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RESEARCH ARTICLE

The blunted loop diuretic response in acute heart failure is driven by reduced tubular responsiveness rather than insufficient tubular delivery. The role of furosemide urine excretion on diuretic and natriuretic response in acute heart failure

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Aims Diuretic response in heart failure is blunted when compared to healthy individuals, but the pathophysiology underlying this phenomenon is unclear. We aimed to investigate whether the diuretic resistance mechanism is related to insufficient furosemide tubular delivery or low tubular responsiveness.

Methods and results We conducted a prospective, observational study of 50 patients with acute heart failure patients divided into two groups based on previous furosemide use (furosemide naïve: $n = 28$ [56%] and chronic furosemide users: $n = 22$ [44%]). Each patient received a protocol-derived, standardized furosemide dose based on body weight. We measured diuretic response and urine furosemide concentrations. The furosemide naïve group had significantly higher urine volumes and natriuresis when compared to chronic users at all timepoints (all $p < 0.05$). Urine furosemide delivery was similar in furosemide naïve versus chronic users after accounting for differences in estimated glomerular filtration rate (28.02 [21.03–35.89] vs. 29.70 [18.19–34.71] mg, $p = 0.87$). However, the tubular response to delivered diuretic was dramatically higher in naïve versus chronic users, that is the urine volume per 1 μg/ml of urine furosemide at 2 h was 148.6 ± 136.1 versus 50.6 ± 56.1 ml ($p = 0.005$).

Conclusions Patients naïve to furosemide have significantly better diuresis and natriuresis when compared to chronic furosemide users. The blunted diuretic response in patients with chronic loop diuretic exposure is driven by decreased tubular responsiveness rather than insufficient furosemide tubular delivery.

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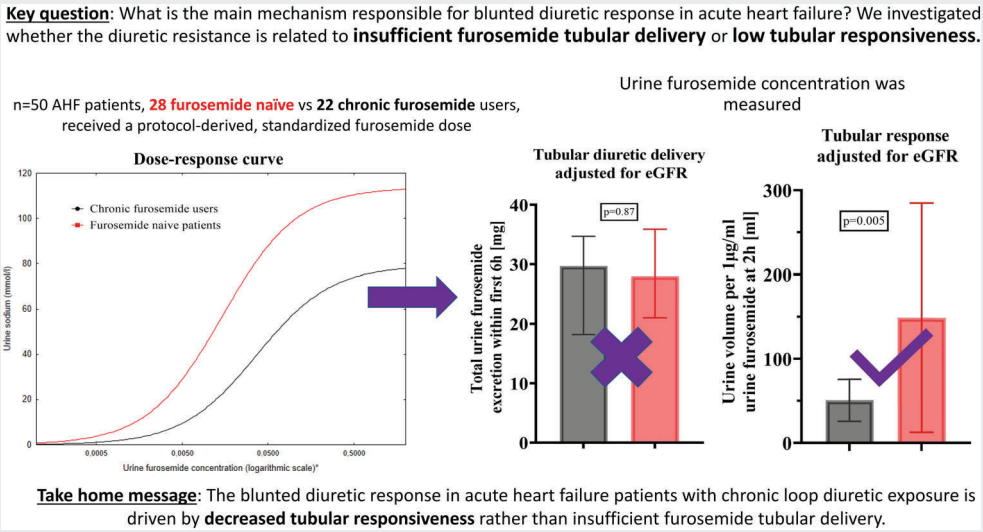
Graphical Abstract



The mechanism of blunted diuretic response in acute heart failure. AHF, acute heart failure; eGFR, estimated glomerular filtration rate.

Keywords Diuretic response • Furosemide • Loop diuretics • Acute heart failure

Introduction

Loop diuretics (i.e. furosemide) are the first-line drugs used to treat congestion in acute heart failure (AHF).[1,2] In general, the diuretic response in heart failure (HF) is blunted (the dose–response curve is moved to the right and downward) when compared to healthy controls.[3–6] The diuretic response to furosemide is believed to be impacted by several factors, including but not limited to diuretic dose, systolic blood pressure, digestive tract absorption (if given orally), glomerular filtration rate (GFR), uraemic anions, neurohormonal activity and degree of fluid overload, all of which make a diuretic response difficult to predict.[7–9] Whether the observed decrease in diuretic response is a result of HF itself or adaptations to chronic use of the loop diuretic remains unanswered.[10,11] Theoretically, there are at least two main mechanisms that may lead to the lower diuretic responsiveness in AHF:

(i) Decreased tubular delivery. To stimulate natriuresis/diuresis, furosemide must reach an adequate concentration at the site of action, the Na^+-K^+-$2Cl^-$ cotransporters (NKCC) in the lumen of the nephron. In healthy individuals, ~65% of the furosemide is excreted unchanged in urine.[9,12] In HF, furosemide may not be delivered to the nephron's lumen due to dysfunction of the organic anion transporter-1 or 2 (OAT 1 or 2) (lower number of transporters or their low efficacy), which actively transport furosemide across the tubular lumen to its site of action. If this is a predominant diuretic resistance mechanism, the urine furosemide excretion should be decreased via a pharmacokinetic resistance mechanism.

(ii) Decreased tubular responsiveness. In HF, the kidney may undergo pathological adaptations (both functional and structural) that drive diuretic resistance such as decreased number of NKCC in the loop of Henle, lower efficiency of the NKCC or alterations of the distal convoluted tubule.[11] Moreover, furosemide also blocks NKCC in the juxtaglomerular cells, which provokes chloride undersensing and further activates the renin–angiotensin–aldosterone system that promotes sodium avidity. If those are a predominant diuretic resistance mechanism, furosemide urine excretion should not be affected and the diuresis is blunted by pharmacodynamic resistance mechanisms.

To investigate these diuretic resistance mechanisms, we aimed to test whether the diuretic response to furosemide in AHF is different between chronic furosemide users versus furosemide naïve patients and the relationship between diuretic response and furosemide tubular delivery.

Methods

This is a single-centre, prospective, observational study that was conducted between June 2021 and April 2022 at the Institute of Heart Diseases, Wroclaw Medical University, Poland. AHF was defined according to the European Society of Cardiology (ESC) guideline criteria.[2] The

research study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

Study population

All adult patients hospitalized with AHF, willing to participate were screened for the study. Only patients who fulfilled inclusion criteria and did not meet exclusion criteria and who signed an informed consent form were enrolled. Inclusion criteria were: (i) hospital admission for a primary diagnosis of AHF, (ii) clinically overt hypervolaemia defined as lower extremity oedema reaching at least knees, (iii) N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2000 pg/ml, (iv) planned intravenous (IV) furosemide therapy, (v) the study could be initiated within 36 h of hospital admission to ensure all patients were in the active decongestive phase of AHF, as urine Na^+ and diuretic response may vary on different phases of AHF.[13,14] Additionally, the study had to be initiated at least 6 h from the last dose of IV (or oral) furosemide to ensure at least a 6 h washout period. Exclusion criteria included: (i) active infection defined as a C-reactive protein >50 mg/dl, procalcitonin >0.1 pg/ml, or receiving antibiotics, (ii) acute coronary syndrome, (iii) end-stage renal disease requiring renal replacement therapy, (iv) cardiogenic shock, (v) recipient of contrast media within the last 72 h, or (vi) inability to cooperate with study procedures. All patients were instructed to limit their fluid intake to 1.5–2 L per 24 h as well as they were advised to limit their daily sodium intake during hospitalization as part of institutional routine clinical practice. As urine furosemide concentrations were not measured in real time, the treating physicians could not utilize these data to guide treatment decisions.

Study phases

The patient's response to furosemide was divided into two phases. The first phase (from baseline to 6 h after furosemide administration) measured the diuretic effect of furosemide. This phase usually commenced between 7:30 and 8:30 a.m. After baseline assessments patients received a standardized dose of furosemide (see below). All oral (morning) drugs were administered prior to the IV furosemide dose. No other drugs were allowed to be initiated/continued during the phase to reduce the potential confounders. Patients already on sodium–glucose cotransporter 2 inhibitors received the drug without interruptions. Patients were supervised by study personnel during this phase. The blood samples for analyses were collected at baseline, 3, 6 and 24 h. In this phase, exact urine output was recorded hourly. After collecting a baseline urine sample, subsequent urine samples were collected at 1, 2, 3, 6 h from the cumulative urine collected from the previous hour for urine furosemide concentration and composition analyses.

In the second phase of the study (6 to 24 h), the urine collection was continued to create a 24 h urine collection. In this phase, all medications and IV infusions were allowed if needed. Additional furosemide was allowed only in this phase in patients who fulfill the criteria for 'rescue furosemide' (see below).

Furosemide dosage

The study population was divided into two groups based on their previous furosemide use: furosemide naïve (those who did not receive furosemide prior to admission), and patients with chronic furosemide use prior to hospital admission. To ensure comparable doses of furosemide between patients were used, all patients received protocol-driven (standardized) diuretic therapy. The dose of furosemide was calculated as 1 mg/kg of body weight. One-half of total weight-based dose of furosemide was administered as bolus (in order to reach high drug's serum concentration, so-called 'ceiling'), immediately followed by the other 50% of the weight-based dose administered as a 2 h infusion. Thus, a 100 kg patient received an IV bolus of furosemide 50 mg followed by 50 mg as a 2 h intravenous infusion.

Patients, with poor diuretic response during the first phase of the study defined as: urine output <100 ml/h at the 5th and 6th hour of the first study phase were allowed to a 'rescue furosemide' dose of an additional 40 mg of furosemide during the second phase of the study.

Methods of urine furosemide assessment

Each urine sample was assessed for urine composition at the local laboratory, and samples were also frozen for further analyses – including furosemide concentration. The urine samples were analysed according to the modified application note by Camag, Switzerland. All extracts were kept frozen prior to analysis. Directly before the analysis the dry residue was dissolved in 1 ml of methanol and subjected to the quantitative analysis with high performance thin layer chromatography system. Chromatography was carried in horizontal Teflon DS-chambers (Chromdes, Lublin, Poland). The analysis was performed with AS30 applicator (Biostep-Desaga, Wiesloch, Germany) CD60 densitometer (Biostep-Desaga, Wiesloch, Germany) with detection at 220 nm UV light, Desaga Proquant software (Ver. 3.05; Biostep-Desaga, Wiesloch, Germany) and Camag Derivatizer (Camag, Muttenz, Switzerland). For each chromatographic plate, the separate six-point calibration curve was applied. Each sample was analysed in quadruplicate. The urine furosemide excretion was calculated by multiplying the urine furosemide concentration by urine volume. The furosemide excretion adjustments were made based on proportional modelling relative to baseline estimated GFR (eGFR).

Laboratory assessment of blood and urine

Laboratory assessments were performed using standard methods. The baseline eGFR that was used for the further adjustment was calculated by local laboratory based on serum creatinine. Creatinine clearance as well as neurohormones: dopamine, adrenaline and noradrenaline were assessed from the 24 h urine collection that was initiated at the time of study start. In addition, the following laboratory tests were performed:

- Plasma NT-proBNP (method: immunoenzymatic, Siemens, Marburg, Germany) at baseline and 24 h.
- Urine composition (Na^+, K^+, Cl^-, creatinine, urea) at baseline, 1, 2, 3, 6, 24 h.

Fraction excretion of sodium (FENa) was calculated using standard formula:

$$FENa = (\text{urinary } Na^+ \times \text{plasma creatinine})/(\text{plasma } Na^+ \times \text{urinary creatinine}) \times 100.$$

Statistical analysis

Continuous variables with a normal distribution are described using means ± standard deviation, variables with skewed distribution described by medians (with upper and lower quartiles [IQR]). Categorical variables are presented as numbers and percentages. The

Table 1 Baseline characteristics and comparison between chronic furosemide users versus furosemide naïve patients

	All patients ($n = 50$)	Furosemide naïve ($n = 28$)	Chronic furosemide users ($n = 22$)	*p*-value
Patients	50 (100)	28 (56)	22 (44)	
Age (years)	66 ± 14	63 ± 14	69 ± 12	0.09
Male sex	46 (92)	25 (89)	21 (95)	0.42
Ischaemic aetiology of heart failure	15 (30)	4 (14)	11 (50)	<0.01
De novo AHF	22 (44)	20 (71)	2 (9)	<0.0001
Heart rate (bpm)	86 ± 17	91 ± 17	79 ± 13	<0.01
Systolic blood pressure (mmHg)	120 ± 20	123 ± 21	116 ± 19	0.20
Diastolic blood pressure (mmHg)	75 ± 16	81 ± 17	68 ± 12	<0.01
Ejection fraction (%)	36 ± 14	35 ± 16	39 ± 12	0.17
Total dose of furosemide at baseline (mg)	95 ± 20	94 ± 22	95 ± 18	0.92
NT-proBNP (pg/ml)	6872 (4488–12 564)	6972 (4793–15 390)	6645 (3074–12 206)	0.56
Serum creatinine at baseline (mg/dl)	1.49 ± 0.62	1.23 ± 0.47	1.82 ± 0.63	<0.001
eGFR (ml/min/1.73 m^2)	58.4 ± 25.7	69.1 ± 26.6	45.3 ± 17.3	<0.001
Serum blood urea nitrogen (mg/dl)	59 (40–90)	40 (31–61)	80 (59–102)	<0.0001
Haemoglobin (g/dl)	12.7 ± 2.5	13.5 ± 2.3	11.6 ± 2.4	0.01
Concomitant medications				
Beta-blocker	36 (72)	16 (57)	20 (95)	0.01
ACEI	29 (58)	12 (43)	17 (77)	0.01
ACEI[a]	17 (34)	10 (36)	7 (32)	0.70
ARB	9 (18)	5 (18)	4 (18)	0.96
Sacubitril/valsartan	2 (4)	0 (0)	2 (9)	0.11
ACEI/ARB/ sacubitril/valsartan	38 (76)	17 (61)	21 (95)	0.005
MRA	22 (44)	10 (36)	12 (55)	0.22
SGLT2 inhibitors	8 (16)	4 (14)	4 (18)	0.75
Thiazides	4 (8)	1 (4)	3 (14)	0.19

Data are presented as *n* (%), mean ± standard deviation, or median and interquartile range (Q25–Q75).
ACEI, angiotensin-converting enzyme inhibitor; AHF, acute heart failure; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SGLT2, sodium–glucose cotransporter 2.
[a]At 100% target dose.

statistical significance of differences between groups was assessed using *t*-test or Mann–Whitney U test as appropriate for distribution. The interrelations between the variables were examined using Spearman's test, multivariable regressions. The dose–response curves were modelled using Statistica software. A *p*-value <0.05 was considered statistically significant. Statistical analyses were performed using Statistica version 13 (StatSoft, Tulsa, OK, USA).

Results

Baseline characteristics

Baseline characteristic are presented in *Table 1*. The study population ($n = 50$) was predominantly male (92%) with a mean age of 66 ± 14 years. The mean ejection fraction was 36 ± 14%, and the median (IQR) NT-proBNP was 6872 (4488–12 564) pg/ml. There were 28 (56%) patients naïve to furosemide of which 8 (33%) had a prior HF diagnosis and received non-diuretic HF therapies prior to hospital admission. The mean dose of oral furosemide that chronic furosemide users had received before hospital admission was 63.6 ± 32.6 mg. The mean weight-based dose of IV furosemide administered in the study was 95 ± 20 mg.

Comparison of baseline characteristics between chronic furosemide users and furosemide naïve patients

Furosemide naïve patients did not differ from patients on chronic furosemide therapy across most baseline characteristics including mean age (63 ± 14 vs. 69 ± 12 years), mean ejection fraction (35 ± 16% vs. 39 ± 12%), total dose of furosemide administered at baseline (94 ± 22 vs. 95 ± 18 mg), or serum sodium (140 ± 4 vs. 139 ± 7 mmol/L) (all $p > 0.05$). However, serum creatinine and blood urea nitrogen were significantly higher in chronic furosemide recipients compared to furosemide naïve patients (*Table 1*).

The trajectories of diuretic and natriuretic response to furosemide in acute heart failure

The trajectory of diuretic and natriuretic response to furosemide in both groups was similar. There was a steep increase in urine sodium concentration, urine volume, and FENa at 1–2 h after furosemide administration, which decreased over the remaining study period. However, there was a substantial difference in

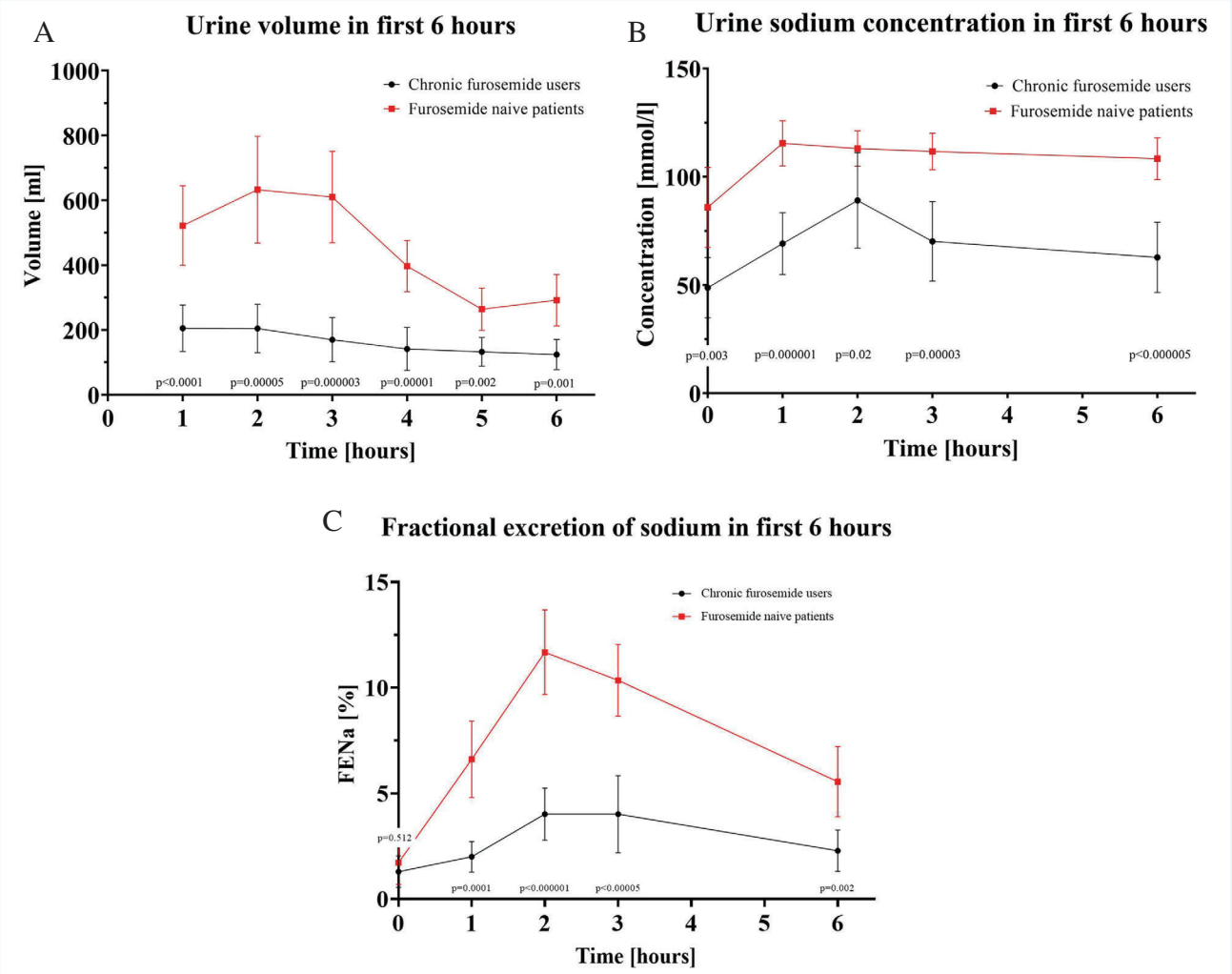


Figure 1 Comparison of diuretic indexes between chronic furosemide users and furosemide naïve patients. (*A*) Urine volumes, (*B*) urine sodium concentration, (*C*) fractional excretion of sodium (FENa).

diuretic and natriuretic response to furosemide between study groups. The furosemide naïve group had significantly higher urine volumes when compared to chronic users at all time points in the first study phase (all $p < 0.05$) (*Figure 1A*, *Table 2*). Patients naïve to furosemide when compared to chronic furosemide users had significantly higher spot urine sodium concentrations at baseline and all timepoints of the first phase of the study (all $p < 0.01$) (*Figure 1B*, *Table 2*). There was no difference in baseline FENa between groups, but patients naïve to furosemide had significantly higher FENa during all subsequent time points of the first phase of the study (*Figure 1C*, *Table 2*).

Urine furosemide concentration

Furosemide was detected in the urine at 1 h with a peak concentration at 2 h. The trajectory of urine furosemide excretion was similar between patients naïve to furosemide and chronic furosemide users. The urine furosemide concentration was significantly lower in patients naïve to furosemide when compared to chronic furosemide users at 1, 2, and 3 h, but this difference was no longer significant at 6 h (*Table 3*). The cumulative absolute furosemide excretion within the first 6 h was significantly higher in furosemide naïve AHF patients (16.51 [13.57–27.34] vs. 11.81 [9.51–15.46] mg, $p = 0.01$) (*Table 3*). However, this difference between groups no longer persisted after adjustment for eGFR. The eGFR-adjusted absolute furosemide excretion was 28.02 (21.03–35.89) versus 29.70 (18.19–34.71) mg/eGFR, ($p = 0.87$) (*Figure 2A*). The propensity score matching by eGFR led to similar results (online supplementary *Table S1*).

Predictors of diuretic response

There was a significant inverse correlation between urinary furosemide concentration at 2 h and the following variables: cumulative 6 h urine output ($r = -0.53$, $p < 0.001$), cumulative 24 h urine output ($r = -0.55$, $p < 0.0001$), cumulative 24 h

Table 2 Diuretic, natriuretic effect and kidney function

	All patients	Furosemide naïve	Chronic furosemide users	*p*-value
Weight at baseline (kg)	96 ± 20	95 ± 21	96.8 ± 18.2	0.93
Weight at 24 h (kg)	95 ± 20	93 ± 21	97.6 ± 17.6	0.61
Weight change at 24 h (kg)	−1.21 (−1.9 to −0.3)	−1.65 (−2.7 to −1.0)	−0.40 (−1.0 to 0.10]	<0.001
Need for rescue furosemide, *n* (%)	10 (20)	1 (4)	9 (41)	<0.001
Urine volume (ml)				
At 1 h	383 ± 302	522 ± 316	205 ± 161	<0.0001
At 2 h	444 ± 397	633 ± 425	205 ± 168	<0.0001
At 3 h	416 ± 363	610 ± 364	170 ± 154	<0.0001
At 4 h	284 ± 221	397 ± 204	141 ± 150	<0.0001
At 5 h	206 ± 156	264 ± 168	133 ± 100	<0.01
At 6 h	218 ± 187	292 ± 204	124 ± 105	0.001
Mean urine nolume 1–6 h (ml)	325 ± 225	453 ± 213	163 ± 104	<0.0001
Urine volume 7–24 h (ml)	1035 ± 572	1187 ± 669	849 ± 357	0.04
Total urine volume (ml)	3000 ± 1585	3943 ± 1486	1842 ± 676	<0.0001
Urine Na^+ (mmol/l)				
Baseline	69 ± 44	86 ± 47	49 ± 32	<0.01
At 1 h	97 ± 36	116 ± 27	69 ± 30	<0.0001
At 2 h	103 ± 36	113 ± 21	89 ± 47	0.02
At 3 h	94 ± 36	112 ± 21	70 ± 39	<0.0001
At 6 h	89 ± 36	108 ± 23	63 ± 34	<0.0001
Total Na^+ excreted at 24 h urine collection (mmol/24 h)	304 ± 248	441 ± 237	118 ± 94	<0.0001
FENa (%)				
Baseline	0.73 (0.34–1.70)	0.74 (0.32–1.72)	0.68 (0.34–1.70)	0.93
At 1 h[a]	3.21 (1.48–6.28)	5.15 (3.12–8.82)	1.44 (0.87–2.88)	<0.0001
At 2 h[a]	7.46 (3.70–13.07)	12.53 (7.91–14.72)	3.70 (2.32–5.58)	<0.0001
At 3 h[a]	7.89 (2.68–12.67)	11.53 (6.59–13.54)	2.61 (0.88–7.45)	<0.0001
At 6 h	3.26 (1.41–5.95)	3.76 (2.50–7.99)	1.60 (0.54–3.30)	<0.01
Serum creatinine (mg/dl)				
Baseline	1.49 ± 0.62	1.23 ± 0.47	1.82 ± 0.63	<0.0001
At 24 h	1.49 ± 0.66	1.23 ± 0.52	1.83 ± 0.68	<0.001
Measured creatinine clearance (ml/min/1.73 m^2)	64.27 ± 39.77	76.45 ± 39.68	48.89 ± 35.08	<0.01
Measured creatinine clearance <60, *n* (%) ml/min/1.73 m^2	25 (50)	10 (36)	15 (68)	<0.01
Serum Na^+ (mmol/l)				
Baseline	140 ± 5	140 ± 4	139 ± 7	0.57
At 6 h	139 ± 6	141 ± 3	136 ± 7	0.05
At 24 h	140 ± 5	141 ± 4	139 ± 6	0.26

FENa, fraction excretion of sodium.
[a]Calculated based on baseline serum creatinine.

sodium excretion ($r = -0.55$, $p < 0.001$), and FENa at 2 h ($r = -0.59$, $p < 0.0001$). In multivariable models, urine furosemide concentration at 2 h (and eGFR) remained inversely and independently associated with the following variables: cumulative 6 h urine output (β-coefficient = −0.38, $p = 0.002$), cumulative 24 h urine output (β-coefficient = −0.46, $p = 0.002$), and cumulative 24 h sodium excretion (β-coefficient = −0.41, $p < 0.005$) (*Table 4*).

Diuretic efficiency

Different definitions of diuretic efficiency were tested (*Table 5*). In general, patients naïve to furosemide exhibited significantly higher tubular response defined as diuretic response per 1 μg/ml of urine furosemide. The tubular responsiveness was 2–3-fold higher among furosemide naïve patients (*Figure 2B*). The naïve group had significantly higher urine sodium concentration and urine volumes as a response to a given urine furosemide concentration and this phenomenon remained significant after adjustments for eGFR (*Figure 3*, *Table 5*). The sensitivity analyses revealed similar results (online supplementary *Table S1*).

Dose–response curves

Modelling of dose–response curves has shown substantial differences between study groups. Patients naïve to furosemide had significantly more robust response in terms of urine volume

Table 3 Urine furosemide excretion during the first 6 h of treatment

	Furosemide naïve	Chronic furosemide users	*p*-value
Raw urine furosemide concentration (μg/ml)			
At 1 h	8.27 (5.76–10.56)	12.43 (8.44–22.14)	0.01
At 2 h	8.05 (5.76–11.34)	13.60 (9.26–22.21)	0.007
At 3 h	7.05 (5.56–9.17)	11.79 (7.39–24.81)	0.009
At 6 h	6.80 (3.73–11.28)	9.99 (7.07–12.70)	0.14
Urine furosemide excretion (mg/h)			
At 1 h	3.78 (1.70–5.01)	2.30 (1.61–4.28)	0.23
At 2 h	4.14 (2.61–8.06)	3.13 (1.50–4.22)	0.04
At 3 h	3.85 (1.92–6.49)	1.74 (1.18–2.78)	<0.0005
At 6 h	1.62 (0.88–2.24)	0.92 (0.78–1.63)	0.09
Urine furosemide excretion adjusted for eGFR (mg/h)[a]			
At 1 h	4.75 (3.57–7.44)	6.77 (2.24–10.02)	0.70
At 2 h	5.46 (4.33–9.89)	5.24 (4.16–8.32)	0.56
At 3 h	6.13 (4.14–8.03)	3.84 (2.21–5.47)	0.03
At 6 h	2.41 (1.13–4.24)	2.09 (1.55–3.73)	0.90
Cumulative urine furosemide excretion			
During the 1st phase of the study (mg/6 h)	16.51 (13.57–27.34)	11.81 (9.51–15.46)	0.01
During the 1st phase of the study (mg/6 h) adjusted for eGFR[a]	28.02 (21.03–35.89)	29.70 (18.19–34.71)	0.87

eGFR, estimated glomerular filtration rate.
[a]Calculated for eGFR of 100 ml/min/1.73 m^2.

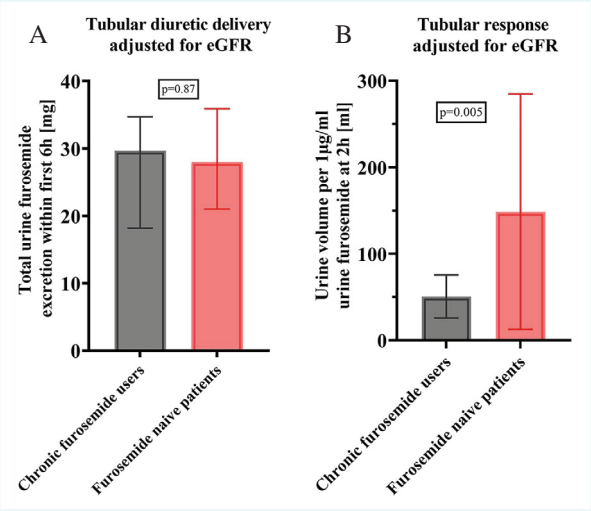


Figure 2 Comparison of major mechanisms leading to differences in diuresis in acute heart failure (adjusted for estimated glomerular filtration rate [eGFR]). (*A*) Total furosemide excretion and (*B*) tubular response between chronic furosemide users and furosemide naïve patients.

(*Figure 4A*), urine sodium excretion (*Figure 4B*) and FENa (*Figure 4C*) when compared to chronic furosemide users.

Discussion

There are several important findings in our study. First, patients with AHF naïve to loop diuretics had more robust diuretic response compared to chronic furosemide users, which was significant within the first hour and led to significantly better cumulative diuretic response. Second, the greater diuretic response in loop diuretic naïve patients was not due to better loop diuretic delivery to the nephron, as both groups had similar eGFR-adjusted cumulative furosemide urinary excretion. Instead, the greater diuretic response in diuretic naïve patients was due to better tubular response to furosemide. Diuretic naïve patients excreted approximately twice the urine sodium for a given urine furosemide concentration compared to patients on chronic loop diuretic therapy. Collectively, these findings indicate diuretic resistance in chronic loop diuretic therapy is driven by decreased tubular responsiveness rather than insufficient furosemide tubular delivery (*Graphical Abstract*).

The observed difference in diuretic response between groups was less likely to be attributed to factors historically believed to impact the diuretic response in AHF, including timing of diuretic treatment initiation, degree of fluid overload, or dose of diuretics, as those potential confounders were balanced between study groups. Similarly, systolic blood pressure and serum sodium were also the same between both groups, and therefore should not be the reason for the observed differences. Importantly, blood urea nitrogen, serum creatinine and eGFR were worse in chronic furosemide users. Furosemide urine delivery should not be directly affected by eGFR, as furosemide is predominantly secreted into the nephron rather than filtered by the glomerulus.[5,15] However, eGFR and tubular secretion are related, since eGFR reflects nephron mass and therefore it is a strong determinant of urine output. Thus, we adjusted urinary furosemide excretion for eGFR. The furosemide naïve group had higher urine furosemide excretion during the first phase of the study, which led to higher cumulative urine

Table 4 Impact of urine furosemide concentration at 2 h on diuretic and natriuretic response in acute heart failure

	Multivariate regression model (β-coefficient)	*p*-value
Urine output during first 6 h		
R-value of the model = 0.79, $p < 0.0001$		
Urine furosemide concentration at 2 h (μg/ml)	**−0.38**	**0.002**
Serum sodium (mmol/l)	0.08	0.296
Systolic blood pressure (mmHg)	0.10	0.403
Ejection fraction (%)	−0.23	0.121
Total dose of furosemide (mg)	0.14	0.208
eGFR (ml/min/1.73 m^2)	**0.47**	**<0.001**
NT-proBNP (pg/ml)	−0.02	0.86
Total urine output (24 h)		
R-value of the model = 0.72, $p < 0.00025$		
Urine furosemide concentration at 2 h (μg/ml)	**−0.46**	**0.002**
Serum sodium (mmol/l)	0.03	0.834
Systolic blood pressure (mmHg)	0.08	0.570
Ejection fraction (%)	−0.26	0.061
Total dose of furosemide (mg)	0.07	0.637
eGFR (ml/min/1.73 m^2)	**0.32**	**0.027**
NT-proBNP (pg/ml)	−0.06	0.703
Total urine sodium excreted at 24 h		
R-value of the model = 0.71, $p < 0.0009$		
Urine furosemide concentration at 2 h (μg/ml)	**−0.41**	**<0.005**
Serum sodium (mmol/l)	0.22	0.120
Systolic blood pressure (mmHg)	0.07	0.622
Ejection fraction (%)	−0.17	0.167
Total dose of furosemide (mg)	0.07	0.653
eGFR (ml/min/1.73 m^2)	**0.32**	**0.03**
NT-proBNP (pg/ml)	−0.05	0.742

Multivariate regression model consists of the following variables: urine furosemide concentration at 2 h (μg/ml), serum sodium (mmol/l), systolic blood pressure (mmHg), ejection fraction (%), total dose of furosemide (mg), estimated glomerular filtration rate (eGFR, ml/min/1.73 m^2) and N-terminal pro-B-type natriuretic peptide (NT-proBNP, pg/ml).

furosemide excretion. However, the difference disappeared once adjustments for eGFR were performed, indicating the difference in urine furosemide delivery may be a result of lower nephron number (in patients with lower eGFR) rather than true impediments in furosemide delivery.

Moreover, given the differences in baseline characteristics (i.e. kidney function) between both groups, and appreciating their potential importance and possible impact on the results, we have additionally run the propensity score matching (as a sensitivity analyses) and obtained the same final result (online supplementary *Table S1*). All these results suggest that decreased loop diuretic delivery to the nephron is not a major driver of blunted diuretic response in AHF.[16]

Importantly, both groups differed in furosemide urine concentration, which was significantly related to diuretic response in the AHF population. Although it seems initially counterintuitive that patients who were naïve to furosemide had significantly lower urine furosemide concentration, this was as a result of higher urine volume diluting the urine furosemide concentration. Additionally, we speculate that lower urine furosemide concentrations achieved the diuretic threshold, inducing the brisk response in patients with good tubular response. This is further supported by the fact that both eGFR and furosemide urine concentration were independent predictors of urine output, with an inverse relation between furosemide urine concentration and urine volumes measured at 6 h and 24 h.

The diuretic tubular responsiveness defined by different definitions of diuretic efficiency calculated per 1 μg/ml of urine furosemide were then examined. Patients naïve to furosemide have generally shown 2–3-fold higher tubular response in terms of both natriuretic response and urine output per given urine furosemide concentration. Unlike tubular drug delivery, this phenomenon was independent of eGFR, which supports the assumption that low tubular responsiveness is a much more important mechanism responsible for diuretic response in AHF.[16]

We uniquely demonstrated the heterogenicity of diuretic responsiveness within a group of patients with clinically evident AHF and fluid overload. Prior studies have shown that the dose–response curve is moved to the right and downward in stable, chronic HF patients, when compared to healthy controls.[3,6,17,18] Our study has confirmed the same phenomenon in

Table 5 Comparison of diuretic efficiency between furosemide naïve versus chronic furosemide users in acute heart failure

Variable	Furosemide naïve	Chronic furosemide users	*p*-value
Diuretic efficiency defined as urine sodium concentration per 1 μg/ml of urine furosemide concentration (mmol/μg)			
At 1 h	16.8 ± 11.2	6.5 ± 4.9	<0.001
At 2 h	15.9 ± 11.0	8.0 ± 5.9	0.008
At 3 h	17.4 ± 10.5	8.8 ± 9.6	0.007
At 6 h	22.4 ± 17.1	11.3 ± 11.3	0.022
Diuretic efficiency defined as urine sodium excretion per 1 μg/ml of urine furosemide excretion adjusted for eGFR (mmol/μg)[a]			
At 1 h	25.6 (12.8–33.1)	10.3 (5.5–25.5)	0.01
At 2 h	21.7 (14.1–29.2)	15.3 (6.4–34.6)	0.32
At 3 h	27.6 (17.4–37.8)	15.6 (3.9–35.1)	0.04
At 6 h	30.0 (18.5–59.7)	14.4 (5.2–29.0)	0.04
Diuretic efficiency defined by urine volume per 1 μg/ml of urine furosemide concentration (ml)			
At 1 h	75.2 ± 69.2	22.4 ± 18.3	0.003
At 2 h	98.4 ± 94.8	23.8 ± 26.6	0.002
At 3 h	100.6 ± 90.9	26.8 ± 36.1	0.001
At 6 h	70.3 ± 76.0	26.7 ± 33.0	0.028
Diuretic efficiency defined by urine volume per 1 μg/ml of urine furosemide concentration adjusted for eGFR (ml)[a]			
At 1 h	120.0 ± 94.7	56.2 ± 56.8	0.015
At 2 h	148.6 ± 136.1	50.6 ± 56.1	0.005
At 3 h	140.5 ± 115.4	58.5 ± 85.1	0.011
At 6 h	110.5 ± 112.6	55.6 ± 64.4	0.072

eGFR, estimated glomerular filtration rate.
[a]Calculated for eGFR of 100 ml/min/1.73 m^2.

AHF with high diuretic potential (in terms of vast fluid overload). In our study, we demonstrated that there is a significant difference in dose–response curves between two AHF groups. Patients naïve to furosemide had much more robust response, while chronic furosemide users exhibited dampened response similar to previously reported in chronic HF. Notably, the percentage of patients with *de novo* AHF was significantly higher among the furosemide naïve group. Therefore groupings may actually represent different stages of HF, which may also contribute to the differences in diuretic responsiveness.

From a clinical perspective, our study indicates that the pharmacokinetic mechanism of decreased diuretic delivery is not a major driver of inadequate diuretic response. This finding seems to be very timely based on the recent concept of prolonged subcutaneous furosemide administration. This method of drug delivery solves only the issue of furosemide bioavailability, but unfortunately is not able to overcome the true fundamental problem of tubular resistance to the drug. Therefore, we may speculate that the future interventions aimed at increasing the tubular responsiveness rather than increasing tubular delivery hold promise for facilitating decongestion in AHF. Moreover, diuretic dose–response curves demonstrate that furosemide's tubular delivery would need to be significantly increased by an intervention targeting increased diuretic delivery in order to obtain a clinically meaningful improvement in diuretic response.

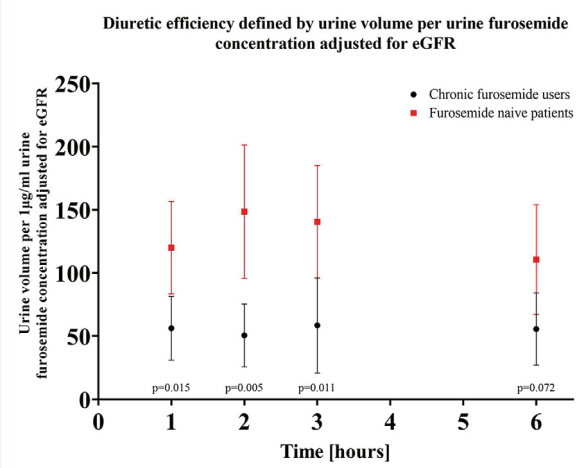


Figure 3 Hourly diuretic efficiency adjusted for estimated glomerular filtration rate (eGFR) between chronic furosemide users and furosemide naïve patients.

Limitations

To ensure a comparable potential for diuretic response between groups, we examined a highly selective patient population, which

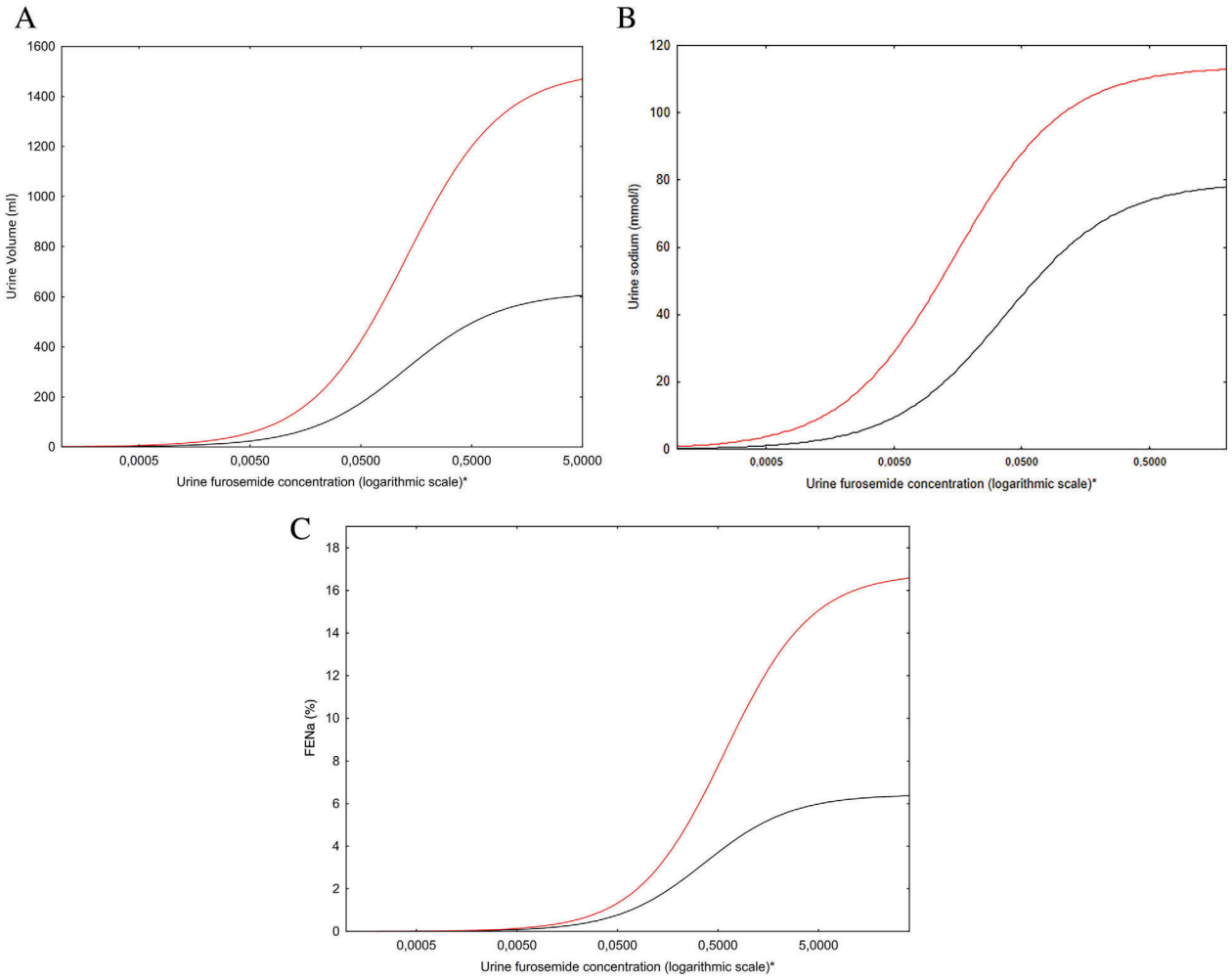


Figure 4 Comparison of the dose–response curves between chronic furosemide users and furosemide naïve patients. (*A*) Urine volume, (*B*) urine sodium concentration, (*C*) fractional excretion of sodium (FENa). Red line: furosemide naïve patients; gray line: chronic furosemide users.

may limit the generalizability of the results. Secondly, to be able to compare the furosemide urine concentrations and diuretic responsiveness, all patients received a standardized (calculated per kg of body weight) dose of furosemide, which is not a standard clinical practice. Although all patients were recruited at the first morning after hospital admission, some could have received IV furosemide before start of the study, which could have influenced some results despite a 6 h washout period. We used urine furosemide concentrations as an imperfect surrogate of the drug's tubular concentration, although this actually represents the end result of the complex process. One needs to remember that urine furosemide concentrations vary at different parts of the nephron, while final urine is being formed. Based on the urine furosemide concentration we are unable to differentiate the part of the nephron where sodium reabsorption/urine concentration takes place, which would be very helpful for better understanding of our results. Moreover, our study was not designed to describe the exact nature of the observed differences in renal responsiveness, as there are several possible explanations like lower number/efficiency of the NKCC or, already described in animal models, unfavorable cellular adaptations to chronic furosemide exposure.[11]

The study population consists of predominantly male patients. We did not monitor the length of loop diuretic use before hospitalization, which might impact the unfavourable tubular cells remodelling in chronic furosemide users. We did not test the furosemide bioavailability in the study. The assessment of eGFR should be ideally performed in a steady state, thus calculation in AHF settings may be biased. The baseline differences (in kidney function and other variables) between groups are known to impact the diuretic response. Despite the fact that we made the adjustments for eGFR as well as the propensity score matching to better cope with those differences, we need to emphasize that there may also still be different mechanisms/confounders like competitive inhibition of furosemide transport by anion uremic toxins, which we are not able to address. The use of sodium–glucose cotransporter 2 inhibitors in our cohort was not restricted and despite the

well-balanced distribution of these drugs in both groups it may have some impact on the results.

Conclusions

Patients naïve to furosemide have significantly better diuretic and natriuretic responses compared to chronic furosemide users. In AHF, the blunted diuretic response is driven mainly by decreased tubular responsiveness rather than insufficient furosemide tubular delivery.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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