Original Article

Hypochloremia and Diuretic Resistance in Heart Failure
Mechanistic Insights

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Background—Recent epidemiological studies have implicated chloride, rather than sodium, as the driver of poor survival previously attributed to hyponatremia in heart failure. Accumulating basic science evidence has identified chloride as a critical factor in renal salt sensing. Our goal was to probe the physiology bridging this basic and epidemiological literature.

Methods and Results—Two heart failure cohorts were included: (1) observational: patients receiving loop diuretics at the Yale Transitional Care Center (N=162) and (2) interventional pilot: stable outpatients receiving ≥80 mg furosemide equivalents were studied before and after 3 days of 115 mmol/d supplemental lysine chloride (N=10). At the Yale Transitional Care Center, 31.5% of patients had hypochloremia (chloride ≤96 mmol/L). Plasma renin concentration correlated with serum chloride (r=–0.46; P<0.001) with no incremental contribution from serum sodium (P=0.49). Hypochloremic versus nonhypochloremic patients exhibited renal wasting of chloride (P=0.04) and of chloride relative to sodium (P=0.01), despite better renal free water excretion (urine osmolality 343±101 mOsm/kg versus 475±136; P<0.001). Hypochloremia was associated with poor diuretic response (odds ratio, 7.3; 95% confidence interval, 3.3–16.1; P<0.001). In the interventional pilot, lysine chloride supplementation was associated with an increase in serum chloride levels of 2.2±2.3 mmol/L, and the majority of participants experienced findings such as hemoconcentration, weight loss, reduction in amino terminal, pro B-type natriuretic peptide, increased plasma renin activity, and increased blood urea nitrogen to creatinine ratio.

Conclusions—Hypochloremia is associated with neurohormonal activation and diuretic resistance with chloride depletion as a candidate mechanism. Sodium-free chloride supplementation was associated with increases in serum chloride and changes in several cardiorenal parameters.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02031354.

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Key Words: cardiorenal syndrome ■ chloride ■ diuretics

The fundamental role of sodium in maintenance of fluid homeostasis and progression of heart failure (HF) has been a central paradigm in medicine for more than a century.[1,2] As a result, a core treatment principal in HF management has been the avoidance of dietary sodium.[3] However, accumulating evidence ranging from the basic sciences to epidemiological studies has challenged this paradigm, suggesting that increased sodium chloride intake may be without deleterious consequences in HF; rather, it may even be beneficial.[4–8]

See Clinical Perspective

From a pathophysiological perspective, a beneficial effect of increasing total body sodium is a challenge to explain. However, there is a possibility that chloride, the counter ion to sodium in salt, might provide benefit; indeed, there is a rapidly accumulating body of science that challenges the assumed ancillary role of chloride in electrolyte homeostasis.[9] In the kidney, renal salt sensing is dependent on chloride rather than sodium, and renin secretion, tubuloglomerular feedback, and regulation of several diuretic-sensitive sodium channels is contingent on chloride rather than sodium.[9–13] The recent discovery of a family of chloride-sensitive kinases that regulate

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several ion transport and neurohormonal pathways represents a plausible mechanistic link between chloride and HF pathogenesis.[14–16] To that end, several recent epidemiological studies have identified low chloride, rather than sodium, as the primary ion driving the poor survival in HF previously attributed to hyponatremia.[17–19] Despite mounting data on the importance of chloride in HF from the basic sciences and large epidemiological studies, we lack an understanding of the mechanisms by which chloride plays a role in disease pathophysiology.

The goal of the current study was to probe the physiological underpinnings bridging this growing basic science and epidemiological literature. Specifically, we sought to (1) determine whether factors such as diuretic responsiveness and renin release would be associated with serum chloride levels as the above-cited literature would suggest, 2) probe the mechanisms underlying hypochloremia, and 3) provide proof of concept that manipulation of chloride homeostasis may be associated with measurable changes in physiological parameters.

Methods

Two HF patient cohorts were studied in this article, as delineated below. All patients provided written, informed consent, and both study protocols were approved by the Yale Institutional Review Board.

Yale Transition Care Center Cohort

The Yale Transition Care Center (YTCC) is an outpatient HF treatment and evaluation center that focuses primarily on fluid and diuretic management (https://www.ynhh.org/services/heart-failure/transitional-care-center.aspx). Consecutive HF patients receiving treatment with loop diuretics in the Transition Care Center were prospectively enrolled. Patient length of stay in the unit is generally 4 to 8 hours, and treatment consists of 1 to 3 doses of diuretic administered compared to that time period based on degree of volume overload and response to therapy. Patients receive either IV bumetanide or PO torsemide at the discretion of the clinician. All urine produced during the treatment period is saved in a cumulative urine collection container, which is sent to the clinical laboratory for measurement of electrolytes at the conclusion of treatment. Spot urine samples are obtained both before and 1 hour after administration of diuretics.

Lysine Chloride Study Cohort

Patients with HF were screened from the outpatient advanced HF clinic at Yale New Haven Hospital. At the screening visit, eligible patients were offered either 500 mg capsules of lysine chloride or lysine chloride powder to meet the 7 g, 3-times-daily lysine chloride requirement. Those that elected powder were asked to sample the lysine chloride powder in at least one diluent to ensure that palatability would not interfere with protocol adherence. Eligibility criteria included a home loop diuretic dose of ≥80 mg oral furosemide equivalents (80 mg furosemide=1 mg bumetanide=20 mg torsemide), and stable HF defined as (1) the absence of signs or symptoms of volume overload as determined by an advanced HF specialist and (2) no changes in HF medications for at least 2 weeks. Exclusion criteria included renal replacement therapy, an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2, significant electrolyte or acid–base disorders (as determined by the treating physician) that could increase risk of adverse effects, or an inability to comply with the study protocol. Study protocol consisted of 3 days of 3-times-daily supplementation with 7 g lysine chloride. Subjects were instructed to follow a low-sodium (<2000 mg) diet for 7 days before beginning the study and continue this throughout the study period. In addition to brief counseling by the study coordinator, patients were provided with an informational pamphlet from the Centers for Disease Control website (http://www.cdc.gov/salt/pdfs/sodium_dietary_guidelines.pdf). A 3-hour timed blood and urine collection protocol was performed in the morning before administration of the first dose of lysine chloride (day 1) and on the morning of administration of the last dose (day 4). A 24-hour urine collection was performed immediately before the beginning of each blood and urine collection (ie, on days 0 and 3). On study day 1 and 4, participants voided on arrival to the study visit, terminating the 24-hour urine collection. After this, an IV line was placed, and subjects reclined to a semirecumbent position for 30 minutes. At this time, blood samples were drawn, patients received a dose of oral torsemide equivalent to their usual home dose of loop diuretic, and a spot urine sample was obtained. A cumulative urine collection was obtained between 30 minutes and 2.5 hours after diuretic administration (allowing 30 minutes for absorption of oral torsemide). Oral torsemide was used for the study on days 1 and 4 because of its reproducible pharmacokinetics; on all other study days, patients were instructed to take their usual home loop diuretic.[20] This portion of the study was registered on the clinicaltrials.gov website (NCT02031354).

Calculations and Definitions

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.[21] Loop diuretic doses were converted to furosemide equivalents with 1 mg bumetanide=20 mg torsemide=40 mg intravenous furosemide=80 mg oral furosemide. Fractional excretions of sodium, chloride, potassium, and lithium were calculated as: Fractional excretion of $X=(X_{urine}/X_{serum})\times(Cr_{serum}/Cr_{urine})\times100$. The fractional excretion of endogenous lithium was used to probe the tubular location for hypochloremia-associated diuretic resistance because this is considered the gold-standard, in vivo measurement of proximal tubular and loop of Henle sodium handling.[22] When directly measured urine osmolality was not available, urine osmolality was calculated as 2×(urine sodium+urine potassium)+urine urea/2.8.

To account for the log-linear relationship between diuretic dose and sodium output,[23] diuretic efficiency was calculated as the increase in sodium output per doubling of the loop diuretic dose, centered on a dose of 40 mg of IV furosemide equivalents: diuretic efficiency=(mmol Na output)/($\log_2$(administered loop diuretic dose)−4.32). Doses ≤20 mg were Winsorized to 40 mg to avoid negative numbers. Early urinary diuretic excretion was estimated by multiplying the concentration of diuretic in the 1-hour spot urine sample by the urine output during the first 3 hours after diuretic administration, to transform from concentration to quantity. The quantity of diuretic excreted was then normalized to administered furosemide equivalents to allow comparison across patients. This normalization was accomplished by dividing the quantity of loop diuretic found in the urine by the published urinary clearance of the drug (50% for bumetanide and 17% for torsemide) and then converting to furosemide equivalents as described above.[20,24]

Hyponatremia was defined as a serum sodium concentration ≤135 mmol/L and hypochloremia as a serum chloride concentration ≤96 mmol/L, consistent with previously published definitions.[25,26] In the Lysine Chloride Study cohort, hemoconcentration was defined as any increase in serum albumin from day 1 to day 3.

Statistical Analysis

Values are reported means±SD, median (quartile 1–quartile 3), and percentiles.

In the YTCC cohort, Student *t* test, the Mann–Whitney test, or the Wilcoxon rank-sum test were used to compare continuous variables. Pearson χ^2 test was used to compare categorical variables. Correlation coefficients reported are Spearman in the case of comparisons of 2 continuous variables. To test for nonlinear relationships between odds for low sodium diuretic efficiency and serum chloride, a logistic model was fit using restricted cubic spline-transformed serum chloride. Predicted odds for low sodium diuretic efficiency were then plotted against serum chloride levels to visually assess this relationship. Multivariable linear regression was used to test for an independent association between serum chloride and total renin after controlling for serum sodium and eGFR. Multivariable logistic regression was used to determine whether an independent association between hypochloremia and low diuretic efficiency was present after controlling for serum sodium, bicarbonate, blood urea nitrogen, eGFR, use of angiotensin-converting enzyme

Table 1. Characteristics of the Study Population

	Overall Cohort (n=162)	Hypochloremia		*P* Value
		No (n=111)	Yes (n=51)	
Demographics				
Age	67.4±14.1	66.3±14.9	69.8±12.2	0.15
Black race	33.6%	39.4%	20.8%	0.02*
Male sex	61.10%	61.30%	60.80%	0.95
Medical history				
Hypertension	84.0%	84.7%	82.4%	0.71
Diabetes mellitus	49.4%	45.9%	56.9%	0.20
Gout	14.8%	14.4%	15.7%	0.83
Ischemic etiology	27.5%	27.5%	27.5%	0.99
Baseline medication use				
ACE or ARB	48.4%	55.5%	33.3%	0.009*
β-Blocker	72.7%	80.0%	56.9%	0.002*
Entry thiazide	12.4%	7.3%	23.5%	0.004*
Entry K-sparing diuretic	25.5%	25.5%	25.5%	>0.99
Digoxin	11.8%	5.5%	25.5%	<0.001*
Home loop diuretic dose (mg furosemide equivalents)	80 (40–160)	80 (40–120)	160 (80–160)	<0.001*
Baseline serum values				
Chloride, mmol/L	99 (96–102)	101 (99–103)	94 (90–96)	<0.001*
Sodium, mmol/L	138 (135–140)	139 (137–140)	135 (130–138)	<0.001*
Potassium, mmol/L	4.1 (3.8–4.4)	4.2 (3.9–4.4)	4.0 (3.7–4.5)	0.21
Bicarbonate, mmol/L	23.4 (21.5–25.7)	22.7 (21.3–24.7)	25.5 (23.0–28.6)	<0.001*
Anion gap, mmol/L	18.9 (16.8–20.9)	18.8 (16.5–20.6)	19.7 (17.9–21.7)	0.03*
Blood urea nitrogen, mg/dL	30.5 (21.0–47.0)	28.0 (20.0–42.0)	40.0 (24.0–65.0)	0.01*
Serum creatinine, mg/dL	1.6±0.8	1.5±0.6	1.7±1.0	0.06
NT pro-BNP, pg/mL	1950 (681–4900)	1850 (467–4380)	2585 (773–6130)	0.32
Albumin, g/dL	3.7±0.4	3.8±0.4	3.7±0.5	0.28
Hemoglobin, g/dL	12.0±2.3	12.0±2.1	11.9±2.6	0.77
GFR estimates				
Creatinine-based, mL/min/1.73 m^2	54.1±29.0	57.2±30.5	47.2±24.2	0.04*
Cystatin C-based, mL/min/1.73 m^2	48.0±32.0	52.5±34.7	38.1±22.5	0.01*
Creatinine and cystatin C-based, mL/min/1.73 m^2	48.5±25.3	52.1±26.4	40.4±20.9	0.01*
eGFR<60 mL/min/1.73 m^2	64.2%	62.2%	68.6%	0.43
Baseline urine values				
Concentrations:				
Sodium, mmol/L	51.5±34.0	56.4±36.3	41.3±26.4	0.02*
Potassium, mmol/L	50.0±24.8	49.5±25.4	50.9±23.8	0.77
Chloride, mmol/L	56.0±34.9	59.8±37.3	48.3±28.4	0.09
Anion gap, mmol/L†	40.8 (28.5–57.2)	42.2 (28.8–59.9)	36.5 (25.9–54.6)	0.30
Chloride/sodium ratio	1.2±0.6	1.2±0.6	1.4±0.6	0.06

(*Continued*)

Table 1. Continued

	Overall Cohort	Hypochloremia		*P* Value
		No	Yes	
	(n=162)	(n=111)	(n=51)	
Fractional excretions				
FENa, %	0.5 (0.2–1.3)	0.5 (0.2–1.1)	0.6 (0.2–1.9)	0.14
FECl, %	0.8 (0.4–1.9)	0.6 (0.3–1.5)	1.1 (0.6–3.4)	0.04*
FECl/FENa ratio	1.5 (1.3–2.0)	1.5 (1.1–1.8)	1.7 (1.4–2.4)	0.01*
FEK, %	18.6 (11.6–35.0)	15.7 (10.3–23.4)	34.1 (17.0–50.3)	<0.001*
FELi, %‡	16.4 (11.5–23.9)	15.9 (9.9–22.9)	16.9 (12.6–24.2)	0.58
Physical examination and LV function				
Weight, kg	98.3±30.9	100.6±31.4	93.5±29.5	0.17
BMI	34.6±10.6	35.1±10.7	33.6±10.6	0.46
Systolic blood pressure, mm Hg	122.9±18.9	125.8±18.7	116.7±18.1	0.005*
Heart rate, beats per min	76.0±13.6	75.4±13.2	77.2±14.5	0.44
Left ventricular ejection fraction, %	45.0 (27.0–57.0)	45.0 (27.0–59.0)	46.5 (25.0–56.0)	0.73
Ejection fraction ≤40%	45.5%	47.2%	42.0%	0.55

ACE indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate, as calculated by the Chronic Kidney Disease Epidemiology Collaboration mixed Cystatin C-creatinine formula; FECl, fractional excretion of chloride; FEK, fractional excretion of potassium; FELi, fractional excretion of lithium; FENa, fractional excretion of sodium; GFR, glomerular filtration rate; and NT pro-BNP, n-terminal probrain natriuretic peptide.

*P value < 0.05.

†Urine anion gap defined as urine sodium plus urine potassium minus urine chloride.

‡Marker of the fractional excretion of sodium from the proximal tubule and loop of Henle.

or angiotensin receptor blockers, thiazide, digoxin, or β-blockers, home loop diuretic dose, and facility-administered loop diuretic dose. Multivariable linear regression was used to test for a relationship between diuretic efficiency and serum sodium and serum chloride as continuous parameters, with adjustment for renal function as measured by eGFR. Cox proportional hazards modeling was used to evaluate time-to-event associations with the end point of all-cause mortality. Multivariable Cox regression models were constructed to evaluate the effect of hypochloremia, hyponatremia, and serum bicarbonate on survival while controlling for kidney function as measured by eGFR. We were not able to detect a statistically significant interaction between the type of diuretic administered and hypochloremia or serum chloride levels in any of these models, and as a result, patients receiving either diuretic were combined and treated as a group.

Changes in continuous parameters from baseline to after lysine chloride supplementation were evaluated using the Wilcoxon signed-rank test.

Statistical analysis was performed with IBM SPSS Statistics version 23 (IBM Corp, Armonk, NY) and Stata version 13 (StataCorp, College Station, TX), and statistical significance was defined as 2-tailed P<0.05 for all analyses except for tests for interaction, where P<0.1 was considered significant.

Assays

Urine and serum electrolytes were measured on a Randox Rx Daytona automated clinical chemistry analyzer using ion selective electrodes, and microalbumin, urea, creatinine, bicarbonate, and cystatin C were measured using Randox reagents as per the manufacturer's instructions (Randox Laboratories, UK). The concentration of interleukin-18, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin was measured using the MesoScale Discovery platform (Meso Scale Diagnostics, Gaithersburg, MD). Amino terminal pro B-type natriuretic peptide levels were measured at the Yale clinical chemistry laboratory on a Roche Elecsys 120 analyzer (Roche Diagnostics, Indianapolis, IN). Plasma renin activity was measured using the commercially available competitive ELISA kit from ALPCO as per manufacturer's instructions (ALPCO, Salem, NH). Total renin (R&D Systems, Minneapolis, MN) and active renin (ALPCO, Salem, NH) were analyzed using commercially available ELISA kits. The total renin immunoassay kit from R&D systems recognizes both active renin and prorenin. The mean detectable limit of the assay is 4.43 pg/mL for total renin and 0.81 pg/mL for active renin.

Bumetanide and torsemide in urine were measured using liquid chromatography mass spectrometry. Ultra high performance liquid chromatography was performed on the Agilent Infinity 1290 UPLC system. Chromatographic separation was achieved on the Zorbax Bonus RP 2.1×50 mm 1.8 µm column at the flow rate of 0.6 mL/min. The mobile phase consisted of 0.1% formic acid (buffer A) and 80% acetonitrile in 0.1% formic acid (buffer B). Mass spectrometry was performed on Agilent Q-TOF system (Agilent, Santa Clara, CA). Detection was performed in positive ion mode.

Endogenous lithium concentrations in serum and urine samples were measured using a Thermo Element2-XR magnetic sector inductively coupled plasma mass spectrometer at Yale Metal Geochemistry Center. Serum/urine samples (300/500 µL) were transferred to acid-cleaned Teflon beakers and evaporated to dryness on a hot plate at 93 °C. When dried, 5 mL of distilled HNO_3 and 100 µL of 30% H_2O_2 were added to the samples, and the solutions were heated sealed on a hotplate at 130 °C for 42 hours. The samples' solutions were then evaporated to dryness. The dried samples were brought up in a 5% HNO_3 and spiked with 10 ppb Sc as internal standard for inductively coupled plasma mass spectrometer analysis. An elemental (SCP Science Plasma) standard solution of 1002±4 µg/mL lithium in 4% HNO_3 was used for the preparation of calibration solutions (with a standard curve between 0.5 and 10 ppb Li). Standards and samples were repeatedly measured throughout the run to monitor instrument stability. Internal (instrument) error was typically <3%. Fifteen percent or more of sample per digest batch were randomly chosen to be run in duplicate. Error based on full protocol duplicates was <6%, and the typical lower limit of detection was 0.013 µg/L.

Table 2. Treatment and Treatment Response Parameters

	Overall Cohort	Hypochloremia		*P* Value
		No	Yes	
	(n=162)	(n=111)	(n=51)	
Referral for treatment of volume overload	37.0%	42.3%	25.5%	0.04*
Diuretic parameters				
Diuretic dose administered (mg furosemide equivalents)	160 (40–280)	120 (40–240)	160 (120–320)	0.02*
Diuretic administered				0.84
IV bumetanide	58.0%	58.6%	56.9%	…
Torsemide	42.0%	41.4%	43.1%	…
Number of doses of IV bumetanide	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	0.86
Estimated urine diuretic excreted (mg furosemide equivalents)	69.6 (30.1–152.2)	80.8 (31.6–142.8)	52.9 (24.8–155.7)	0.54
Administered IV to urine diuretic ratio	0.7 (0.3–1.2)	0.8 (0.5–1.2)	0.4 (0.2–0.9)	<0.001*
Diuretic response parameters				
Diuretic efficiency (mmol Na per doubling of diuretic dose)	31.9 (15.4–58.7)	41.2 (23.4–66.5)	17.1 (8.7–26.3)	<0.001*
Tubular diuretic efficiency (mmol Na per doubling of excreted urinary diuretic)	43.3 (26.8–77.3)	57.4 (33.4–89.4)	28.3 (16.0–43.0)	<0.001*
Postdiuretic urine solute concentrations				
Sodium, mmol/L	90.6±28.6	99.8±25.0	70.5±25.9	<0.001*
Chloride, mmol/L	96.6±29.8	106.2±25.6	76.6±28.2	<0.001*
Urine chloride/sodium ratio	1.1 (1.0–1.2)	1.1 (1.0–1.1)	1.1 (1.0–1.2)	0.86
Potassium, mmol/L	26.7±13.6	23.3±10.9	33.8±16.0	<0.001*
Postdiuretic total urine fluid and solute outputs				
Fluid output, mL	850 (550–1300)	950 (650–1375)	600 (400–1200)	0.01*
Total sodium output, mmol	82.4 (40.2–135.9)	104.1 (57.1–143.3)	48.5 (24.9–85.4)	<0.001*
Total chloride output, mmol	83.0 (47.4–138.0)	102.4 (61.2–158.6)	50.5 (22.2–100.1)	<0.001*
Total potassium output, mmol	20.4 (12.2–30.7)	20.7 (12.2–29.8)	20.1 (11.8–36.9)	0.54
Urine osmolality	345.4±122.3	366.4±139.3	301.2±53.5	0.002*
Postdiuretic fractional excretions				
FENa, %	5.1 (2.8–7.5)	5.4 (3.3–7.8)	3.5 (1.9–5.9)	0.003*
FECl, %	7.4 (4.3–10.9)	7.8 (5.6–11.1)	5.2 (2.8–10.0)	0.01*
FECl/FENa ratio	1.5 (1.4–1.6)	1.5 (1.3–1.6)	1.5 (1.4–1.8)	0.08
FEK, %	38.1 (27.2–63.7)	36.2 (26.1–57.8)	47.5 (28.3–81.7)	0.02*
FELI, %	27.5 (19.7–43.5)	29.4 (19.6–42.6)	27.1 (19.8–43.6)	0.92

FECl indicates fractional excretion of chloride; and FENa, fractional excretion of sodium.

Results

YTCC Cohort

In total, 162 patients receiving loop diuretics in the YTCC were included in the analysis, and baseline characteristics are shown in Table 1. The average age was 67.4±14.1 years; 38.9% of the population was of female sex, and left ventricular ejection fraction was reduced (<50%) in 53.9% of patients. Referral to YTCC was for treatment of volume overload in 37.0% of the population or routine posthospitalization follow-up in the remainder, as described in Table 2. Intravenous bumetanide was administered in 58% of patients (n=94) and oral torsemide in 42% (n=68). Choice of diuretic was not different between patients with and without hypochloremia (P=0.84). A median of 160 mg (40–280 mg) of intravenous furosemide equivalents was administered resulting in a median total sodium output of 82.4 mmol (40.2–135.9 mmol).

The average serum chloride level in the population was 98.4±5.0 mmol/L with 31.5% meeting criteria for hypochloremia (serum chloride ≤96 mmol/L). Mean serum sodium was 137.1±4.1 mmol/L with a similar 31.5% of patients hyponatremic (serum sodium ≤135 mmol/L). However, the correlation between sodium and chloride was modest (r=0.62; P<0.001) and 41.1% of hypochloremic patients were not hyponatremic. Characteristics of patients with versus without hypochloremia can be found in Table 1. In general, demographics and

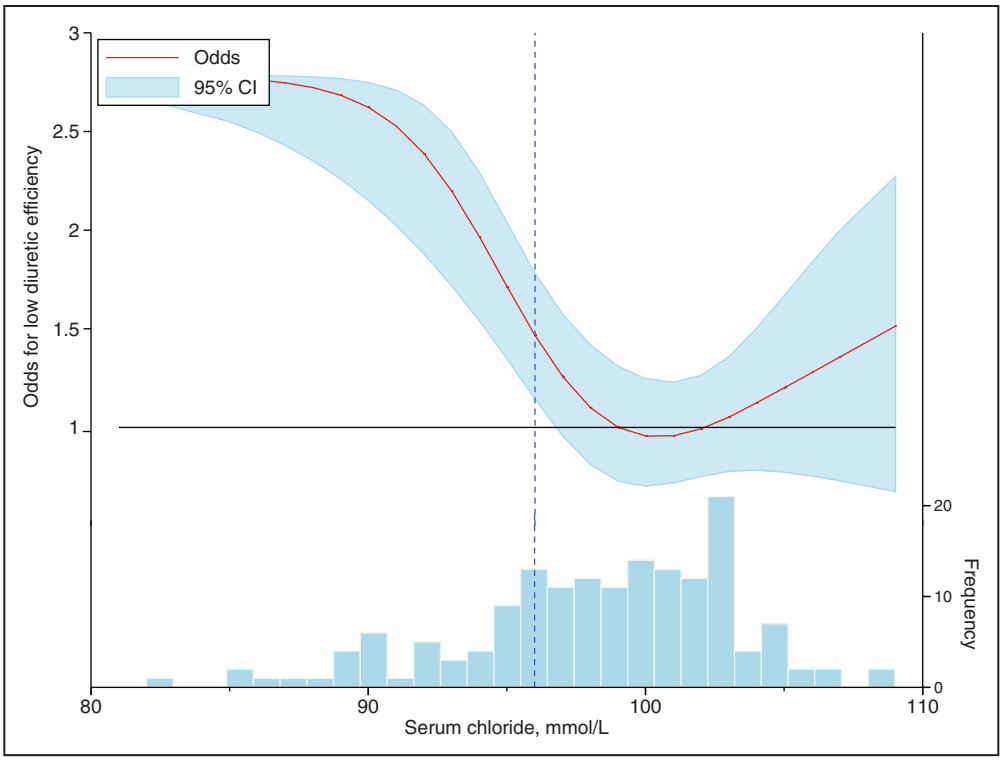

Figure 1. Relationship between serum chloride and odds of low diuretic efficiency. Red line represents the odds for low diuretic efficiency as a function of serum chloride relative to a reference value of 99 mmol/L (the median value in the population). Blue dashed line indicates a serum chloride value of 96, the cutoff value for hypochloremia. Blue bars at the bottom are the frequency of serum chloride levels in the population.

comorbidities tended to be similar between patients with and without hypochloremia. Blood urea nitrogen was higher in patients with hypochloremia and eGFR was lower, particularly when equations using cystatin C were used to estimate eGFR. The use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in addition to β-blockers was less common with hypochloremia, but the use of digoxin was significantly higher. There was no difference in the use of mineralocorticoid antagonists. Home loop diuretic dose was significantly higher as was the use of adjuvant thiazide diuretics in patients with hypochloremia.

Mechanisms Relevant to Maintenance of Hypochloremia

The baseline fractional excretion of sodium was not significantly different between patients with and without hypochloremia. However, there seemed to be wasting of chloride compared with sodium at baseline because both the fractional excretion of chloride and the ratio of fractional excretion of chloride to fractional excretion of sodium were significantly higher in patients with hypochloremia (Table 1). Furthermore, despite comparability in baseline fractional excretion of sodium between with and without hypochloremia, baseline fractional excretion of potassium was higher in patients with hypochloremia (Table 1) and the ratio of urine chloride to urine (sodium+potassium) was not different between those with and without hypochloremia (P=0.77). Calculated urine osmolality at baseline was lower in hypochloremic patients (342.9±101.2 versus 474.9±135.5 mOsm/kg; P<0.0001). Similarly, in the subset of patients with measured baseline urine osmolality available (n=52, correlation with calculated urine osmolality r=0.97; P<0.001) osmolality was lower in the hypochloremic patients (355.0±121.7 versus 477.3±136.1; P=0.002). After loop diuretic administration, both the total and fractional excretions of chloride were lower in hypochloremic patients, whereas the fractional excretion of potassium was significantly higher (Table 2). Measured postdiuretic urine osmolality was lower in hypochloremic patients (available n=149), but this was less pronounced than the differences at baseline (Table 2).

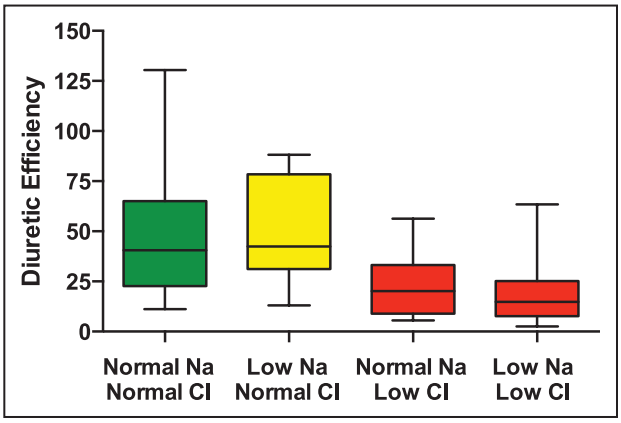

Figure 2. Diuretic efficiency in groups defined by presence or absence of hyponatremia and hypochloremia. Diuretic efficiency is expressed in mmol of sodium excreted per doubling of loop diuretic dose. Whiskers extend from 10th to 90th percentile.

Diuretic Efficiency

Median diuretic efficiency was 31.9 (15.4–58.7) mmol of sodium per doubling of loop diuretic dose. There was a moderate strength association between serum chloride and diuretic efficiency (r=0.38; P<0.0001). There was a weaker association with serum sodium (r=0.16; P=0.04), which was eliminated after adjustment for serum chloride (P=0.29). Serum bicarbonate had no statistically significant relationship with diuretic efficiency (P=0.12). The relationship between serum chloride and odds for low diuretic efficiency was nonlinear (Figure 1), with the majority of the association with poor diuretic efficiency isolated to patients with hypochloremia. As such, the odds for low diuretic efficiency in patients with hypochloremia were substantially increased (odds ratio, 7.3; 95% confidence interval [CI], 3.3–16.1; P<0.001). Addition of serum sodium and bicarbonate to the model did not significantly change the association between hypochloremia and low diuretic efficiency (odds ratio, 12.5; 95% CI, 4.0–38.9; P<0.001). After adjustment for baseline characteristics (serum sodium, bicarbonate, blood urea nitrogen, eGFR, home loop diuretic dose, YTCC administered diuretic dose, and use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, thiazide, digoxin, or β-blocker) hypochloremia remained significantly associated with low diuretic efficiency (odds ratio, 7.6; 95% CI, 2.0–29.7; P=0.003). Importantly, this association seemed to be independent of variation in serum sodium, as patients with isolated hyponatremia and normal serum chloride had comparable diuretic efficiency to patients with normal serum sodium and chloride (Figure 2). Similarly, in a linear model evaluating the relationship between serum chloride, serum sodium, and diuretic efficiency with adjustment for baseline eGFR, serum chloride was significantly associated with diuretic efficiency (β=2.8; P=0.001), whereas serum sodium was not (P=0.71).

Mechanisms for Reduced Diuretic Efficiency in Hypochloremia

The quantity of loop diuretic excreted in the urine (and thus reaching the tubular site of action) tended to be lower in patients with hypochloremia, but this did not reach statistical significance (Table 2). However, the dose of diuretic administered was significantly higher in hypochloremic patients (Table 2). As a result, the ratio of administered to excreted diuretic (ie, relative excretion) was reduced in hypochloremic patients (Table 2; P<0.001). Diuretic efficiency calculated using the quantity of diuretic actually reaching the site of action (ie, total diuretic found in the urine) was also substantially decreased in patients with hypochloremia (Table 2). The

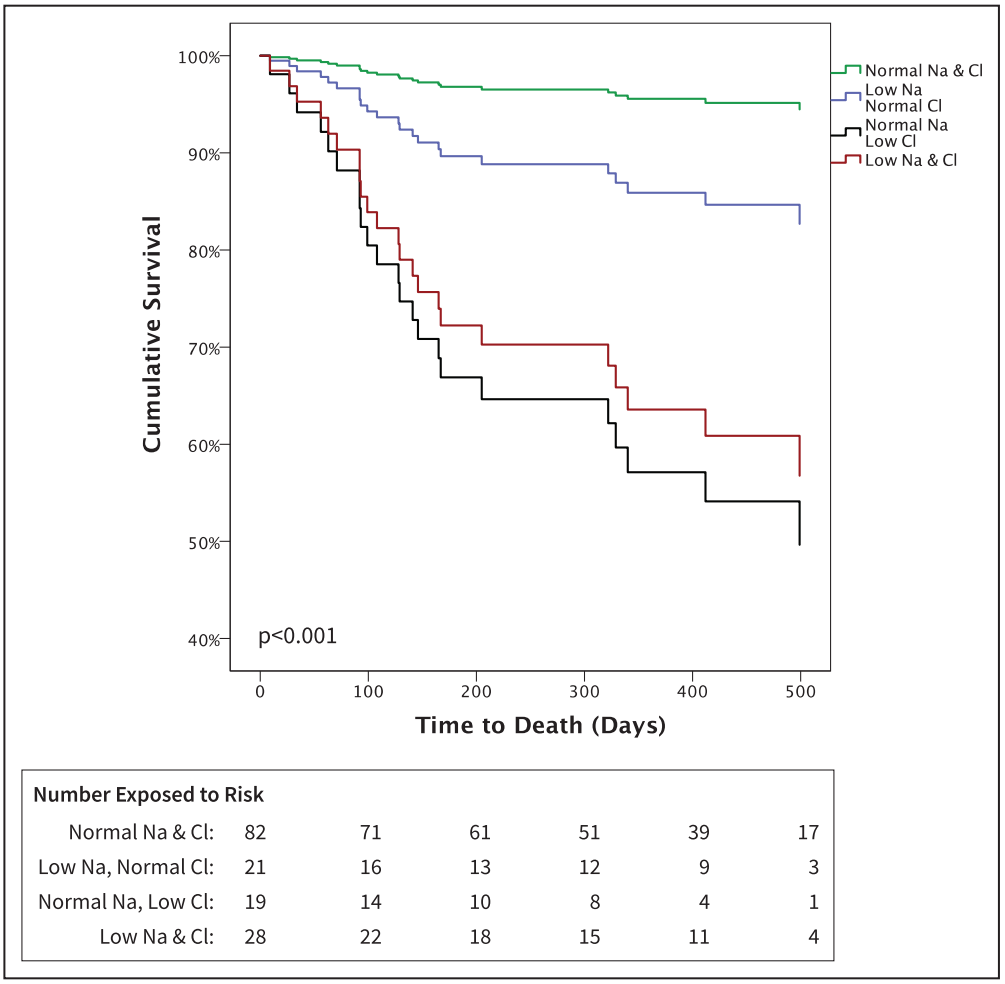

Figure 3. Survival curves for patients with combinations of hypochloremia and hyponatremia. Hyponatremia was defined as <135 mmol/L and hypochloremia as ≤ 96 mmol/L. Plots are adjusted for serum bicarbonate levels.

increase in sodium exit from the proximal tubule and loop of Henle after diuretic administration (ie, response of these nephron segments to the diuretic) was similar between patients with hypochloremia (diuretic induced increase in fractional excretion of lithium 11.4% [4.8–18.6]) and without hypochloremia (diuretic induced increase in fractional excretion of lithium 11.7% [4.5–21.8]; *P*=0.90).

Renin Levels

Total renin levels were higher in patients with hypochloremia compared with those without hypochloremia (Table 1). Renin levels were negatively correlated with serum chloride (r=−0.46; P<0.001), whereas this correlation was less pronounced with serum sodium (r=−0.30; P<0.001). In a multivariable model containing both serum chloride and serum sodium, chloride remained significantly associated with renin levels (β=−0.08; P<0.001), whereas sodium was no longer associated with renin levels (β=−0.02; P=0.49); these results persisted after adjustment for baseline eGFR (β=−0.08; P<0.001 for serum chloride; P=0.37 for serum sodium).

Associations With Survival

A total of 23 patients died during a median follow-up of 384 days (159–543 days). On univariable analysis, both hypochloremia (hazard ratio [HR], 4.8; 95% CI 2.0–11.3; P<0.001) and hyponatremia (HR, 3.5; 95% CI, 1.5–8.1; P=0.003) were strongly associated with worsened survival, whereas serum bicarbonate was not (P=0.64). However, when hypochloremia, hyponatremia, and bicarbonate were entered into the same model, hypochloremia remained associated with survival (HR, 5.7; 95% CI, 1.9–17.2; P=0.002), whereas hyponatremia (P=0.49) and serum bicarbonate (P=0.09) were not (Figure 3). Similar results were found when eGFR was included as a covariate (HR associated with hypochloremia, 4.5; 95% CI, 1.4–14.0; P=0.01; P for hyponatremia and serum bicarbonate >0.1). Higher plasma total renin concentration was also associated with reduced survival (HR, 2.1 per log increase; CI, 1.4–3.0; P<0.001). In a model containing hypochloremia (HR, 3.1; 95% CI, 1.1–8.3; P=0.03) and plasma renin concentration (HR, 1.7 per log increase; 95% CI, 1.1–2.6; P=0.01), both parameters remained significantly associated with survival.

Lysine Supplementation Pilot

Ten stable outpatients were studied before and after 3 days of 115 mmol/d supplemental chloride in the form of lysine chloride. Baseline characteristics of the participants are described in Table 3. Median time from the last hospitalization to study enrollment was 432 days (57–850). The mean baseline serum chloride in the population was 97±4 mmol/L. Patients received an average of 29±15 mg of torsemide on each of the study days which resulted in a sodium output of 78±48 mmol and a diuretic efficiency of 63±41 mmol sodium per doubling of the loop diuretic dose on the prechloride supplementation study day. All study participants elected lysine chloride powder, reported a 100% intake of all study drug doses with the most common diluent being ginger ale, and denied any significant side effects.

After 3 days of chloride supplementation, serum chloride increased by 2.2±2.3 mmol/L (P=0.01; Figure 4A) with 8 out of 10 participants experiencing an increase in serum chloride and only 1 of 10 a decrease in serum chloride. Venous pH decreased slightly by 0.03±0.02 (Figure 4B; P=0.007), whereas urine anion gap decreased by a much larger amount (28.2±14.2 mEq/L; P=0.008), indicating increased net renal acid excretion. Interestingly, 24-hour fractional chloride excretion did not significantly differ between presupplementation and day 3 of chloride supplementation (P=0.07) and only

Table 3. Baseline Characteristics of the LCS Cohort

	(n=10)
Demographics	
Age	49.3±15.8
Black race	20%
Male sex	80%
Past medical history	
Hypertension	80%
Diabetes mellitus	20%
Gout	10%
Ischemic etiology	10%
Time since last hospital discharge, d	432 (57–850)
Baseline medication use	
ACE or ARB	100%
β-Blocker	100%
Thiazide	10%
Digoxin	30%
Home loop diuretic dose (mg furosemide equivalents)	115.6±61.5
Baseline laboratory values	
Serum chloride, mmol/L	96.5±3.9
Serum sodium, mmol/L	138.1±2.9
Serum potassium, mmol/L	4.5±0.4
Blood urea nitrogen, mg/dL	23.8±10.0
Serum creatinine, mg/dL	1.1±0.4
eGFR, mL/min/1.73 m^2	78.4±37.6
BUN/creatinine ratio	21.5±4.5
NT pro-BNP, pg/mL	433.4±358.9
Albumin, g/dL	4.0±0.2
Physical examination and LV function	
Weight, kg	118.7±18.6
BMI	38.5±6.8
Heart rate, beats per min	76.5±8.6
Systolic blood pressure, mm Hg	105.7±10.3
Left ventricular ejection fraction, %	37.3±11.8

ACE indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate, as calculated by CKD-EPI mixed cystatin C-creatinine equation; and NT pro-BNP, n-terminal probrain natriuretic peptide.

2 patients had a large increase in the fractional excretion of chloride (Figure 4C).

Primary End Points

Day 4 total urine volume did not increase with chloride supplementation (P=0.72) with a trend toward worsening in patients with the highest pre-chloride supplementation urine output (Figure 5A). Plasma renin activity increased with chloride treatment in all patients (Figure 5B; P=0.005) with a median increase of 119% (33%–560%). Urinary total and active renin levels tended to increase, but this did not reach statistical significance (P=NS for both).

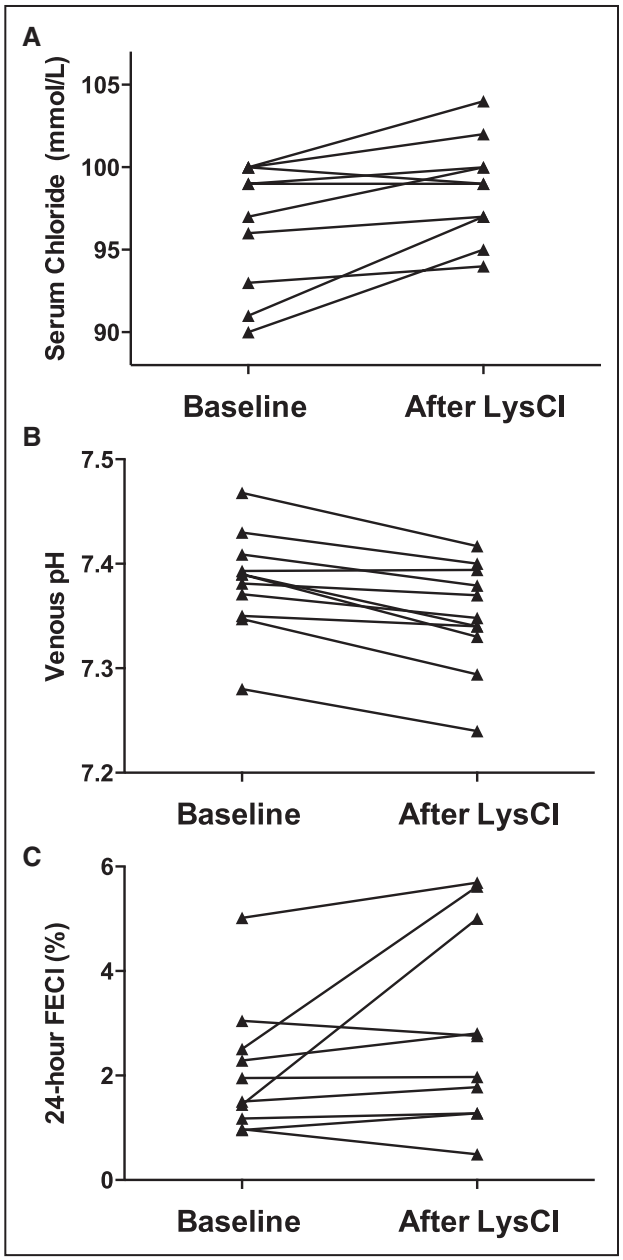

Figure 4. Serum chloride, venous pH, and 24-h fractional chloride excretion before and after chloride supplementation. Twenty-four hour fractional excretion of chloride was determined the 24 h before the other baseline parameters, and the last 24 h of the lysine supplementation period. FECl indicates fractional excretion of chloride; and LysCl, lysine chloride.

Change in Markers of Volume Status

In addition to the rise in plasma renin activity, the majority of metrics available suggested a decrease in intravascular volume associated with chloride supplementation. Notably, hemoconcentration occurred in 8 of 10 patients with an average increase in serum albumin of 2.4±3.4% (Figure 6A; P=0.036). Similarly, the majority of patients lost weight with 3 patients losing >5 pounds (Figure 6B; P=0.11). Amino terminal pro B-type natriuretic peptide levels decreased in 8 of 10 patients (Figure 6C). The 2 patients without an improvement in natriuretic peptide levels were the 2 patients who did not have improvement in serum chloride levels. Among those with improvement in serum chloride levels, the average decrease in amino terminal pro B-type natriuretic peptide was 25±21% (P=0.01). The blood urea nitrogen to creatinine ratio increased by 22% ± 15% (P=0.007; Figure 6D). There was no significant change in creatinine, cystatin C, or the kidney injury markers neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and interleukin-18 (P=NS for all).

Discussion

There are several findings from the current study: (1) Hypochloremic patients exhibited renal chloride wasting with an accompanying increase in potassium excretion. However, they did not show evidence of reduced renal free water clearance as they had lower urine osmolality. (2) Diuretic response by several metrics was strongly associated with serum chloride

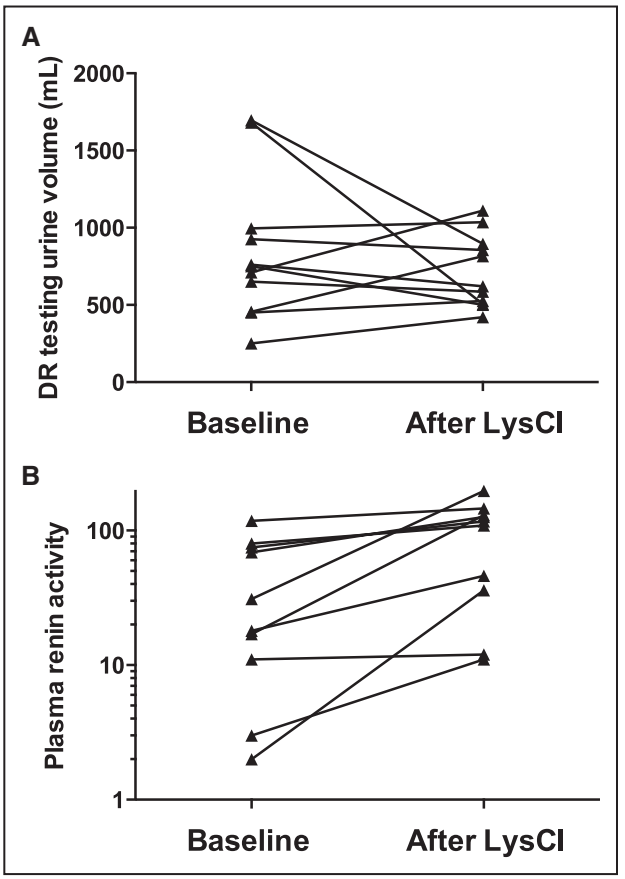

Figure 5. Diuretic induced urine volume and plasma renin activity before and after chloride supplementation.

but not with sodium levels. (3) Although some of this reduced diuretic efficiency was mediated by reduced tubular delivery, inadequate renal tubular response to delivered diuretic seemed to be an important driving mechanism because diuretic efficiency calculated using the quantity of diuretic reaching the tubular site of action was also decreased in hypochloremic patients. The specific location for the reduced diuretic efficiency seemed to be primarily distal tubular, given that there was no difference in sodium exit from the proximal tubule and loop of Henle between patients with and without hypochloremia. (4) Plasma total renin levels were independently correlated with serum chloride but not with serum sodium levels. (5) Supplementation of 115 mmol/d of lysine chloride was associated with an increase in serum chloride levels and changes in multiple cardiorenal, neurohormonal, and volume-related parameters. These results suggest that hypochloremia in HF might represent more than just a marker of disease severity; rather, it may be amenable to therapeutic modification.

Accumulating evidence has highlighted the importance of chloride in a multitude of important cardiorenal processes. Notably, chloride is also responsible for modulation of renin secretion and tubular glomerular feedback.[10–13] In vitro studies have identified a family of WNK (With-No-Lysine) serine–threonine kinases that play an essential role in regulating the actions of the renin–angiotensin–aldosterone system and the transporters on which loop and thiazide diuretics work.[16] Chloride seems to bind directly to the catalytic site of these kinases and regulates their ability to phosphorylate important sodium-regulatory pathways.[14–16] Analogous direct regulatory functions of sodium have not been described. Our findings that diuretic response and total renin levels are strongly linked to chloride rather than sodium levels are therefore not surprising. Because chloride regulates diuretic-sensitive tubular sodium transporters, the finding that the predominant mechanism for diuretic resistance with hypochloremia was at the renal tubular level, rather than because of impaired diuretic delivery, is also consistent. Furthermore, that chloride activates the sodium-potassium-2-chloride cotransporter through the WNK system is in line with the observed greater degree of baseline potassium wasting in hypochloremic patients. Some support for the biological importance of the above in humans is provided by the growing epidemiological literature, which suggests that hypochloremia is prognostically important in HF, independent of hyponatremia, a finding also observed in the current cohort (Figure 3).[17–19]

On the basis of this pathophysiological construct, we hypothesized that chloride supplementation would be associated with improved diuretic response and reduced plasma renin activity. However, what we observed was no change in diuretic response and a significant paradoxical increase in plasma renin activity. Notably, we also found that the majority of patients experienced hemoconcentration, weight loss, reduction in amino terminal pro B-type natriuretic peptide levels, and an increase in the blood urea nitrogen to creatinine ratio. Importantly, previous research has found that even in the setting of hospitalized acute decompensated heart failure patients undergoing diuresis with intravenous loop diuretics, only ≈50% experience hemoconcentration.[27] However, 80% of patients in the pilot study hemoconcentrated despite being stable outpatients without recent hospitalization and on stable doses of oral diuretics. Given that there is no biologically

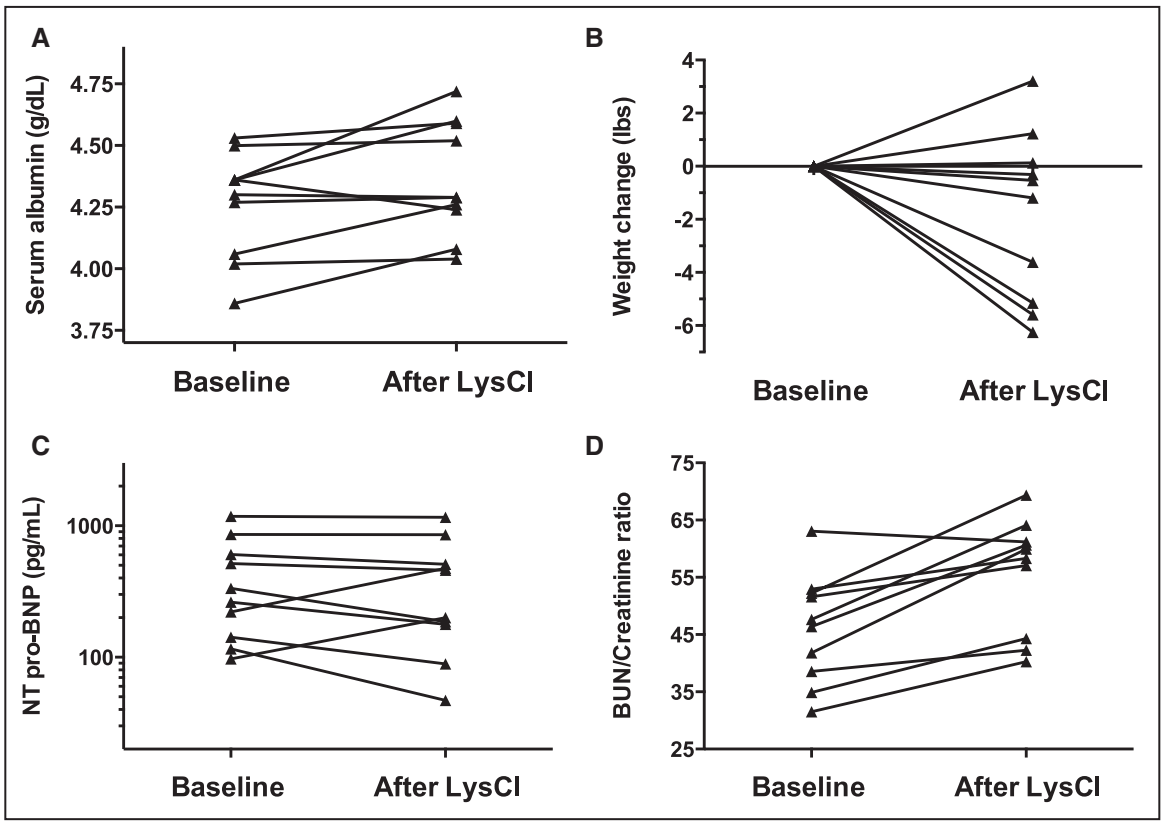

Figure 6. Markers of volume status before and after chloride supplementation.

plausible reason that chloride supplementation would increase plasma renin activity, an optimistic interpretation of the current pilot study findings may be that chloride supplementation worked too well, causing a meaningful reduction in total body/blood volume and thus the increase in plasma renin activity and the other changes in surrogates for volume status. By the third day of supplementation, when diuretic response was reassessed, we simply missed the increase in diuresis, and diuretic braking had already set in with the reduction in blood volume as has been well described.[23] That said, the more appropriate and conservative interpretation of the above findings would be that 3 days of modest-dose chloride supplementation was associated with a multitude of changes in potentially important cardiorenal parameters, and in light of the previously published epidemiological data, these findings should motivate additional research on the genesis, maintenance, and treatment of hypochloremia.

The cross-sectional nature of the observational arm of the article prevents us from commenting on the mechanism for the genesis of hypochloremia; however, we can comment on the mechanism for its maintenance. The first important observation was that patients with hypochloremia exhibited a lower propensity for renal conservation of free water. Loop diuretics impair the ability of the kidney to reabsorb free water via washout of the medullary concentration gradient.[23] As such, improvement in free water clearance at the time of loop diuretic exposure would be expected, even in hypochloremic patients. However, we noted that prediuretic urine osmolality was actually significantly lower in hypochloremic patients. Although it is possible/probable that hypochloremic patients have greater thirst and thus drink more, driving lower urine osmolality, it is notable that even in the diuretic-free compensatory period (ie, prediuretic), there was not an exaggerated renal predilection to retain free water. Additional notable findings were the relative greater wasting of both chloride alone and sodium compared with chloride in hypochloremic patients in the YTCC cohort; even on the third day of supplementation of 115 mmol/d of additional chloride in the pilot study, fractional chloride excretion did not meaningfully increase in the majority of patients. These findings point toward depletion rather than dilution as the cause for hypochloremia in these patients. This is an important point because vasopressin antagonists should be effective treatment for dilutional hypochloremia, but these agents were not found to improve outcomes.[28] As such, further research into the mechanisms underlying hypochloremia in HF and therapies to reduce chloride wasting and replete chloride stores should be prioritized for clinical trials.

Limitations

The findings from the YTCC cohort are cross-sectional and observational, thus making it impossible for us to confirm causality. Furthermore, because associations with serum and urine chloride were only determined at one point in time, factors related to the genesis, worsening, or resolution of hypochloremia cannot be determined. Because of the inherent association between pH and chloride levels, we could not fully separate the effects of changes in chloride versus changes in pH status between groups. Further research is necessary to determine whether pH may affect the role of chloride in diuretic response. Additionally, the study was not powered to detect interactions, and as a result, we cannot rule out the possibility that the use of 2 different diuretic agents in the YTCC cohort may have added heterogeneity to the results. A major limitation applies to our use of calculated eGFR as a marker of kidney function; the assumption of steady-state creatinine kinetics in an unstable condition such as acute decompensated HF is not likely to be met, and as a result, the accuracy of calculated eGFR is limited in our population because of its reliance on a single serum creatinine measurement. The largest limitations apply to the interventional pilot study, which was halted after an interim analysis yielding the present findings. Although multiple internally consistent signals suggestive of significant biological effects were identified, the primary end points were negative. The observation of biologically plausible associations within these 2 different experimental designs supports the notion that the importance of chloride in diuretic response is worth additional research; however, it must be particularly emphasized that the pilot study was not a blinded, randomized trial, and therefore, it is not possible to conclude that all observed changes were directly caused by administration of lysine chloride. Although the pilot study was designed to attempt to avoid some of these confounding issues, we cannot rule out the possibility of some changes between the pre- and post-lysine chloride time points occurring because of increased adherence to a low-sodium diet, Hawthorne effect, or the use of a diluent to ingest the lysine chloride powder. Furthermore, the sample size of this study was small at n=10 with a large number of comparisons, and it is possible that some of the observations arose from chance alone. Therefore, these results should serve as hypothesis generating only and should be used in conjunction with the growing body of data to design future studies.

Conclusions

Lower chloride levels are linked to reduced loop diuretic response. Hypochloremic patients had a greater relative wasting of chloride compared with sodium, whereas renal free water clearance did not seem to be impaired, suggesting that depletion rather than dilution may be the responsible mechanism. Oral supplementation of sodium-free chloride to patients chronically receiving high doses of loop diuretics was associated with increases in serum chloride levels and changes in several cardiorenal parameters. Additional studies on the mechanisms underlying hypochloremia in HF and the therapeutic value of manipulating chloride homeostasis are warranted.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Recent epidemiological studies have implicated chloride, rather than sodium, as the driver of the poor survival previously attributed to hyponatremia in heart failure. Accumulating evidence from more basic lines of investigation has identified chloride as a critical factor in renal salt sensing. The goal of the current study was to probe the physiological underpinnings bridging this growing epidemiological and basic science literature. We studied 2 outpatient heart failure cohorts: (1) an observational cohort of outpatients presenting for IV or oral diuretic treatment and (2) an interventional pilot study of stable outpatients receiving 3 days of oral lysine chloride supplementation and presenting for diuretic response testing at the beginning and end of this 3-day period. In the observational study, lower chloride levels were linked to reduced loop diuretic response. Hypochloremic patients had a greater relative wasting of chloride compared with sodium, whereas renal free water clearance did not seem to be impaired, suggesting that depletion rather than dilution may be the driving force behind their hypochloremia. In the pilot study, supplementation with lysine chloride was associated with an increase in serum chloride levels and changes in multiple cardiorenal, neurohormonal, and volume-related parameters. These results suggest that hypochloremia in heart failure might represent more than just a marker of disease severity; rather, it may be amenable to therapeutic modification. However, additional research is needed to more fully elucidate the mechanisms underlying hypochloremia in heart failure, and the therapeutic value of manipulating chloride homeostasis.

Hypochloremia and Diuretic Resistance in Heart Failure: Mechanistic Insights

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