Heart Failure

Volume Status and Diuretic Therapy in Systolic Heart Failure and the Detection of Early Abnormalities in Renal and Tubular Function

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Objectives
This study sought to determine the pharmacodynamic effect of modulation of volume status by withdrawal and reinstitution of diuretic treatment on markers of renal and tubular function.

Background
Decreased renal perfusion and increased congestion are associated with renal dysfunction in patients with heart failure.

Methods
In this study, 30 patients with chronic systolic heart failure in a presumed euvolemic state and on standard oral furosemide therapy (40 to 80 mg) were examined. At baseline, subjects were withdrawn from their loop diuretics. After 72 h, their furosemide regimen was reinstated, and patients were studied again 3 days later. Serum creatinine, atrial and B-type natriuretic peptide, urinary kidney injury molecule (KIM)-1, urinary N-acetyl-beta-D-glucosaminidase (NAG), and serum as well as urinary neutrophil gelatinase–associated lipocalin (NGAL) were determined at various time points.

Results
Diuretic withdrawal resulted in increases in atrial and B-type natriuretic peptide (both p < 0.05). Serum creatinine was unaffected. Both urinary KIM-1 (p < 0.001) and NAG (p = 0.010) concentrations rose significantly, after diuretic withdrawal, whereas serum and urinary NGAL were not significantly affected. After reinitiation of furosemide, both urinary KIM-1 and NAG concentrations returned to baseline (both p < 0.05), but NGAL values were unaffected.

Conclusions
Subclinical changes in volume status by diuretic withdrawal and reinstitution are associated with increases and decreases of markers of tubular dysfunction in stable heart failure. Diuretic therapy may favorably affect renal and tubular function by decreasing congestion. (J Am Coll Cardiol 2011;57:2233–41) © 2011 by the American College of Cardiology Foundation

Impaired renal function is one of the most important prognostic factors in both acute and chronic systolic heart failure (HF) (1,2). When reduced renal function is present or worsening renal function occurs, prognosis in patients with HF is extremely poor (1,3,4). Renal impairment in systolic chronic HF is mainly linked with reduced renal perfusion and systemic arterial pressure. In particular, in situations of already reduced renal perfusion, venous congestion, as shown by a high central venous pressure (CVP), is independently associated with renal function (5–7). Diuretics are important drugs in patients with systolic HF because they may control volume status by reducing extracellular body water (8). However, these drugs can result in volume depletion, thereby leading to renal dysfunction and suboptimal cardiac loading, and may be associated with reduced survival (9). On the other hand, they may also reduce venous congestion, which might theoretically im-

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Diuretic Resumption

In the present study, we therefore set out to investigate the impact of subtle changes in volume status on parameters of renal clearance and tubular function in patients with chronic systolic HF using a model of staged diuretic withdrawal and reinstitution. Because serum creatinine is not a very sensitive marker to detect such changes, we investigated new biomarkers of tubular function that may be more sensitive to subtle changes (10).

Methods

Study population. Patients were recruited from the Heart Failure Services at City and Sandwell Hospitals, both in Birmingham, United Kingdom. Patients had to have a left ventricular ejection fraction of <40% (on quantitative echocardiography or rest radionuclide scintigraphy) and were symptomatic but clinically stable on chronic oral furosemide dosing (40 to 80 mg daily). Patients also had to be on optimal (maximum tolerated) HF medication according to current guidelines, including an angiotensin-converting enzyme inhibitor, a beta-blocker, and/or spironolactone. Exclusion criteria included a recent hospitalization for any major illness 3 months before enrollment into the study; poor treatment compliance; uncontrolled hypertension or diabetes; clinically significant other illness such as malignancy, unstable stroke disease, or neurodegenerative illness; and history of ongoing alcohol or substance abuse.

Study design. Table 1 shows the timeline for the study design. Patients took part in a 7-day diuretic manipulation protocol, whereby on day 1 (Tuesday) they received their usual chronic diuretic dose and omitted diuretic on days 2, 3, and 4. On day 4 (at 72 h), they were given a bolus dose of intravenous furosemide (50 mg), followed by reinstatement of their usual diuretic on days 5 to 7. Other cardiac medication was continued as usual. Patients attended the study center on days 1, 2, 3, 4, and 7 at the same time on each occasion (between 7:00 AM and 8:30 AM). Patients fasted from 9:00 PM the prior evening and refrained from alcohol, caffeine, and smoking. However, they were allowed normal fluid intake to avoid dehydration. On arrival to the study center, they rested supine for 30 min before bladder voiding and blood collection for biomarker analyses. A total of 20 ml of blood was collected and transferred into chilled tubes containing ethylenediaminetetraacetic acid (1.5 mg/ml) and aprotinin (500 IU/ml, Trasylool, Bayer, Newbury, Berks, United Kingdom) and serum separator tubes at room temperature. Both serum and ethylenediaminetetraacetic acid/aprotinin tubes were centrifuged at 4°C at 1,200 rpm for 20 min. Samples were aliquoted into respective plastic tubes and stored at −70°C for subsequent analyses. Urine samples were also stored in plastic tubes at −70°C. Samples were gently thawed and vortexed before analysis. Patients underwent daily symptom review and physical examination during the diuretic withdrawal phase to ensure that there were no signs of acute decompensation. To ensure further patient safety, one of the investigators (M.J.N.K.C.) was available by telephone 24 h per day for any emergencies. In case of suspected decompensation, patients would be admitted to the acute cardiology ward for treatment and/or observation under the care of the attending consultant. All patients gave informed consent, and the study was approved by the Local Research and Ethics Committee (West-Birmingham).

Atrial and B-type natriuretic peptide. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) were used to track changes in volume status leading to changes in atrial and ventricular stretch, respectively, in response to volume and pressure changes (11). Both ANP and BNP have shorter half-lives, and thus greater observed variability over time, than their respective inactive forms, N-terminal proANP and N-terminal proBNP. Furthermore, although BNP is the more widely studied biomarker in patients with HF, ANP may be more sensitive to acute volume changes (12). ANP was analyzed by enzyme-linked immunosorbent assay (ELISA, USCN Life Science Inc., Wuhan, China) at St. George’s Hospital. The detection limit was 0.14 pg/ml, intra-assay coefficient of variation (CV) was <5%, and interassay CV was <14%. The assay range was 0 to 25 pg/ml, with the normal range being 10 to 70 pg/ml. BNP was measured using the ADVIA Centaur automated chemiluminescence system (Siemens Medical Solutions Diagnostics, Newbury, Berks, United Kingdom). The interas-

### Table 1 Study Design

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<th>Baseline</th>
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<td>Usual dose of furosemide</td>
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<td>50 mg IV furosemide</td>
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<td>Blood and urine biomarkers</td>
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IV = intravenous.
say CV was 13.6% for low (4.26 pg/ml), 10.0% for medium (36.9 pg/ml), and 11.2% for high (133.0 pg/ml) values. The interassay CV was 22.3% for low, 10.9% for medium, and 11.6% for high values. The sensitivity of the assay was 6.9 pg/ml, and the upper detection limit was 17,301 pg/ml.

**Urinalysis and markers of tubular damage.** Urinary creatinine was determined to correct for concentration of urine. Neutrophil gelatinase–associated lipocalin (NGAL) was determined by means of a commercially available ELISA test kit from Antibody Shop (Gentofte, Denmark). Samples were diluted 500 times in dilution buffer supplied with the test kit. The NGAL was first expressed in nanograms per milliliter (limit of detection 0.093 ng/ml), and values were then corrected for urinary creatinine concentration. The enzyme N-acetyl-beta-D-glucosaminidase (NAG) was evaluated using the substrate p-nitrophenyl N-acetyl-β-D-glucosaminide (Sigma, St. Louis, Missouri) in citrate buffer at pH 4.5. After 60 min at 37°C, 1 M of Na2CO3 was added to the mixture to terminate the reaction and to develop a yellow color released from the converted substrate. Controls were obtained from each sample by addition of Na2CO3 at time = 0. The color was measured at 405 nm by a microtiter plate reader, and controls were subtracted. A standard curve was made with N-acetyl-β-D-glucosaminidase. Urinary NAG activity was first expressed as units per milliliter (limit of detection 0.113 U/ml) and then adjusted for urinary creatinine (units per gram of creatinine). Urinary kidney injury molecule (KIM)-1 measurements were performed using ELISA (R&D Systems, Minneapolis, Minnesota). For measurements, 50 μl of urine samples were analyzed in duplicate. The lowest limit of detection for this assay is 0.125 ng/ml. The interassay and intra-assay variability was 20%. The urinary KIM-1 level was normalized to the urinary creatinine concentration (in nanograms per gram of creatinine).

**Statistical analysis.** Data are given as mean ± SD when normally distributed, as median (interquartile range) when skewed distributed, and as frequencies and percentages for categorical variables. Multiple repeated measurement testing was carried out using the Friedman test for non-normally distributed variables and the repeated measured analysis of variance for normally distributed variables, where appropriate. Differences were subsequently determined using the Wilcoxon signed-rank test for non-normally distributed variables and the paired-samples t test for normally distributed variables, where appropriate. All reported probability values are 2-tailed, and a p value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (version 12.0, SPSS Inc., Chicago, Illinois) and STATA (version 10.0, STATA Corp., College Station, Texas).

## Results

A total of 30 patients were included in the present study. Table 2 shows the baseline characteristics. The majority of the patients were male, with a mean age of 70 ± 7 years. The main cause of systolic HF was coronary artery disease, accounting for two thirds of all patients. All patients were on either ACE inhibitor or angiotensin receptor blocker therapy. Markers of glomerular and tubular function were increased in this cohort of patients with HF. Serum creatinine was 1.66 (1.32 to 2.28) mg/dl, and mean estimated glomerular filtration rate (eGFR) was 47 (27 to 54) ml/min/1.73 m². Urinary KIM-1 at 562 (132 to 1,357) ng/gCr and serum NGAL at 470 (333 to 599) ng/ml were also increased. Median urinary NGAL levels at 1.4 (0.5–26.0) ng/ml were also increased. Median urinary NAG levels at 8.5 (5.8–13.4) U/gCr were also increased. Medically NGAL values were <20 ng/ml, and mean estimated glomerular filtration rate (GFR) was 47 (27 to 54) ml/min/1.73 m². Urinary KIM-1 at 562 (132 to 1,357) ng/gCr (normal value <200 ng/gCr), urinary NAG at 8.5 (5.8 to 13.4) U/gCr (normal value <3 U/gCr), and serum NGAL at 470 (333 to 599) ng/ml (normal <20 ng/ml) were also increased. Median urinary NGAL levels at 1.4 (0.5 to 26) μg/gCr (normal <60 μg/gCr) were not elevated in the overall study population and were undetectable in 2 of 30 patients.

**Effect of diuretic withdrawal.** All patients tolerated the 3-day diuretic withdrawal protocol, and none of the patients experienced an episode of cardiac decompensation. During diuretic withdrawal, both ANP and BNP levels increased.

### Table 2 Baseline Characteristics of the Study Population

| Age, yrs | 70 ± 7 |
| Male    | 26 (87) |
| LVEF, % | 25 ± 8 |
| NYHA functional class, % (I/II/III) | 3/87/10 |
| Systolic blood pressure, mm Hg | 136 ± 22 |
| Diastolic blood pressure, mm Hg | 77 ± 12 |
| Etiology of heart failure | Ischemic: 20 (67) |
| | Idiopathic: 5 (17) |
| | Other: 5 (17) |
| eGFR, ml/min/1.73 m² | 46 (27–54) |
| BNP, pg/ml | 157 (104–292) |
| ANP, pg/ml | 794 (264–2,543) |
| Hemoglobin, g/dl | 9.1 ± 1.2 |

**Tubular markers**

| Urinary KIM-1, ng/gCr | 562 (132–1,357) |
| Urinary NAG, U/gCr | 8.5 (5.8–13.4) |
| Urinary NGAL, μg/gCr | 1.4 (0.5–26.0) |
| Serum NGAL, ng/ml | 470 (333–599) |

**Medication**

| ACE inhibitors | 20 (67) |
| ARBs | 10 (33) |
| Beta-blockers | 23 (77) |
| Lipid-lowering agents | 23 (77) |
| Loop diuretics | 30 (100) |
| Aldosterone receptor antagonists | 10 (33) |

**Medical history**

| Diabetes | 10 (33) |
| Hypertension | 14 (47) |
| Atrial fibrillation | 11 (37) |
| Cerebrovascular/peripheral artery disease | 3 (3) |
ANP showed a significant increase as early as 8 h after the last dose of oral diuretics, whereas BNP was only elevated at day 4. There was a nonsignificant increase in body weight during diuretic withdrawal (+1.6 kg, p = 0.149). As a positive control, urinary creatinine concentrations increased during diuretic withdrawal, reaching maximum urinary concentration at day 2 (Fig. 2). In contrast, median serum creatinine levels did not significantly change in this period (mean change from baseline to day 4 = 0.04 ± 0.91 mg/dl). Of the measured tubular markers, both urinary KIM-1 and urinary NAG levels rose significantly after diuretic discontinuation (Fig. 3A). Urinary KIM-1 levels rose significantly as early as 8 h after the last dosing of oral diuretics, with the concentration further increasing up until day 2 when a plateau phase was reached, with persisting elevation at day 4 (995 [401 to 1,545] ng/gCr, p < 0.001). Urinary NAG levels rose much slower and less extensively, but showed similar patterns as the urinary KIM-1 concentrations (day 4: 9.6 [4.8 to 15.5] U/gCr, p = 0.010). In total, 20 (67%) and 17 (57%) of patients showed an increase at day 4 for KIM-1 and NAG, respectively. In contrast, neither plasma nor urinary concentrations of NGAL changed significantly during diuretic withdrawal (Fig. 3B), which resulted in 14 (47%) and 15 (50%) patients showing an increase in urinary and serum NGAL at day 4, respectively. Of those patients who showed an increase in urinary KIM-1 and NAG levels at day 4, baseline serum creatinine was higher, whereas hemoglobin levels and mean arterial pressure were significantly lower. Estimated GFR showed a trend toward lower values at baseline in these patients.

**Effect of diuretic reinitiation (after 3 days).** After the intravenous furosemide bolus and reinstatement of oral furosemide therapy, urinary creatinine and plasma BNP levels returned to their baseline values before the start of the study. ANP levels further increased after reinitiation of the diuretic regimen, resulting in a plasma concentration of 1,183 (430 to 2,674) pg/ml at day 7 (p = 0.006 for difference from baseline). In parallel to the finding with diuretic withdrawal, mean serum creatinine concentrations did not change after the reinitiation of diuretic therapy (mean change 0.05 ± 0.80 mg/dl from reinitiation to day 7). With diuretic reinitiation, both urinary KIM-1 and urinary NAG levels returned to their baseline values before the study (Fig. 4A). Similarly to the quick response to diuretic withdrawal, mean serum creatinine concentrations did not change after the reinitiation of diuretic therapy (mean change 0.05 ± 0.80 mg/dl from reinitiation to day 7).

In total, 23 (77%) and 21 patients (70%) experienced a decrease in either KIM-1 or NAG from day 4 to day 7, respectively. Neither serum nor urinary NGAL levels were influenced by the reinitiation of diuretic therapy (Fig. 4B), with 15 (50%) and 16 patients (53%) showing a decrease at day 7 for urinary and serum NGAL, respectively.
Online Figures 2A and 2B show the mean change in tubular markers from day 4 and forward.

Discussion

In the present study, we found that a subtle volume increase after diuretic withdrawal in patients with clinically stable chronic systolic HF, as shown by an early elevation of ANP and subsequent elevation of BNP, resulted in a significant increase in urinary levels of the tubular markers KIM-1 and NAG, but not NGAL. After reinstitution of the oral diuretic regimen, urinary levels of both markers returned to their baseline values.

Modulation of volume status and renal function in HF.

In (chronic) HF, reduced renal blood flow is the most important determinant of the GFR (6,13,14). However, we and others have recently shown that not only decreased perfusion is important in determining renal function, but also increased renal venous congestion as indicated by increased CVP (5–7). In these cross-sectional studies, CVP affected GFR, especially when perfusion was already compromised. Yet, to date, no study has evaluated the effect of modulation of volume by diuretic treatment on parameters of renal function in patients with systolic HF.

Indirect evidence suggests, however, that changes in volume status may change renal function. In early experimental studies, induction of increased renal venous pressure resulted in impairment of renal function (15). Recently, Mullens et al. (16) showed that decreasing CVP by decreasing intra-abdominal pressure improves renal function. On the other hand, in the UNLOAD (Ultrafiltration versus IV Diuretics for Patients Hospitalized for Acute Decompensated Congestive Heart Failure) trial, fluid removal by ultrafiltration did not result in significant changes in renal function in patients with acute HF (17).

In our present study, we found that during withdrawal of diuretics, there was a subtle increase in both ANP and BNP, as well as a nonsignificant increase in weight, which may suggest a small increment in CVP. However, the accompanied change in serum creatinine was negligible. On the other hand, the change in serum creatinine showed a bell-shaped frequency curve, suggesting that individual patients may react differently to changes in CVP. Some patients may be characterized by an asymptomatic intravascular dehydratic state, whereas others may be euvoletic or even volume-expanded, all without overt decompensation or symptoms. Nevertheless, serum creatinine may not be the most accurate and sensitive parameter for early changes in renal function, as the full effect on serum creatinine may only occur hours or even days after the initiation of renal damage (10).

Markers of tubular dysfunction. Tubular markers might be able to detect changes in renal function earlier than serum creatinine. Diuretic withdrawal resulted in an increase in urinary KIM-1 as early as 4 h after the last oral dose of chronic diuretic therapy, which persisted after 72 h of diuretic withdrawal. Urinary NAG levels showed a similar pattern, but the increase was only apparent after 48 h. Both markers showed a significant decrease to baseline levels when diuretic therapy was reinitiated. Indeed, BNP concentrations decreased to
their baseline values after diuretic reinitiation, which may suggest that a decrease in CVP accompanied the reinitiation of diuretics and the fall in both BNP and urinary KIM-1/NAG. We also found that patients with compromised kidney function, lower hemoglobin levels, and lower blood pressures at baseline were especially at increased risk for deteriorating tubular function. This group of patients may be more susceptible to volume/diuretic changes that can alter renal perfusion and oxygen delivery and may have limited reserve capacity to preserve tubular function.

These tubular markers are sensitive and are known to respond extremely quickly after induction of tubular dysfunction or the occurrence of acute kidney injury (10,18,19). We recently showed that the concentrations of these markers were increased in congestive heart failure, which may suggest that this patient group also suffers from chronic hypoxic tubular damage in addition to reduced glomerular
function (20). KIM-1 is thought to be expressed in the urine when (hypoxic) tubular dysfunction develops, whereas the response time of urinary NAG is somewhat slower and less specific (21,22). The absence of any alteration in NGAL levels in urine or serum remains obscure. NGAL levels may not only be dependent of tubular dysfunction, but also of other comorbid organ dysfunction and inflammation (23). Furthermore, urinary NGAL levels are more dependent on production of NGAL in the distal tubule after injury, whereas both KIM-1 and NAG are markers that represent proximal tubular injury (23). Although proximal tubular injury may also result in higher urinary NGAL levels, this is a reflection of serum NGAL that has been filtered through the glomerulus and not reabsorbed in the proximal tubule. Given the absence of changes in serum NGAL, this may be a reason for a lack of an effect on NGAL. Together, this
may suggest that especially proximal tubular injury occurs (rise in KIM-1 and NAG), whereas distal tubular injury is limited (no effect on NGAL). Finally, it is possible that NGAL may not be susceptible to changes in volume status, but more to substantial changes in renal perfusion. The present study does not allow us to investigate this.

**Direct effects of diuretics.** The effects of diuretics on renal function might not only be related to changes in CVP, but diuretics might have a direct effect on renal function as well. Diuretic therapy has been indirectly related to worsening renal function. Gottlieb et al. (24) showed that although furosemide is a well-known and potent natriuretic agent, it also induces a significant fall in GFR. This impact on renal function may be greater in patients with systolic HF. More recently, Metra et al. (4) found that patients who receive higher dosing of diuretics were especially at risk for development of worsening renal function. In addition, via the tubuloglomerular feedback mechanism, higher sodium concentrations in the distal tubules may trigger afferent vasoconstriction, inducing reduced GFR (25).

Only a few studies have previously explored the impact of programmed diuretic withdrawal in patients with systolic HF. One small study showed that diuretic withdrawal in 4 patients with HF implanted with a hemodynamic monitor led to individual changes in hemodynamics, with high interindividual variability (26). Galve et al. (27) showed that with diuretic withdrawal, renal function parameters improved after 3 months of withdrawal. However, no previous study has evaluated the short-term effect of diuretic withdrawal on renal function parameters or the effect of reinitiation of diuretic therapy.

In our present study, diuretic withdrawal led to increased concentrations of markers of tubular dysfunction, an effect that was attenuated by the reinitiation of diuretic therapy. Loop diuretics have been shown to preferentially improve medullary but not cortical oxygen tension (28). In addition, furosemide treatment leads to reduced renal oxygen consumption in patients admitted to the intensive care unit after cardiac surgery (29). Therefore, diuretics may improve medullary oxygen tensions by decreasing the need for sodium and water retention in this region of the kidney, resulting in improved oxygen availability. Considering the extreme salt-retaining state in patients with systolic HF, this may be an important mechanism to prevent hypoxic tubular damage.

**Implications.** Our results suggest that loop diuretic therapy may favorably affect makers of tubular function in HF patients with a presumed euvoletic state. Whether this is due to changes in volume status or a more direct effect is unclear. However, our findings may suggest a balance between diuretic use, volume status, and renal function. Each of these factors will be subject to variations that may disturb this balance, which consequently may trigger further alterations by a treating physician. This could be either withdrawing or (re)initiating diuretics, depending on the clinical characteristics. However, we have shown that some tubular markers may give important information on the course of renal and tubular function during these adjustments in therapy. Although some evidence suggests that (aggressive) loop diuretic therapy may increase mortality and renal insufficiency, our present short-term data suggest that, at least in some patients, this seems not to be the case (9). Our results further underline the need for individualized medicine and the need for more research on the interaction between diuretics, volume status, and renal function in patients with HF.

**Study limitations.** The patient population consisted of patients in an approximate euvoletic state, which was assessed largely on the basis of symptomatic stability, clinical examination, and echocardiography. Median baseline BNP levels were not extremely elevated, supporting these assessments. However, it should be noted and would be expected that the results would have been different in patients with pronounced hypervolemia or perhaps even more rigorous hypovolemia. We used ANP and BNP as markers of filling pressures, but we did not assess CVP or renal venous pressure directly. We observed a sustained elevation in ANP levels, even after reinitiation of diuretics. Because ANP is a strong marker of hypervolemia, it would have been expected to decrease with volume depletion. However, from our study we cannot derive an explanation for this unexpected finding. During our study, protein and sodium intake was not tightly controlled, which could be a source of bias in our analyses. Although our study was tightly controlled within subjects, it consisted of a limited number of patients and should be replicated in a larger study. It must be noted, however, that the sequential measurements (before, during, and after withdrawal of diuretics) of renal function and tubular markers is an important strength of our study.

**Conclusions**

Our present results indicate that subtle changes in volume status by modulation of diuretic therapy lead to parallel changes in markers of tubular dysfunction in patients with systolic HF. Diuretic therapy may favorably affect tubular function by decreasing congestion or a direct pharmacological effect. Withdrawal of diuretic therapy in stable patients may be associated with recordable rises in biomarkers of renal injury.

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Key Words: diuretics  heart failure  kidney  tubular damage.

APPENDIX

For supplementary figures, please see the online version of this article.