of hemoconcentration has been reproducibly observed in large SGLT2i trials.[15,19,20] This apparent decrease in intravascular volume occurs despite these agents being substantially weaker natriuretics than loop diuretics, drugs that do not reduce blood volume in euvolemic subjects.[21] Another important difference compared with loop diuretics is the proximal tubular location action of the SGLT2is, which leads to increased rather than decreased sodium chloride delivery to the macula densa. This physiology may explain the small or absent neurohormonal response to SGLT2is compared with what would normally be expected with the observed reduction in blood volume.[22] The purpose of the current investigation was to study the immediate and intermediate (14-day) effects of SGLT-2is on natriuresis, volume status, and neurohormonal activation in patients with HF.

METHODS

Deidentified data that support the findings of this study are available from the corresponding author on reasonable request. Because of the small sample size and single-center nature of the study, creating a higher potential for reidentification, data will be provided only to qualified researchers with training in human subject confidentiality protocols.

Study Oversight

The study was an investigator-initiated trial that was conceived of and designed by the investigators with funding support from Boehringer-Ingelheim. Approval was obtained by the Yale University Institutional Review Board, and written informed consent was obtained from all patients. The study was registered on ClinicalTrials.gov (NCT03027960).

Study Population

Inclusion criteria included the following: (1) stable HF (diagnosed by an advanced HF cardiologist) as defined by no hospitalizations during the preceding 60 days, stable HF medications for at least 2 weeks and stable diuretics for 4 weeks, and opinion of the HF cardiologist that the patient is at optimal volume status; (2) diagnosis of type 2 diabetes mellitus; (3) regular home monitoring of blood glucose; (4) estimated glomerular filtration rate (eGFR) ≥45 mL·min^{-1}·1.73 m^{-2}; and (5) ≥18 years of age. Exclusion criteria included the following: (1) active titration of long-term HF medications expected during the study period; (2) use of a nonloop diuretic aside from an aldosterone antagonist (≤25 mg spironolactone or ≤50 mg eplerenone); (3) critical stenotic valvular disease, complex congenital heart disease, or previous heart transplantation; (4) history of diabetic ketoacidosis, brittle diabetes mellitus or frequent hypoglycemia, or severe hypoglycemic episodes requiring emergency intervention in the last 6 months; (5) history of bladder dysfunction, incontinence, pyelonephritis, urosepsis, or frequent urinary tract infections; (6) anemia with hemoglobin <8 g/dL; (7) pregnant or breastfeeding; (8) history of serious hypersensitivity; (9) participation in another trial with an investigational drug within 30 days before informed consent; (10) use of another SGLT2i; (11) anticipated inability to

participate in the required study procedures; and (12) inability to give written informed consent. In an effort to improve enrollment rate and the cumulative safety experience gained in the ongoing large SGLT-2i trials, the eGFR inclusion criterion was modified to ≥20 mL·min^{-1}·1.73 m^{-2} in July 2018.

Study Design

The study was a randomized, double-blind, placebo-controlled crossover study consisting of treatment with either 10 mg empagliflozin or matched placebo daily for 14 days followed by a 2-week washout period and crossover to 14 days of treatment with the alternate therapy (Figure I in the Data Supplement). Randomization was performed in permuted blocks by the Yale–New Haven Hospital Investigational Drug Service.

Study Procedures

On days 1 and 14 of each study arm, patients underwent body fluid space measurements followed by biospecimen collection. On arrival to the clinical research unit, an intravenous catheter was placed, and the participant then underwent a 1-hour period of quiet recumbency. The angle of the bed was noted at visit 1, and the same bed angle was set for visit 2. Blood was collected from the existing intravenous catheter into chilled potassium EDTA tubes and immediately placed on ice. All biomarkers, including neurohormones, were measured at the start of each study visit before study drug administration.

Patients were asked to perform an overnight fast the night before the study visit. At the beginning of each study visit, baseline body weight, vital signs, and blood and urine samples were collected. After a 60-minute recumbency period, blood volume was determined via indicator dilution with I-131 albumin (Daxor Inc, New York, NY). Next, empagliflozin or matched placebo and 10 g of 99.9% deuterium oxide were administered orally. All patients then received a 500-mL bolus of 5% dextrose in sterile water administered intravenously over 30 minutes followed by a continuous infusion of 100 mL/h to optimize the fidelity of urine clearance periods throughout the early part of the study visit. In 12 patients, because of a national shortage of 5% dextrose in water, the 500-mL bolus was replaced with consumption of 500 mL of an oral sports beverage (Gatorade) over 30 minutes followed by 100 mL/h. Each patient received the same hydration route for testing during his or her crossover treatment.

Vital signs, blood and urine samples, blood glucose levels, and sonographic postvoid residual volumes were obtained at 1.5, 3, 4.5, and 6 hours after empagliflozin or matched placebo administration. During this time, all urine produced was collected in 1.5-hour cumulative collections, ending with each specified time point. Three hours after the patient was given empagliflozin or matched placebo, intravenous bumetanide was administered in a dose equivalent to the patient's home loop diuretic dose, up to 4 mg. Patients who were not on a long-term loop diuretic (n=1) received 0.5 mg IV bumetanide. At 6 hours, body weight was measured. Patients also had a safety visit on days 3 and 7 of each study arm to ensure stable renal function, electrolytes, and blood pressure. After a 2-week washout, participants were crossed over to the opposite therapy, and the above protocol was repeated in an identical fashion.

Trial End Points

The study was designed to evaluate both the immediate (day 1) and 14-day diuretic and cardiorenal effects of empagliflozin. The primary short-term end point was the natriuretic effect of empagliflozin both as monotherapy and in combination with loop diuretics. The primary 14-day end point was to understand if these acute natriuretic effects would translate into improved volume status after 14 days of therapy, as assessed by change in blood volume. Secondary end points were the change in neurohormones within 14 days of therapy. We also sought to extensively characterize the cardiorenal effects of empagliflozin in patients with HF; thus, a number of exploratory end points and biomarkers were also included.

Assays and Calculations

Throughout this article, fractional excretion of sodium (FENa) is the primary metric used to describe sodium handling. FENa was chosen because it offers the best instantaneous assessment of sodium excretion, which is required given the various natriuretic experimental conditions during each study visit. When referring to other metrics such as absolute sodium excretion (millimoles of sodium excretion during a timed collection), we explicitly specify the metric used. We defined HF with reduced ejection fraction as a left ventricular ejection fraction ≤40%. eGFR was calculated with the cystatin-based and creatinine-based Chronic Kidney Disease Epidemiology formulas.[23]

Blood volume, plasma volume, and red cell mass were determined on the BVA 100 semiautomated blood volume machine (Daxor Inc) by I-131 albumin indicator dilution and spun hematocrit values. Plasma renin activity, norepinephrine, and aldosterone were measured with the commercially available ELISA kit from ALPCO according to the manufacturer's instructions (ALPCO, Salem, NH). Total renin was measured with ELISA kits from R&D Systems (Minneapolis, MN). The total renin immunoassay kit from R&D Systems recognizes both active renin and prorenin. Further assay description can be found in Methods in the Data Supplement. All neurohormonal and inflammatory biomarkers were log-transformed before analysis.

Statistical Analysis

Descriptive analysis and statistical tests were performed with SPSS, version 26 (IBM, Armonk, NY), SAS software, version 9.4 (SAS Institute Inc, Cary, NC), and Stata version 13.1 (StataCorp LP, College Station, TX). Data with a normal distribution are presented as mean±SD. Categorical values are presented as frequencies and percentages, and data with a skewed distribution are shown as median with interquartile ranges (IQRs). We examined the difference between 2 interventions over time using the linear mixed model with random intercepts that incorporate correlated outcomes within subjects. The interactions between time (categorized as either within-visit time point or between-visit time point) and intervention (empagliflozin versus placebo) were tested for the intervention effects at a 5% significance level. To account for crossover design effect, the linear mixed model was adjusted for the interaction between time and the order of the interventions.

RESULTS

A CONSORT (Consolidated Standards of Reporting Trials) diagram is shown in Figure II in the Data Supplement. The cohort was predominately male (75%) and obese (body mass index, 37±7 kg/m^2), with an average age of 60±12 years. The median hemoglobin A_{1c} was 7.1% (6.2%–10.5%), and 40% (8 of 20) were administering insulin at home. Patients with HF with reduced ejection fraction made up 45% of the cohort (9 of 20). Participants were largely diuretic resistant; the mean daily home loop diuretic dose was 244±306 mg furosemide equivalents and the peak FENa after receiving 1.9±1.4 mg bumetanide during the placebo period was 3.9±1.9% (normal response would be an increase in FENa of >20%).[24] Full baseline characteristics can be found in Table 1. To check for carryover effect, we examined the differences in serum and urinary electrolytes, in addition to the primary end points. We found no difference with respect to those who received empagliflozin or placebo first (P>0.18 for all).

Effect of Empagliflozin on Renal Glucose Handling

Oral empagliflozin was rapidly absorbed and delivered to the renal tubular site of action, as evidenced by a 27-fold increase in urinary glucose excretion that peaked at 3 hours and remained stable at that level for the remainder of the 6-hour study visit (Figure 1). After 14 days of empagliflozin therapy, there was no evidence of either tachyphylaxis to or intensification of the glucosuric effect; repeated dosing yielded similar peak levels of glucose excretion at both the day 1 and day 14 visit (P=0.43; Figure 1). There was also no evidence of a carryover effect because glucose excretion before empagliflozin was similar between empagliflozin and placebo, regardless of randomization order (P=0.82). As previously reported, patients with a higher eGFR had substantially greater glucose excretion than patients with a lower eGFR (P for interaction=0.001; Figure III in the Data Supplement).

Effect of Empagliflozin on Renal Sodium Handling

A modest natriuretic effect was observed with empagliflozin monotherapy (P<0.001), with a greater natriuresis over the 3-hour period after empagliflozin during both the day 1 (P<0.001) and day 14 (P=0.02) visits (Figure 2). The natriuresis demonstrated a temporal pattern similar to that of glucose excretion, with the 3-hour FENa highest during the day 1 visit but a more sustained increase observed at the day 14 visit. However, there was no significant attenuation in FENa

Table 1. Characteristics of the Patients at Baseline

Characteristic	Cohort (n=20)
Age, y	60±12
Female sex, n (%)	5 (25)
Body mass index, kg/m^2	37±7
Race, n (%)	
Black	8 (40)
NYHA functional classification, n (%)	
3	6 (30)
4	0 (0)
Heart rate, bpm	74±12
Systolic blood pressure, mm Hg	126±18
Left ventricular ejection fraction, %	42.9±15.0
HFrEF, n (%)*	9 (45)
NT-proBNP (IQR), pg/mL	399 (139–2000)
Principal cause of heart failure, n (%)	
Ischemic	5 (25)
Nonischemic	15 (75)
Medical history, n (%)	
Hypertension	19 (95)
Coronary artery disease	12 (60)
Arrhythmia	11 (55)
Hyperlipidemia	16 (80)
Hemoglobin A_{1c} (IQR), %	7.1 (6.2–10.5)
Renal function	
eGFR by CKD-EPI, mL·min^{-1}·1.73 m^{-2}	69.1±19.0
<60 mL·min^{-1}·1.73 m^{-2}, n (%)	7 (35)
Device therapy, n (%)	
Implantable cardioverter-defibrillator	10 (50)
Cardiac resynchronization therapy	2 (10)
Heart failure medication, n (%)	
Loop diuretic	19 (95)
Oral furosemide equivalents, median (IQR), mg	80 (40–300)
ACE inhibitor, ARB, or ARNi	17 (85)
β-Blocker	19 (95)
Mineralocorticoid receptor antagonist	10 (50)
Digoxin	2 (10)
Glucose-lowering medications, n (%)	
Any form of insulin†	8 (40)
Metformin	12 (60)
Sulfonylurea	3 (15)
DPP-4 inhibitor	2 (10)

ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ARNi, angiotensin receptor-neprilysin inhibitor; CKDEPI, Chronic Kidney Disease Epidemiology Collaboration; DPP-4 = dipeptidyl peptidase 4 inhibitor; eGFR, estimated glomerular filtration rate; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

*HFrEF was defined as ejection fraction ≤40%.

†Includes short-, intermediate-, and long-acting forms of insulin.

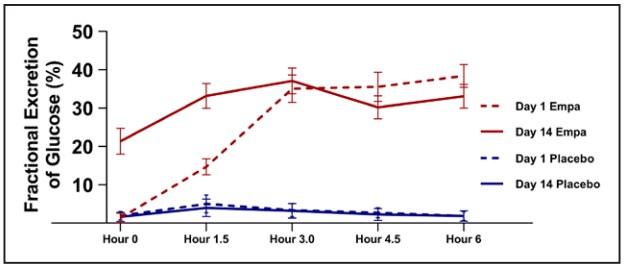

Figure 1. Fractional excretion of glucose on day 1 (dotted line) and day 14 (solid line) of treatment.
Empa indicates Empagliflozin.

with monotherapy between the day 1 visit and after 14 days of therapy (*P*=0.44). In addition, natriuresis was greater with empagliflozin at the day 3 and day 7 safety visits (FENa, 2.4±2.6% versus 1.6±1.6%; *P*=0.041). After the administration of bumetanide, a significant synergistic effect on natriuresis was observed in patients receiving empagliflozin during both the day 1 (*P*<0.001) and day 14 (*P*=0.008) visits (Figures 2 and 3). Similarly, there was no detectable attenuation of the synergistic natriuretic effect after 14 days of empagliflozin therapy (*P*=0.15). With respect to FENa, there was no difference between patients who received empagliflozin first or second (*P*=0.74). Over the 6-hour study visits, urine output (1735 mL [IQR, 1365–2225 mL] versus 1405 mL [IQR, 1150–2065 mL]; *P*=0.001), net fluid balance (–732 mL [IQR, –382 to –1086 mL] versus –377 mL [IQR, –75 to –933 mL]; *P*=0.001), and total sodium output (137 mmol [IQR, 87–180 mmol] versus 111 mmol [IQR, 68–174 mmol]; *P*=0.03) were greater with empagliflozin than placebo, with no difference in this effect between the day 1 and day 14 visits (*P*=NS for all comparisons).

Unlike the strong effect of eGFR on glucose excretion noted above, eGFR had a much smaller effect on the natriuretic effect of empagliflozin. For example, among patients receiving empagliflozin, the cumulative glucose excreted during the 6-hour study visits was linearly related to eGFR (*r*=0.61, *P*<0.001), but total sodium output was not (*r*=0.20, *P*=0.24), findings true in both the pre– and post–loop diuretic periods. Similarly, in patients with an eGFR <60 mL·min^{-1}·1.73 m^{-2}, total sodium output was not significantly different from

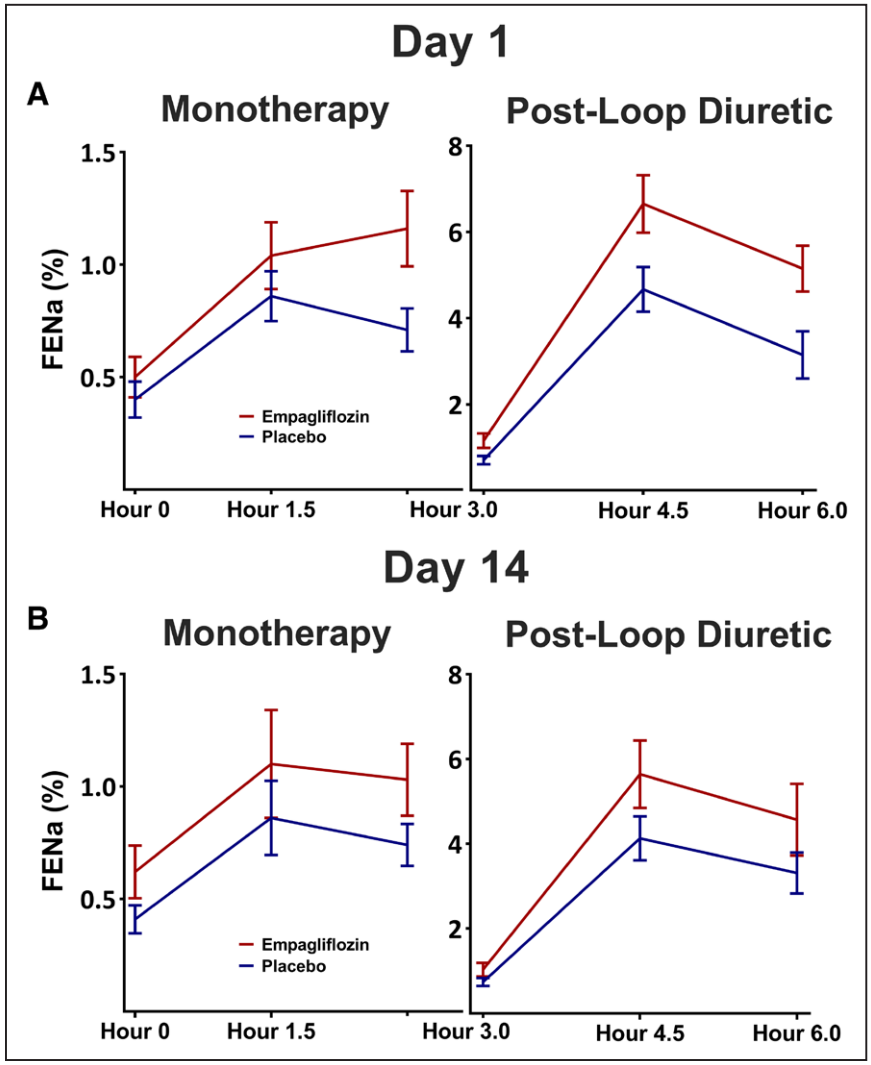

Figure 2. Empagliflozin increased natriuresis as monotherapy (A) and in combination with a loop diuretic (B) both with the first dose (top) and after 14 days of therapy (bottom).
FENa indicates fractional excretion of sodium.

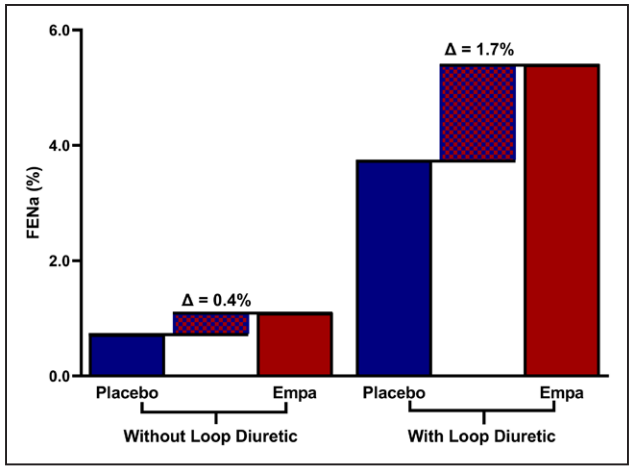

Figure 3. Natriuresis at the day 1 visit.
Solid bars represent measured sodium excretion under the 4 different experimental conditions. Thatched area represents the difference in natriuresis between the empagliflozin (Empa) period and the placebo period. The enhancement during the loop diuretic period is >4-fold greater than what is observed during monotherapy, illustrating the synergistic effect between loop diuretics and empagliflozin. FENa indicates fractional excretion of sodium.

that in patients with an eGFR ≥60 mL·min^{-1}·1.73 m^{-2} (*P*=0.36), in contrast to the large differences noted in glucose excretion (Figure III in the Data Supplement).

Mechanism of Sodium Excretion

To better understand the mechanism of increased sodium excretion (ie, direct effect versus passive osmotic diuresis from glucosuria), we evaluated the relationship between the glucose osmotic load and metrics of sodium output and natriuresis. During the monotherapy period (when osmotic effects should be most prominent), urine osmolarity was not significantly increased by empagliflozin (401±158 mOsm/L versus 399±179 mOsm/L; *P*=0.92), and there was only a small increase in total urine osmolarity (221±121 mOsm versus 180±119 mOsm; *P*=0.04). To further explore any possible contribution of urine osmolarity to sodium excretion, we examined the associations between several metrics of glycosuria (ie, fractional excretion of glucose, total glucose excretion, urinary glucose concentration) and natriuresis and found no significant positive correlation (Figure 4).

Changes in Volume Status and Red Cell Indexes

Fourteen days of empagliflozin therapy resulted in a greater reduction in total blood volume (*P*=0.035) and plasma volume (*P*=0.04) compared with placebo (Figure 5 and Figure IV in the Data Supplement). With respect to change in blood volume, there was no difference between patients who received empagliflozin first and those who received empagliflozin second (*P*=0.79).

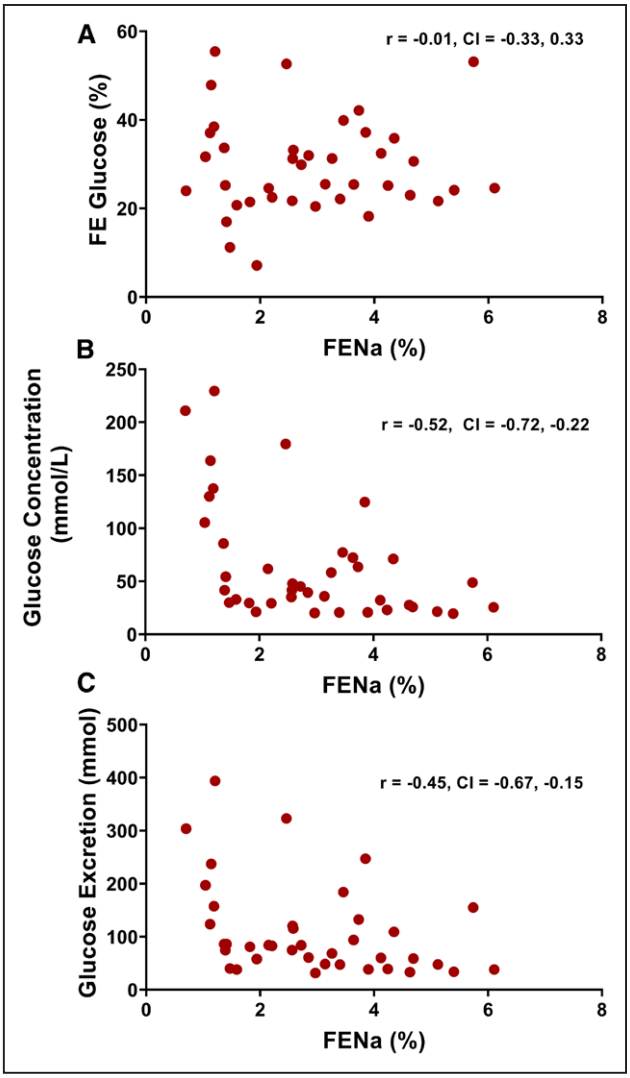

Figure 4. Relationship between natriuresis and metrics of glucosuria.
During the empagliflozin period, there was no association seen between fractional excretion of sodium (FENa) and fractional excretion of glucose (**A**). There was an inverse correlation between both FENa and urinary glucose concentration (**B**) and glucose excretion (**C**) over the 6-hour study visits.

Baseline blood volume and change in blood volume with empagliflozin did not differ between patients with HF with reduced ejection fraction and all others (*P*>0.65 for both), and there was no difference in the primary end points when patients were stratified above or below the median baseline blood volume (Table I in the Data Supplement). Total body water (*P*=0.001) and body weight (*P*=0.005) decreased during empagliflozin compared with placebo (Figure 6 and Figure IV in the Data Supplement). There was a moderately strong correlation between both weight loss and plasma volume loss (*r*=0.50, *P*<0.001) and weight loss and total body water loss (*r*=0.61, *P*<0.001).

At 14 days, erythropoietin increased more in patients receiving empagliflozin compared with those receiving placebo (*P*=0.037). Empagliflozin appeared to be protective against decreasing hematocrit during the 14-day

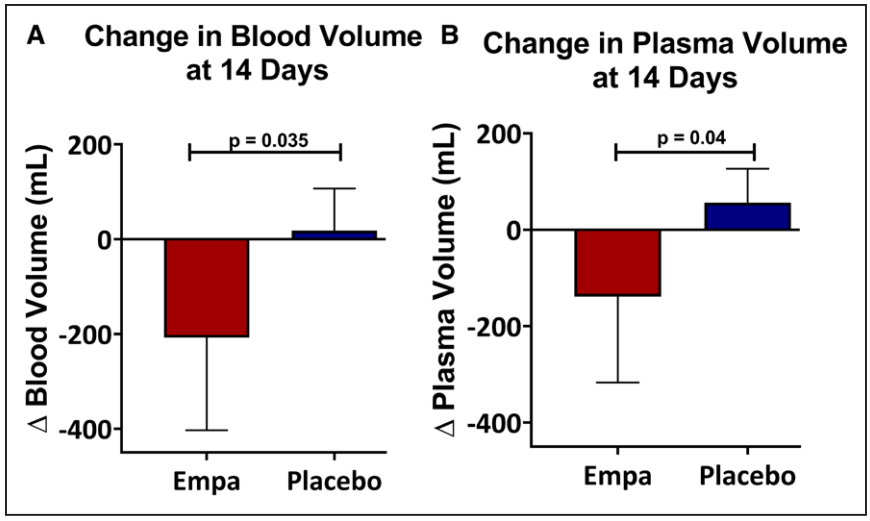

Figure 5. Effects of empagliflozin (Empa) on total blood volume and plasma volume. During the empagliflozin period, patients lost significantly more blood volume (**A**) and plasma volume (**B**) than during the placebo period.

phlebotomy-intensive study (total of 193 mL blood removed over the 6-week study); subjects had a 0.7±2.1% decrease in hematocrit during placebo, whereas hematocrit was stable (0.0±2.2% change) during empagliflozin (treatment by randomization order, *P*=0.011). Change in measured red cell volume was not significantly different between the empagliflozin and placebo periods (*P*=0.64). Change in NT-proBNP (N-terminal pro-B-type natriuretic peptide) was not different between the empagliflozin (–20.8 [IQR, –230.3 to 369.4 pg/mL) and placebo (40.9 [IQR, –316.4 to 382.9]; *P*=0.67) periods.

Effect of Empagliflozin on Potassium, Magnesium, and Uric Acid Handling

There was no change in total 6-hour potassium excretion with empagliflozin versus placebo (28.9±11.2 mmol versus 26.6±8.9 mmol; *P*=0.20). Serum potassium levels were not different between the empagliflozin and placebo periods (4.3±0.5 mEq/L versus 4.3±0.5 mEq/L; *P*=0.51). On day 1, there was a trend toward a reduction in total urine magnesium excretion (3.8±1.5 mmol versus 4.6±1.2 mmol, *P*=0.08) with empagliflozin, and the urine sodium-to-magnesium ratio substantially increased (95±56 versus 55±23; *P*=0.002). At the 14-day visit, the increase in sodium/magnesium ratio had significantly attenuated (64±27, *P*=0.002; versus 56±24, *P*=0.037; time by treatment *P*=0.006). The increase in magnesium excretion after 14 days of empagliflozin therapy was likely related to re-establishment of magnesium balance because the plasma magnesium level significantly increased with empagliflozin (14-day magnesium level, 2.4±0.3 mEq/L versus 2.1±0.3 mEq/L at baseline; *P*<0.001). Empagliflozin therapy increased the fractional excretion of uric acid (*P*<0.001), with the largest effect surprisingly seen during the combination therapy with loop diuretic (9.7±3.1% versus 7.7±3.3%; *P*=0.001). This uricosuric effect resulted in a reduction in serum uric acid levels after 14 days of therapy with empagliflozin versus placebo (7.0±2.0 µmol/L

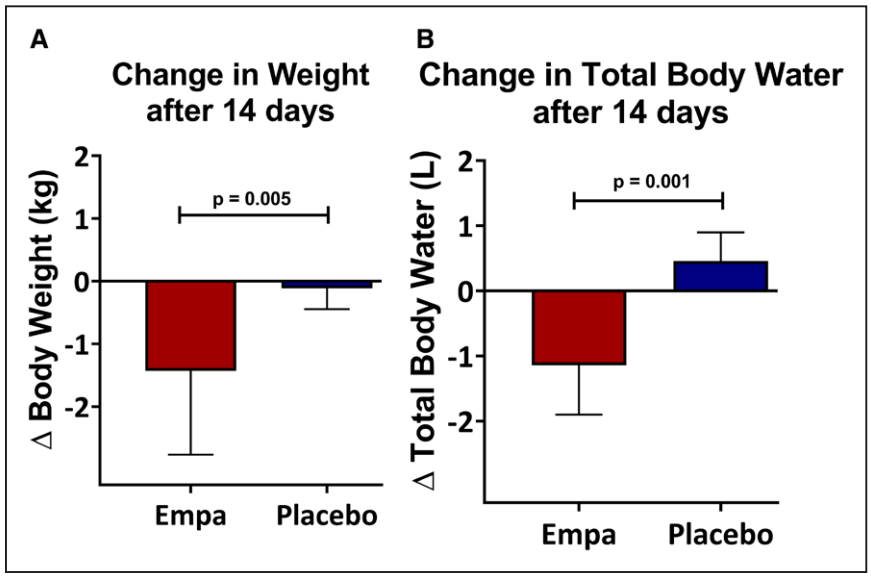

Figure 6. Effects of Empagliflozin on weight and total body water. During the empagliflozin (Empa) period, patients lost significantly more weight (**A**) and total body water (**B**).

versus 7.6±2.4 µmol/L; *P*=0.008). Last, we examined the effect of urine urea concentrations and found no difference between empagliflozin and placebo periods during either the day 1 (69±29 mmol/L versus 99±38 mmol/L; *P*=0.13) or day 14 (87±31 mmol/L versus 101±35 mmol/L; *P*=0.57) visit.

Neurohormonal Activation and Inflammatory Biomarkers

Despite a reduction in blood volume, there was no detectable activation of the renin-angiotensin-aldosterone system, with stable levels of plasma renin activity, total renin, and aldosterone (Table 2). Changes in plasma norepinephrine levels were significantly better with empagliflozin compared with the placebo period (*P*=0.023; Table 2). There were no differences in inflammatory biomarkers tested, including C-reactive protein, interleukin-6, interleukin-10, interleukin-18, tumor necrosis factor receptors 1 and 2, or growth/differentiation factor-15 (Table 2). Fasting ketone levels tended to increase after 14 days of empagliflozin therapy compared with placebo (0.046±0.131 mmol/L versus −0.012±0.105 mmol/L), a finding of borderline statistical significance (*P*=0.05). Absolute levels of biomarkers are reported in Table II in the Data Supplement.

Effects on Glomerular Filtration, Tubular Injury, and Renal Secretory Capacity

Overall, empagliflozin was well tolerated from a renal perspective. Changes in creatinine-based eGFR (−5.2±6.5 mL·min^{-1}·1.73 m^{-2} versus −1.2±7.6 mL·min^{-1}·1.73 m^{-2}; *P*=0.11), cystatin C eGFR (−4.1±11.0 mL·min^{-1}·1.73 m^{-2} versus −1.8±8.6 mL·min^{-1}·1.73 m^{-2}; *P*=0.50), β2-microglobulin (0.81±1.81 µg/mL versus −0.21±1.77 µg/mL; *P*=0.13), 6-hour creatinine clearance (6.3±49.5 mL·min^{-1}·m^{-2} versus −2.8±20.5 mL·min^{-1}·m^{-2}; *P*=0.46),

Table 2. Biomarkers

Biomarker	Change From Beginning to End of Treatment Period		*P* Value
	Empagliflozin	Placebo	
Neurohormones			
Norepinephrine, nmol/L	0.09 (−1.39 to 0.71)	0.7 (0.02 to 2.33)	0.023*
Plasma renin activity, ng·mL^{-1}·h^{-1}	0.84 (−4.90 to 13.81)	0.56 (−2.07 to 10.69)	0.63
Total renin, pg/mL	241.3 (−252.8 to 744.9)	368.5 (−56.3 to 1062.1)	0.50
Aldosterone, pg/mL	18.3 (−3.0 to 41.4)	1.7 (−16.4 to 23.9)	0.35
Copeptin, pg/mL	−8.19 (−45.15 to 14.81)	−4.04 (−19.09 to 16.31)	0.08
Cardiac			
NT-proBNP, pg/mL	−20.8 (−230.3 to 369.4)	40.9 (−316.4 to 382.9)	0.67
Endothelin, pg/mL	−0.13 (−0.68 to 0.41)	−0.15 (−0.66 to 0.31)	0.95
Troponin I, ng/mL	−5.6 (−71.5 to 226.0)	41.7 (−71.6 to 127.6)	0.17
Renal			
Urine albumin, mg/g	0.4 (−10.0 to 7.4)	0.02 (−4.6 to 6.5)	0.39
Urine NGAL, pg/mg	−67 (−948 to 1751)	305 (−1246 to 1890)	0.61
Urine KIM-1, ng/mg	90 (−514 to 1211)	226 (−132 to 894)	0.023*
Inflammatory			
IL-6, pg/mL	0.0 (−2.32 to 4.88)	−1.24 (−10.25 to 1.24)	0.43
IL-10, pg/mL	0.0 (0.0 to 3.56)	0.0 (−3.56 to 20.6)	0.62
IL-18, pg/mL	−4.5 (−15.8 to 13.9)	−0.3 (−17.6 to 9.8)	0.99
GDF-15, pg/mL	370.1 (−479.1 to 1194.0)	183.6 (−299.5 to 877.3)	0.96
CRP, mg/L	0.07 (−0.14 to 0.41)	−0.01 (−0.65 to 0.24)	0.48
TNF-R1, pg/mL	78.5 (−141.7 to 205.6)	45.4 (−137.5 to 224.3)	0.52
TNF-R2, pg/mL	410.8 (−807.9 to 824.9)	588.8 (−334.4 to 1319.4)	0.22
Other			
Erythropoietin, mIU/mL	3.44 (−2.46 to 11.87)	0.95 (−2.97 to 4.79)	0.037*

Values are reported as median (IQR). Renal biomarkers were normalized to urine creatinine. CRP indicates C-reactive protein; GDF-15, growth/differentiation factor-15; IL, interleukin; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TNF-R1, tumor necrosis factor receptor 1; and TNF-R2, tumor necrosis factor receptor 2.

*$P<0.05$.

and albuminuria (0.4 mg/g creatinine [IQR, −10.0 to 7.4 mg/g] versus 0.02 mg/g creatinine [IQR, −4.6 to 6.4]; *P*=0.20) did not differ significantly between 14 days of empagliflozin versus placebo. The change in the renal tubular injury biomarker urine KIM-1 (kidney injury molecule-1) was significantly better during empagliflozin therapy (90 ng/mg creatinine [IQR, −514 to 1211]) than the placebo period (226 ng/mg creatinine [IQR, −132 to 894; *P*=0.023]). Urine neutrophil gelatinase-associated lipocalin was not significantly different (*P*=0.61) during the empagliflozin versus the placebo period (Table 2).

Vital Signs, Electrolytes, and Adverse Events

In general, empagliflozin was well tolerated with few reported adverse events. Specifically, there were no instances of genitourinary infections, symptomatic hypoglycemia, or diabetic ketoacidosis. There were no significant differences in systolic blood pressure (121±12 mm Hg versus 127±20 mm Hg), diastolic blood pressure (74±12 mm Hg versus 74±11 mm Hg), mean arterial pressure (90±10 mm Hg versus 92±13 mm Hg; *P*>0.45 for all), or heart rate (76±12 bpm versus 78±12 bpm; *P*=0.79) between the empagliflozin and placebo periods. In addition, there was no significant effect on serum sodium (136±7 mEq/L versus 136±6 mEq/L), bicarbonate (22.0±4.0 mEq/L versus 22.8±5.7 mEq/L), chloride (101±4 mEq/L versus 99±4 mEq/L), calcium (9.4±0.4 mg/dL versus 8.8±2.0 mg/dL), or phosphorus (4.2±1.1 mg/dL versus 3.7±0.7 mg/dL) levels (*P*=NS for all).

DISCUSSION

The primary findings of this study of empagliflozin in patients with type 2 diabetes mellitus and HF are as follows: (1) Empagliflozin modestly enhanced natriuresis as monotherapy and, when combined with loop diuretics, exerted a meaningful synergistic natriuretic effect. (2) Unlike traditional diuretics, potassium wasting did not worsen, and both magnesium and uric acid handling improved. (3) The natriuretic effect of empagliflozin was not driven by the urinary glucose load, indicating a direct natriuretic effect rather than an "osmotic" diuresis. (4) Unlike the effect on glucose excretion, the degree of renal dysfunction had limited importance in determining the natriuretic effect. (5) A natriuretic effect was sustained through 14 days of therapy with empagliflozin, leading to a reduction in blood and plasma volume. (6) This intravascular volume contraction did not come at the expense of a significant renin-angiotensin-aldosterone system or sympathetic nervous system activation, hypotension, or reflex tachycardia. (7) Empagliflozin therapy was not associated with a significant decline in glomerular filtration rate across multiple filtration markers, and biomarkers of renal tubular injury were either unchanged or improved.

This study provides evidence that empagliflozin functions as a loop diuretic adjuvant with a clinically significant effect on natriuresis and what appears to be an excellent neurohormonal/renal/electrolyte safety profile. The most frequently used loop diuretic adjuvants in patients with HF are the thiazide and thiazide-type diuretics.[25] However, these agents are known to substantially increase potassium and magnesium wasting, worsen uric acid excretion, and commonly lead to a deterioration in renal function.[26] Notably, the electrolyte abnormalities associated with combined loop-thiazide therapy are often clinically significant/severe, and the resultant hypokalemia may play a role in the signals for worsened survival associated with adjuvant thiazide use.[27] In the present study, despite a significant adjuvant natriuretic effect, there was no change in potassium excretion and improvement in both magnesium and uric acid levels at 14 days. Furthermore, despite the significant reduction in plasma volume, we did not see any detectable worsening in renal function by several metrics, with evidence of some parameters actually improving. Overall, these findings suggest that empagliflozin may prove to be a preferred diuretic adjuvant over thiazides. Additional research to confirm these findings and to understand the performance of empagliflozin in the setting of acute HF with active IV diuretics is needed.

It is well described that despite days of aggressive intravenous dosing of loop diuretics, many patients hospitalized with decompensated HF do not experience an improvement in intravascular volume.[13,28–31] This inability of traditional diuretics to consistently improve intravascular volume is not unique to HF. For example, in otherwise healthy hypertensive volunteers, 30 days of furosemide 40 mg twice daily did not reduce plasma volume.[21] The lack of change in plasma volume is driven by the vigorous renal compensatory response to defend intravascular volume triggered by loop diuretics. Within minutes of loop diuretic administration, induction of neurohormonal systems and rapid deployment of mechanisms to conserve sodium occur, leading to the development of diuretic resistance, which is detectable within the first dose of diuretic.[21,32,33] Some of this effect (eg, renin secretion) is mediated by antagonism of sodium chloride entry into the primary salt sensor of the kidney, the macula densa.[34] This effect theoretically could be observed with any proximally acting natriuretic agent and may not be specific to the SGLT2i class.

In contrast to the effect of loop diuretics, empagliflozin resulted in a persistent natriuresis at 14 days with a reduction in plasma volume. Counterintuitively, both occurred without triggering a detectable increase in neurohormonal activation. Several previous studies of SGLT2i in animal models or humans have identified a minimal increase or even suppression in neurohormonal levels.[19,35–39] One candidate mechanism that may explain

the contrasting observations with SGLT-2is versus loop diuretics is the resultant sodium chloride delivery to the macula densa with these drugs. Although loop diuretics directly antagonize sodium chloride entry into the macula densa, increasing renin-angiotensin-aldosterone system and sympathetic nervous system activity, SGLT2is inhibit sodium transport proximally, thus increasing sodium chloride delivery.[40] This increased sodium chloride delivery appears to be durable and of a physiologically relevant magnitude in humans, as evidenced by the decrease in glomerular filtration rate with initiation and increase in glomerular filtration rate with cessation of long-term SGLT2i therapy.[41] These changes in glomerular filtration rate are thought to be secondary to tubuloglomerular feedback, which is also a response of the macula densa to increased salt delivery.[42,43] As a result, although a reduction in blood pressure and plasma volume would be expected to activate neurohormonal and sodium retaining systems, the increased sodium chloride delivery to the macula densa may counterbalance this effect.

Limitations

Our study has several limitations. Because this was a mechanistic study using an intensive protocol, the sample size is small, and patients were highly selected from a single center. To facilitate the crossover design, only stable, euvolemic patients with chronic HF were enrolled, so it remains unknown how these results may be applied to patients with acute decompensated HF and significant volume overload. Similarly, to ensure that patients would have stable volume status and medical therapy for crossover, the treatment and washout periods were short by necessity. Given the lack of significant attenuation in the monotherapy natriuretic effect at 14 days, we can conclude that these patients most likely had not re-established sodium balance. As a result, the ultimate long-term, fully adapted effects of empagliflozin on renal electrolyte handling and cardiorenal function cannot be determined from this study. Although the natriuretic effects were unrelated to the glucosuric effects, the study enrolled only patients with diabetes mellitus; thus, the results in patients without diabetes mellitus may differ. All patients in this study were fasting from the night before the study visit. It is known that sodium intake is a primary determinate of sodium output, in both the basal and diuretic-induced states. As a result, it remains unknown how a dietary sodium load would influence empagliflozin-induced natriuresis. Last, although this study focused on natriuretic effects, multiple alternative hypotheses for the mechanism of benefit to SGLT2is have been proposed. Although our study highlighted the positive effects of empagliflozin on natriuresis and plasma volume, we cannot confirm or exclude these other mechanisms as important drivers of improved outcomes.

Conclusions

In this randomized, placebo-controlled crossover study of diabetic patients with chronic stable HF, empagliflozin resulted in a significant augmentation of natriuresis when combined with loop diuretics. This synergistic natriuresis persisted over the 14-day study period, resulting in an improvement in plasma volume, but did not occur at the expense of off-target electrolyte wasting, renal dysfunction, or neurohormonal activation. This seemingly ideal diuretic profile may offer significant advantages in the management of volume status in patients with HF and may represent an important mechanism underlying the superior long-term HF outcomes observed with these agents.

ARTICLE INFORMATION

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Dr Testani reports personal fees from Reprieve Medical, grants and personal fees from BMS, personal fees from AstraZeneca, personal fees from Novartis, grants and personal fees from 3ive Labs, personal fees from Cardionomic, personal fees from Bayer, grants and personal fees from Boehringer Ingelheim, personal fees from MagentaMed, grants from Otsuka, grants and personal fees from Sanofi, grants and personal fees from FIRE1, grants from Abbott, personal fees from W.L. Gore, personal fees from Windtree therapeutics, and grants from Merck outside the submitted work; in addition, Dr Testani has patents for treating diuretic resistance filed and issued. Dr Riello reports consulting fees from Janssen, Johnson & Johnson, Pfizer, and Portola and serves on advisory boards at AstraZeneca, Janssen, Johnson &Johnson, Medicure, and Portola. Dr Inzucchi serves on committees at Boehringer-Ingelheim and Lexicon/Sanofi; serves as a consultant for

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Supplemental Materials

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