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ORIGINAL ARTICLE
Heart Failure

Empagliflozin Administration Can Decrease the Dose of Loop Diuretics and Prevent the Exacerbation of Renal Tubular Injury in Patients With Compensated Heart Failure Complicated by Diabetes

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Background: Whether the dose of loop diuretics can be decreased by administration of a sodium-glucose cotransporter 2 (SGLT2) inhibitor in diabetic outpatients with compensated heart failure (HF) is unclear.

Methods and Results: This study prospectively enrolled 60 diabetic outpatients with compensated HF. Patients were randomly divided into 2 groups: those administered the SGLT2 inhibitor empagliflozin (n=28) and those not (n=30). Changes in the daily dose of loop diuretics, blood sampling data, and urinary renal tubular biomarkers were evaluated 6 months after the intervention. The median (interquartile range) furosemide dose decreased significantly over the 6-month follow-up period in the empagliflozin group (from 40 [20–40] to 20 [10–20] mg), but not in the non-empagliflozin group (from 23 [20–40] to 40 [20–40] mg). Hemoglobin levels increased significantly in the empagliflozin group (from 13.2 [11.9–14.6] to 14.0 [12.7–15.0] g/dL). In addition, excretion of acetyl-β-D-glucosaminidase decreased significantly over the 6-month follow-up in the empagliflozin group (from 4.8 [2.6–11.7] to 3.3 [2.1–5.4] IU/L), especially in the group in which the dose of loop diuretics decreased (from 4.7 [2.5–14.8] to 3.3 [2.1–4.5] IU/L).

Conclusions: Empagliflozin administration decreased the dose of loop diuretics and increased the production of erythropoietin, which may help prevent renal tubular injury in diabetic outpatients with HF.

Key Words: Acetyl-β-D-glucosaminidase; Chronic heart failure; Furosemide; Sodium-glucose cotransporter 2 (SGLT2) inhibitors

The number of heart failure (HF) patients has been rapidly increasing worldwide in what is being called an "HF pandemic".[1] Although the number of HF patients is increasing in aging societies,[1,2] few medical approaches for managing HF were established in Japan in the 2010s.[3] However, sodium-glucose cotransporter 2 (SGLT2) inhibitors were proposed as a new medical therapy for treating diabetes in the 2010s. SGLT2 inhibitors are glucose-lowering drugs that increase urinary glucose excretion by inhibiting the reabsorption of blood glucose. Given their mechanism of action, SGLT2 inhibitors were expected not only to be a novel treatment for diabetes, but also for HF.

Two major multivariate studies, namely the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) and the CANagliflozin cardioVascular Assessment Study (CANVAS), suggested that the administration of SGLT2 inhibitors may reduce HF hospitalization in patients with diabetes.[4,5] Although the efficacy of SGLT2 inhibitors was suggested in an experimental model of HF,[6] and an ongoing prospective randomized study protocol has been proposed,[7,8] few clinical trials of SGLT2 inhibitors have targeted HF patients. The clinical benefit of SGLT2 inhib-

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Table 1. Patient Characteristics at the Study Start Date

	All patients (n=58)	Empagliflozin (n=28)	Control (n=30)	P value
Status and vital signs				
Age (years)	74 [70–79]	73 [66–77]	76 [71–79]	0.167
Male sex	48 (82.8)	26 (92.9)	22 (73.3)	0.051
LVEF (%)	55 [39–64]	55 [39–64]	53 [39–61]	0.825
SBP (mmHg)	125 [112–144]	127 [118–146]	120 [104–139]	0.411
HR (beats/min)	77 [66–83]	79 [65–86]	74 [69–80]	0.689
History of admission due to HF	31 (53.4)	16 (57.1)	15 (50.0)	0.389
Etiology (ischemia)	25 (43.1)	13 (46.4)	12 (40.0)	0.410
Atrial fibrillation	23 (39.7)	8 (28.6)	15 (50.0)	0.081
Medical history				
Hypertension	40 (69.0)	21 (75.0)	19 (63.3)	0.250
Dyslipidemia	45 (77.6)	22 (78.6)	23 (76.7)	0.557
Hyperuricemia	34 (58.6)	19 (67.9)	15 (50.0)	0.133
CKD	27 (46.6)	15 (53.6)	12 (40.0)	0.220
Laboratory data				
Uric acid (mg/dL)	5.8 [5.1–7.0]	5.7 [5.0–7.1]	5.9 [5.1–6.6]	0.791
Total bilirubin (mg/dL)	0.7 [0.5–1.0]	0.7 [0.4–0.8]	0.8 [0.5–1.0]	0.323
BUN (mmol/L)	20.3 [16.1–26.4]	22.1 [15.7–29.6]	18.0 [16.2–25.0]	0.606
Creatinine (g/dL)	1.03 [0.87–1.49]	1.29 [0.92–1.85]	0.96 [0.85–1.15]	0.086
eGFR (mL/min/1.73 m^2)	51.2 [34.2–62.5]	43.3 [28.5–61.1]	56.7 [43.6–64.6]	0.192
Sodium (mmol/L)	141 [139–143]	140 [139–142]	141 [140–143]	0.622
Potassium (mmol/L)	4.3 [4.0–4.7]	4.2 [4.0–4.6]	4.3 [4.1–4.7]	0.676
Hemoglobin (g/dL)	13.4 [12.1–14.7]	13.2 [11.9–14.6]	13.4 [12.3–14.8]	0.738
CRP (mg/dL)	0.09 [0.03–0.20]	0.10 [0.05–0.28]	0.08 [0.00–0.18]	0.425
BG (mg/dL)	127 [106–154]	126 [103–183]	127 [110–145]	0.773
HbA1c (%)	6.7 [6.2–7.6]	7.2 [6.3–7.8]	6.6 [6.2–6.8]	0.193
Diabetes medications				
Insulin	9 (15.5)	7 (25.0)	2 (6.7)	0.058
Sulfonylureas	5 (8.6)	3 (10.7)	2 (6.7)	0.467
Biguanides	21 (36.2)	13 (46.4)	8 (26.7)	0.098
α-Glucosidase inhibitor	9 (15.5)	4 (14.3)	5 (16.7)	0.546
Thiazolidinediones	2 (3.4)	2 (7.1)	0 (0.0)	0.229
Glinides	8 (13.8)	4 (14.2)	4 (13.3)	0.607
DPP-4 inhibitor	40 (69.0)	17 (60.7)	23 (76.7)	0.152
GLP-1 receptor agonist	4 (6.9)	3 (10.7)	1 (3.3)	0.280
Medication for HF				
Loop diuretics	58 (100.0)	28 (100.0)	30 (100.0)	–
Tolvaptan	9 (15.5)	6 (21.4)	4 (13.3)	0.320
Thiazide diuretics	2 (3.5)	0 (0.0)	2 (6.7)	0.263
MRA	21 (36.2)	10 (35.7)	11 (36.7)	0.579
ACEI/ARB	34 (58.6)	16 (57.1)	18 (60.0)	0.518
β-blockers	44 (75.9)	19 (67.9)	25 (83.3)	0.143
Statins	35 (60.3)	18 (64.3)	17 (56.7)	0.373
Antithrombotic agents	17 (29.3)	7 (25.0)	10 (33.3)	0.342
Antiplatelet agents	30 (51.7)	14 (50.0)	16 (53.3)	0.504
Cardiac biomarkers				
HFABP (ng/mL)	6.6 [4.4–10.0]	7.8 [4.5–10.1]	5.8 [4.4–9.8]	0.722
BNP (pg/mL)	91 [52–194]	68 [47–144]	107 [66–288]	0.169
Renal urinary biomarkers				
NGAL (ng/mg)	11.8 [10.0–25.5]	13.8 [10.0–43.4]	10.0 [10.0–20.4]	0.379
LFABP (ng/mg)	1.07 [0.50–3.55]	1.23 [0.74–11.88]	0.83 [0.50–1.76]	0.156
NAG (U/mg)	3.40 [1.80–8.60]	4.80 [2.60–11.70]	2.25 [1.45–7.20]	0.136

Unless indicated otherwise, data are given as the median [interquartile range] or n (%). The significance of differences between the empagliflozin and control (non-empagliflozin) groups was determined using the Mann-Whitney U-test or the Chi-squared test. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BG, blood glucose; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CKD, chronic kidney disease; CRP, C-reactive protein; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HF, heart failure; HFABP, heart-type fatty acid-binding protein; HR, heart rate; LFABP, liver-type fatty acid binding protein; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NAG, *N*-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; SBP, systolic blood pressure.

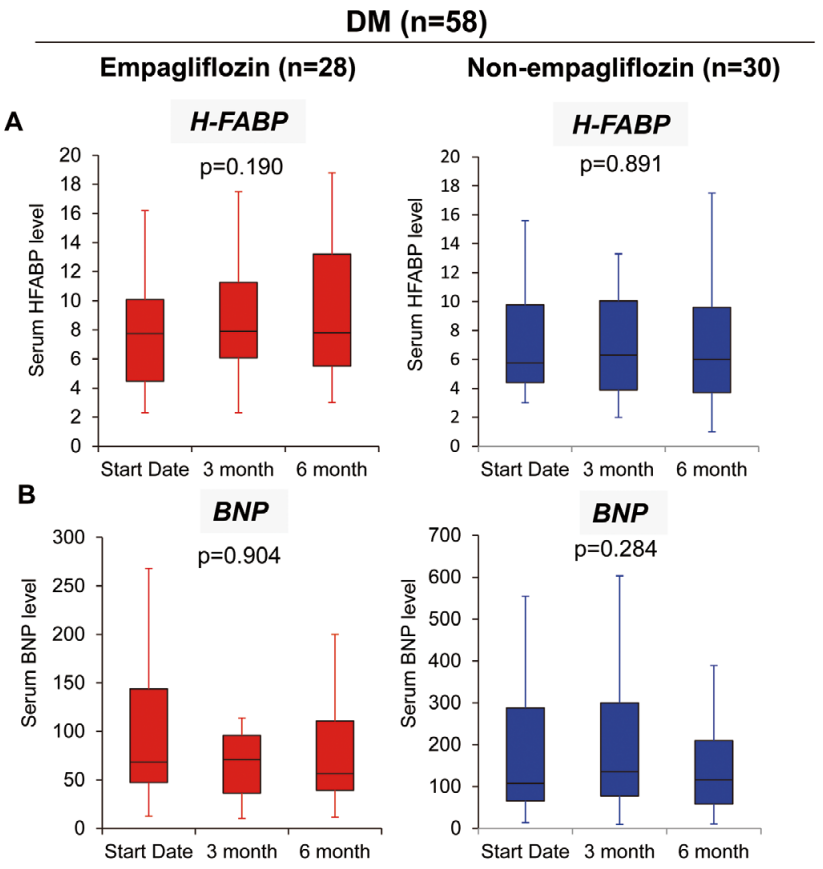

Figure 1. Changes in cardiac biomarkers in the empagliflozin and control (non-empagliflozin) groups. (**A**) There was no significant changes in serum heart-type fatty acid-binding protein (HFABP) concentrations over the 6-month follow-up period in either the empagliflozin (from 7.6±3.7 to 7.7±4.0 ng/mL) or control (from 7.8±4.8 to 8.0±6.0 ng/mL) group. (**B**) There were no significant differences in changes in serum B-type natriuretic peptide (BNP) concentrations between the empagliflozin (from 152.2±165.5 to 173.9±351.3 pg/mL) and control (from 106.7±89.2 to 107.2±119.0 pg/mL) groups. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

itors in patients with drug-refractory HF, particularly those with right-side HF, was demonstrated in a Japanese institution.[9] In addition, a Spanish study suggested improved exercise capacity at 1 month in HF patients after SGLT2 inhibitor administration.[10]

Subgroup analysis of the EMPA-REG OUTCOME trial suggested that the SGLT2 inhibitor empagliflozin is associated with a decreased rate of post-acute HF re-hospitalization and improved mortality.[11] Therefore, the Empagliflozin Outcome Trial in Patients With Chronic Heart Failure (EMPEROR-Reduced), which is expected to lead to better long-term outcomes through treatment with SGLT2 inhibitors, is now ongoing.[7] However, the mechanisms underlying the beneficial effects of SGLT2 inhibitors in HF remain controversial and unclear.[12] SGLT2 inhibitors have also been reported to reduce the risk of adverse renal outcomes and to increase hematocrit levels.[13,14] These effects may help clarify the mechanisms through which SGLT2 inhibitors improve outcomes in patients with HF.

One of the effects of SGLT2 inhibitors is a diuretic effect, induced by increases in urinary glucose and sodium content. Therefore, we posed the clinical question of whether the dose of loop diuretics could be reduced in HF patients being treated with an SGLT2 inhibitor. We hypothesized that an SGLT2 inhibitor would help prevent renal dysfunction, particularly renal tubular injury, by enabling a reduction in the dosage of loop diuretics in HF patients.

To test this hypothesis, we conducted a multicenter center prospective study in diabetic patients with compensated HF who were already being treated with loop diuretics.

Methods

Subjects

This study prospectively enrolled 60 diabetic patients with compensated HF who visited the outpatient clinics of Nippon Medical School Chiba Hokusoh Hospital, Hasegawa Hospital, and Tohokamagaya Hospital Knamachidaiichi Hospital between August 2018 and October 2019. All patients had been diagnosed with type 2 diabetes. HF was diagnosed by the treating physician at the outpatient clinic according to the European Society of Cardiology (ESC) and Japanese guidelines for the diagnosis of HF.[15,16] Physicians first considered HF based on the patient's symptoms, medical history, physical findings, electrocardiogram, and chest X-ray findings and then definitively diagnosed HF based on N-terminal pro B-type natriuretic peptide (NT-proBNP) and B-type natriuretic peptide (BNP) concentrations, echocardiogram findings, and a cardiac catheter test.

Patients enrolled in the study were diagnosed as having chronic HF or a history of acute HF at the date of enrolment and were assessed as having compensated HF. All patients were receiving loop diuretics (furosemide and/or trasemide and/or azosemide) at the study start date. Patients with a history of hypersensitivity to SGLT2 inhibitors, diabetic coma, or severe infectious disease, those in whom SGLT2 inhibitor administration was deemed

Table 2. Differences in Patient Characteristics Between the Start Date and the 6-Month Time Point

	Empagliflozin (n=28)			P value	Control (n=30)			P value
	Start Date	3 months	6 months		Start date	3 months	6 months	
Laboratory data								
Uric acid (mg/dL)	5.7 [5.0–7.1]	5.1 [4.0–5.8]	5.0 [4.4–5.7]	0.018	5.9 [5.1–6.6]	6.3 [5.2–6.9]	6.0 [5.2–7.0]	0.316
Total bilirubin (mg/dL)	0.7 [0.4–0.8]	0.5 [0.4–0.7]	0.6 [0.5–0.8]	0.732	0.8 [0.5–1.0]	0.8 [0.6–1.1]	0.9 [0.7–1.0]	0.165
BUN (mmol/L)	22.1 [15.7–29.6]	20.5 [16.9–28.7]	21.0 [16.0–28.1]	0.622	18.0 [16.2–25.0]	19.1 [14.6–22.0]	20.5 [17.0–27.8]	0.785
Creatinine (g/dL)	1.29 [0.92–1.85]	1.32 [0.96–1.72]	1.37 [1.05–1.67]	0.269	0.96 [0.85–1.15]	0.93 [0.85–1.21]	1.00 [0.83–1.19]	0.703
eGFR (mL/min/1.73 m^2)	43.3 [28.5–61.1]	39.8 [31.5–55.8]	38.2 [32.1–56.4]	0.143	56.7 [43.6–64.6]	51.0 [44.3–65.9]	51.3 [40.6–61.2]	0.688
Sodium (mmol/L)	140 [139–142]	140 [139–140]	140 [138–142]	0.433	141 [140–143]	142 [140–143]	141 [138–143]	0.256
Potassium (mmol/L)	4.2 [4.0–4.6]	4.5 [4.2–4.7]	4.4 [4.1–4.8]	0.242	4.3 [4.1–4.7]	4.2 [3.8–4.5]	4.5 [4.0–4.7]	0.753
Hemoglobin (g/dL)	13.2 [11.9–14.6]	14.1 [12.6–15.0]	14.0 [12.7–15.0]	0.003	13.4 [12.3–14.8]	13.4 [12.2–15.0]	13.6 [12.1–14.7]	0.574
CRP (mg/dL)	0.10 [0.05–0.28]	0.14 [0.10–0.28]	0.14 [0.05–0.40]	0.449	0.08 [0.00–0.18]	0.10 [0.09–0.20]	0.09 [0.02–0.21]	0.475
BG (mg/dL)	126 [103–183]	148 [115–193]	133 [110–186]	0.568	127 [110–145]	126 [110–156]	127 [114–160]	0.178
HbA1c (%)	7.2 [6.3–7.8]	7.2 [6.6–7.7]	6.9 [6.6–7.4]	0.991	6.6 [6.2–6.8]	6.4 [6.3–6.8]	6.6 [6.2–7.1]	0.888
Diabetes medications								
Insulin	7 (25.0)	–	8 (28.6)	1.000	2 (6.7)	–	2 (6.7)	1.000
Sulfonylureas	3 (10.7)	–	2 (7.1)	1.000	2 (6.7)	–	2 (6.7)	1.000
Biguanides	13 (46.4)	–	13 (46.4)	1.000	8 (26.7)	–	11 (36.7)	0.580
α-Glucosidase inhibitor	4 (14.3)	–	1 (3.6)	0.352	5 (16.7)	–	4 (13.3)	1.000
Thiazolidinediones	2 (7.1)	–	2 (7.1)	1.000	0 (0.0)	–	0 (0.0)	–
Glinides	4 (14.3)	–	4 (14.3)	1.000	4 (13.3)	–	3 (10.0)	1.000
DPP-4 inhibitor	17 (60.7)	–	17 (60.7)	1.000	23 (76.7)	–	23 (76.7)	1.000
GLP-1 receptor agonist	3 (10.7)	–	3 (10.7)	1.000	1 (3.3)	–	1 (3.3)	1.000
HF medications								
Loop diuretics	28 (100.0)	–	25 (89.3)	0.236	30 (100.0)	–	30 (100.0)	–
Tolvaptan	6 (21.4)	–	4 (14.3)	0.729	4 (13.3)	–	4 (13.3)	
Thiazide diuretics	0 (0.0)	–	0 (0.0)	–	2 (6.7)	–	2 (6.7)	1.000
MRA	10 (35.7)	–	9 (32.1)	1.000	11 (36.7)	–	11 (36.7)	1.000
ACEI/ARB	16 (57.1)	–	16 (57.1)	1.000	18 (60.0)	–	18 (60.0)	1.000
β-blockers	19 (67.9)	–	19 (67.9)	1.000	25 (83.3)	–	26 (86.7)	1.000
Statins	18 (64.3)	–	18 (64.3)	1.000	17 (56.7)	–	18 (60.0)	1.000

Unless indicated otherwise, data are given as the median [interquartile range] or n (%). The significance of differences between the start date and the 6-month time point was determined using the Wilcoxon test or the Chi-squared test. Abbreviations as in Table 1.

impossible by the physician, and those who did not provide informed consent were excluded from the study.

The present is a prospectively randomized clinical trial, and patients were divided into 2 groups: those administered empagliflozin and those not. Patients were randomly allocated to one of the 2 groups using the envelope method. Empagliflozin was started at a dose of 10 mg/day and increased to 25 mg/day after the first evaluation of tolerability. All patients were titrated to a dose of 25 mg/day empagliflozin during the study period. There were no limitations on HF therapy except for empagliflozin use, and the treatment strategy for individual patients was determined by each patient's doctor. Two patients in the empagliflozin group dropped out during the 6-month follow-up; thus, 58 patients (28 in the empagliflozin group and 30 in the non-empagliflozin [control] group) were analyzed.

Laboratory and urinary data (including cardiac and urinary biomarkers) were obtained at the start date, and 3 and 6 months after the intervention. Time-dependent changes in laboratory and urinary data (including cardiac and urinary biomarkers), as well as medications for diabetes and HF (including the dose of loop diuretics), were evaluated between the start date and the 6-month time point in both the empagliflozin and control groups. For statistical analyses, the doses of azosemide and trasemide were converted as follows: azosemide 60 mg = furosemide 40 mg; and trasemide 8 mg = furosemide 40 mg.

A subgroup analysis was performed in the empagliflozin-treated group. The patients in the empagliflozin-treated group were further divided into 2 groups according to the presence or absence of chronic kidney disease (CKD) and a decrease in the dose of loop diuretics.

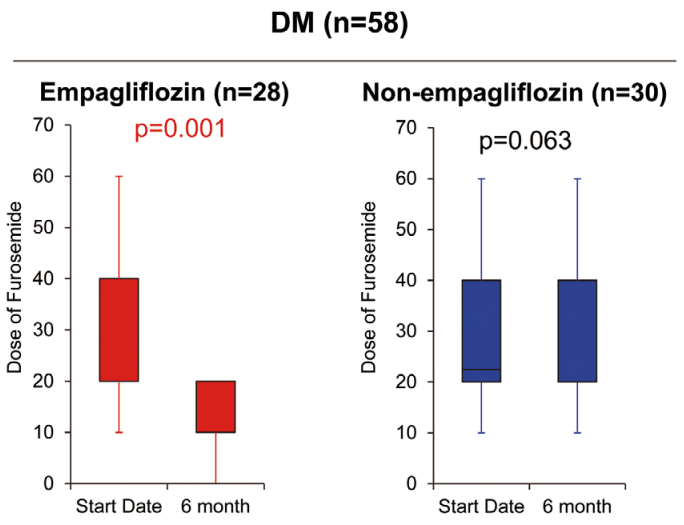

Figure 2. Changes in the dose of diuretics in the empagliflozin and control (non-empagliflozin) groups over the 6-month follow-up period. The furosemide dose decreased significantly (P<0.001) in the empagliflozin group (from a median [interquartile range] of 40 [20–40] to 20 [10–20] mg), but not in the control group (from 23 [20–40] to 40 [20–40] mg). The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range. *Azosemide 60 mg=Furosemide 40 mg; Trasemide 8 mg=Furosemide 40 mg.

Urinary Biomarker Excretion and Measurement of Serum Biomarkers

Urine and blood samples were collected on the day when consent was obtained (start date) and at the follow-up examination after 6 months. Samples were centrifuged within 5 min of collection (2,000 g, 7 min, 4°C), and the supernatant was collected immediately frozen at −80°C until analysis. Serum concentrations of the cardiac biomarkers heart-type fatty acid-binding protein (HFABP) and BNP were measured. In addition, neutrophil gelatinase-associated lipocalin (NGAL), urine liver fatty acid-binding protein (LFABP), and acetyl-β-D-glucosaminidase (NAG) excretion were measured as markers of urinary renal tubular function. These urine and serum biomarkers were measured by the Special Reference Laboratory (SRL; Tokyo, Japan). Urinary concentrations of LFABP were measured using a chemiluminescent enzyme immunoassay (Lumipulse Presto L-FABP; FujiRebio, Tokyo, Japan), urinary NGAL concentrations were measured using a chemiluminescent immunoassay (U-NGAL; Abbott Laboratories, Abbott Park, IL, USA), and urinary NAG was measured using a colorimetric assay (L-Type NAG; FUJIFILM Wako Pure Chemical, Tokyo, Japan). The lower and upper limits of detection were 10 and 1,000 ng/mL, respectively, for urinary NGAL; 0.2 and 400 ng/mL, respectively, for urinary LFABP; and 0.4 and 1,000 IU/L, respectively, for urinary NAG.

Statistical Analyses

Data were analyzed using SPSS 22.0 J (SPSS Japan Institute, Tokyo, Japan). All numerical data are expressed as median values with the interquartile range (IQR). Median values were compared between the empagliflozin-treated and control groups using the Mann-Whitney U-test. Wilcoxon's test was used to evaluate the significance of differences in parameters between the start date and 6 months later. Comparisons of all proportions were made using Chi-squared tests. Two-sided P<0.05 was considered significant.

Ethical Considerations

The Institutional Review Board of the Nippon Medical School Chiba Hokusoh Hospital approved the study protocol. Written informed consent was obtained from all participants before they started in the study. This study is registered with the UMIN Clinical Trials Registry (UMINID 000040347). All procedures were performed in accordance with the Declaration of Helsinki.

Results

Patient Characteristics

The median age of the HF patient cohort was 74 years, and 48 patients (82.8%) were male. The median LVEF upon registration was 55.0%, and 27 patients (46.6%) had CKD (**Table 1**). Twenty-five patients (43.1%) had an ischemic etiology, 23 (39.7%) had atrial fibrillation (persistent or paroxysmal), and 31 (51.4%) had a history of hospitalization due to HF. There were no significant differences patient characteristics between the empagliflozin-treated and control groups (**Table 1**).

Differences in Time-Dependent Changes in the Empagliflozin-Treated and Control Groups

For most laboratory findings, including HFABP and BNP, there were no significant differences between the start date and the 6-month time point (**Figure 1**; **Table 2**). Hemoglobin levels increased significantly from the start date to the 6-month time point in the empagliflozin group (from 13.2 [11.9–14.6] to 14.0 [12.7–15.0] g/dL, respectively), but not in the control group (**Table 2**).

With regard to medications, the dose of furosemide decreased significantly (P<0.001) from the start date to the 6-month time point in the empagliflozin group (from 40 [20–40] to 20 [10–20] mg), but not in the control group (from 23 [20–40] to 40 [20–40] mg; **Figure 2**). There were no significant differences in the rate of administration of other diabetes and HF medications between the start date and the 6-month time point in the empagliflozin-treated or control groups (**Table 2**). In the empagliflozin group, the dosage of loop diuretics decreased over the 6-month period in 15 patients. Information regarding the timing and reasons for the reduction are given in **Table 3**. Almost all 15 patients (80.0%) had the dose of loop diuretics decreased within 3 months, with reasons for the reduction including

Table 3. Changes in Loop Diuretics and Reasons for Changes in Patients Administrated Empagliflozin

Patient no.	Age (years)	Sex	Furosemide dose (mg)			Timing of change (month)	Reason for change
			At start date	At 6 months	Change		
1	68	Male	10	10	0	–	–
2	79	Female	10	20	10	–	–
3	60	Male	40	20	–20	5	Decrease in serum BNP
4	73	Male	10	10	0	–	–
5	75	Male	40	10	–30	3	Increase in urinary volume
6	93	Male	40	40	0	–	–
7	66	Male	10	10	0	–	–
8	71	Male	80	80	0	–	–
9	73	Male	40	20	–20	1	Increase in urinary volume
10	72	Male	40	20	–20	3	Decrease in serum BNP and increase in Scr
11	65	Male	40	0	–40	1	Increase in urinary volume
12	84	Male	20	0	–20	1	Increase in urinary volume
13	74	Male	20	20	0	–	–
14	71	Male	40	10	–30	2	Increase in urinary volume
15	51	Male	60	10	–50	1	Increase in urinary volume
16	67	Male	20	20	0	–	–
17	62	Male	40	20	–20	2	Decrease in serum BNP and increase in Scr
18	73	Male	40	10	–30	3	Decrease in serum BNP and increase in Scr
19	78	Male	20	40	20	–	–
20	78	Male	40	20	–20	3	Increase in Scr
21	71	Male	40	20	–20	1	Increase in urinary volume
22	75	Male	20	10	–10	5	Decrease in serum BNP and increase in Scr
23	37	Male	20	20	0	–	–
24	76	Female	20	20	0	–	–
25	92	Male	80	60	–20	4	Decrease in serum BNP
26	64	Male	20	10	–10	3	Increase in Scr
27	83	Male	40	40	0	–	–
28	47	Male	10	10	0	–	–

BNP, B-type natriuretic peptide; Scr, serum creatinine.

an increase in urine volume, decreased serum BNP concentrations, and increases in serum creatinine concentrations. Decisions regarding the dose of loop diuretics were made by each patient's physician.

With regard to urinary biomarkers, there was no significant difference in urinary NGAL excretion between the start date and the 6-month time point in either the empagliflozin group (from 13.8 [10.0–43.4] to 10.0 [10.0–24.8] ng/mL) or the control group (from 10.0 [10.0–20.4] to 11.9 [10.0–31.0] ng/mL). Urinary LFABP excretion did not differ significantly between the start date and the 6-month time point in the empagliflozin group (from 1.23 [0.74–11.88] to 2.16 [0.72–10.95] ng/mL), but was significantly higher in the control group (from 0.83 [0.50–1.76] to 1.45 [0.61–4.51] ng/mL). Urinary NAG excretion decreased significantly from the start date to the 6-month time point in the empagliflozin group (from 4.8 [2.6–11.7] to 3.3 [2.1–5.4] IU/L), but did not change significantly in the control group (from 2.3 [1.5–7.2] to 4.3 [2.0–7.2]; **Figure 3**).

In the subgroup analysis of the empagliflozin-treated group, urinary NAG excretion decreased significantly from the start date to the 6-month time point in the group of patients in which the dose of loop diuretics was reduced (n=14; from 4.7 [2.5–14.8] to 3.3 [2.1–4.5] IU/L), as well as in patients without CKD (n=13; from 4.1 [2.4–9.8] to 1.9 [1.3–3.4] IU/L; **Figure 4**; **Table 4**).

Discussion

The dose of loop diuretics was significantly decreased by the addition of SGLT2 inhibitor therapy in diabetic patients with compensated HF. Renal tubular injury markers, such as NAG, and hemoglobin levels showed time-dependent differences between the empagliflozin and control (non-empagliflozin) groups. Renal tubular injury may have been alleviated by the reduction in the dose of loop diuretics and the production of erythropoietin following administration of the SGLT2 inhibitor. These mechanisms may be associated with HF prognosis (e.g., mortality and HF rehospitalization). More studies are needed to investigate this further.

Diuretics and SGLT2 Inhibitor Treatment in HF Patients

Loop diuretics (e.g., furosemide, trasemide, and azosemide) are the mainstay HF treatment at present. Certain kinds of loop diuretics are administered as fundamental therapy in most large-scale clinical trials of acute and/or chronic HF. However, the volume reduction caused by loop diuretics can sometimes lead to a decrease in renal blood flow, thereby activating the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system. This may be associated with exacerbation of renal function and may induce adverse effects in patients with HF.[17] There-

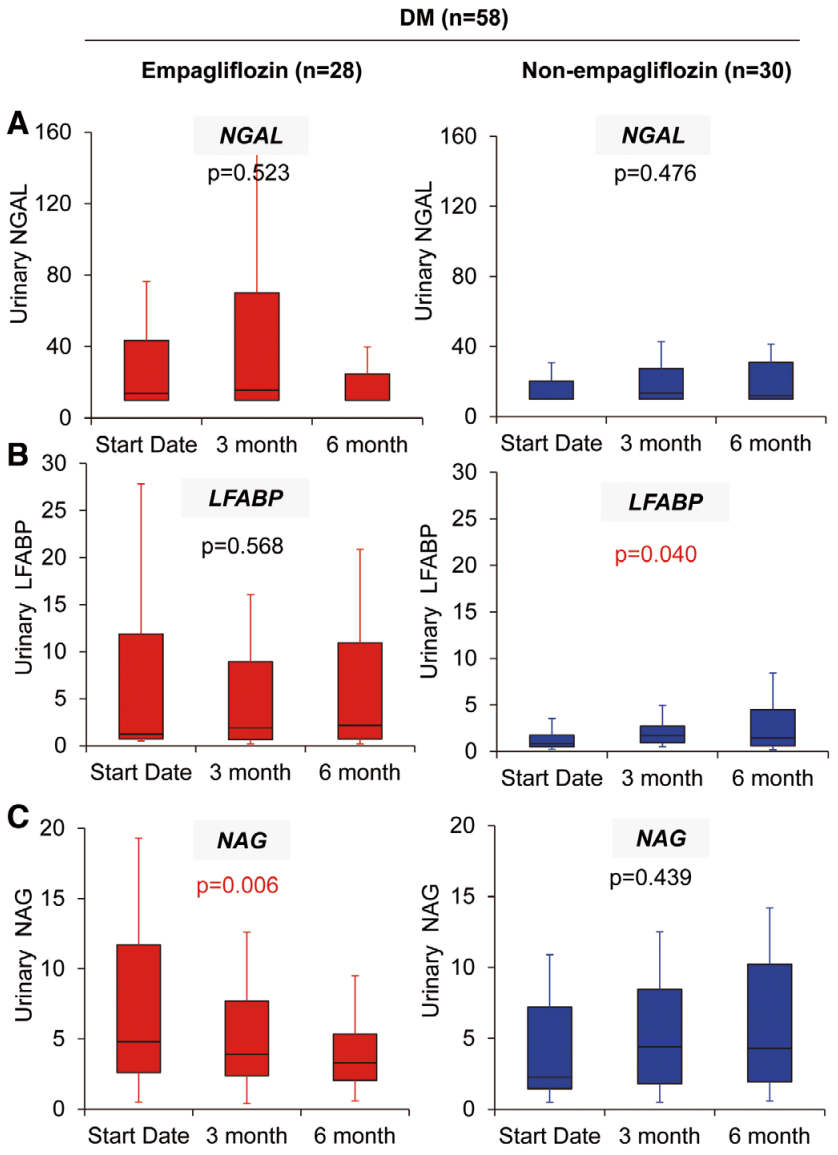

Figure 3. Changes in urinary biomarkers in the empagliflozin and control (non-empagliflozin) group. (**A**) There were no significant changes in the median (interquartile range [IQR]) urinary excretion of neutrophil gelatinase-associated lipocalin (NGAL) between the start date and the 6-month time point in either the empagliflozin (from 13.8 [10.0–43.4] to 10.0 [10.0–24.8] ng/mL) or control (from 10.0 [10.0–20.4] to 11.9 [10.0–31.0] ng/mL) group. (**B**) Similarly, there were no significant changes in the median (IQR) urinary excretion of liver fatty acid-binding protein (LFABP) over the 6-month period in the empagliflozin group (from 1.23 [0.74–11.88] to 2.16 [0.72–10.95] ng/mL), although LFABP excretion increased significantly in the control group (from 0.83 [0.50–1.76] to 1.45 [0.61–4.51] ng/mL). (**C**) Median (IQR) urinary acetyl-β-D-glucosaminidase (NAG) excretion decreased significantly over the 6-month period in the empagliflozin group (from 4.8 [2.6–11.7] to 3.3 [2.1–5.4] IU/L), but did not change significantly in the control group (from 2.3 [1.5–7.2] to 4.3 [2.0–7.2]). The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

fore, a clinical approach to reducing the dose of diuretics while preventing worsening of renal function is required. Tolvaptan may be a candidate agent for achieving such effects in Japan.[3]

Although SGLT2 inhibitors are primarily considered glucose-lowering drugs due to their inhibition of the reabsorption of blood glucose and sodium, they also sometimes act as diuretics. Indeed, it was reported that urine volume increased by an average of 300 mL/day in the first several weeks after the administration of SGLT2 inhibitors.[17] The mechanism underlying the diuretic effect of SGLT2 inhibitors differs completely from that of conventional diuretics, such as loop diuretics and/or thiazides, because, unlike conventional diuretics, SGLT2 inhibitors reduce the intravascular volume. It was therefore suggested that SGLT2 inhibitors may be able to prevent the neurohormonal and sympathetic activation that leads to HF exacerbation, improve abnormal electrolyte levels, and decrease activation of the RAAS.[18] A previous small-cohort Japanese study previously reported a reduction in the original dose of diuretics following the administration of SGLT2 inhibitors in patients with advanced HF.[9] The diuretic effect of SGLT2 inhibitors is limited, because this is merely a secondary effect of the drugs. Because SGLT2 inhibitors would not markedly increase urine volume,[17] it may be appropriate to prescribe them to outpatients with compensated HF who are not in the acute phase.

Biomarkers Indicating Renal Tubular Injury

The mechanism underlying tubular injury in HF patients is unclear. Renal tubular injury in such cases may be caused by various conditions (e.g., activation of the RAAS, increased sympathetic nervous system activity, renal vasoconstriction, ischemic damage, chronic inflammation, and activation of reactive oxygen species). Many urinary biomarkers have been suggested as useful for evaluating renal tubular injury in patients with HF. It may be important to be used properly case-by-case.

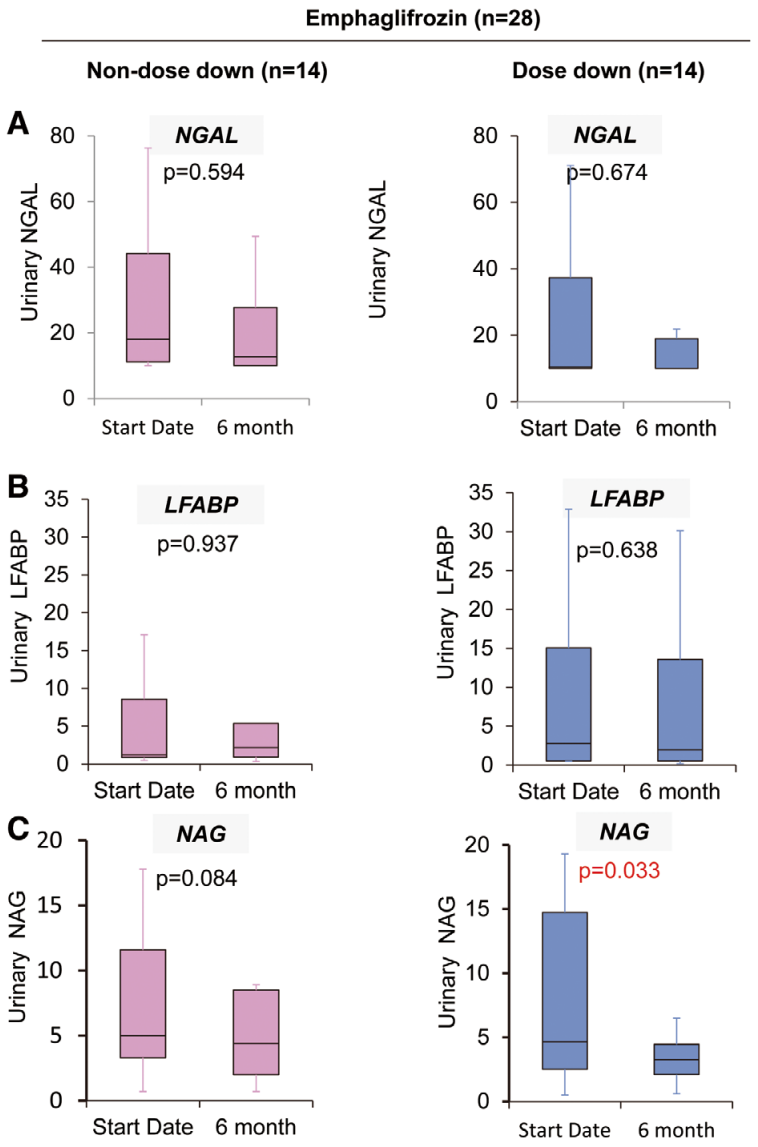

Figure 4. Changes in urinary biomarkers in empagliflozin-treated patients according to whether the dose of loop diuretics was decreased over the follow-up period. (**A**) There were no significant changes in the median (interquartile range [IQR]) urinary excretion of neutrophil gelatinase-associated lipocalin (NGAL) between the start date and the 6-month time point in either the group with no change in loop diuretic dose (from 18.0 [11.2–44.1] to 12.7 [10.0–27.7] ng/mL) or the group in which the dose of loop diuretics was decreased (from 10.5 [10.0–37.3] to 10.0 [10.0–19.0] ng/mL). (**B**) Similarly, there were no significant changes in the median (IQR) urinary excretion of liver fatty acid-binding protein (LFABP) over the 6-month period in group with no change in loop diuretic dose (from 1.22 [0.85–8.57] to 2.16 [0.91–5.38] ng/mL) or the group in which the dose of loop diuretics was decreased (from 2.80 [0.55–15.10] to 1.98 [0.56–13.57] ng/mL). (**C**) Median (IQR) urinary acetyl-β-D-glucosaminidase (NAG) excretion decreased significantly over the 6-month period in the group in which the dose of loop diuretics was decreased (from 4.7 [2.5–14.8] to 3.3 [2.1–4.5] IU/L), but did not change significantly in the group with no change in loop diuretic dose (from 5.0 [3.3–11.6] to 4.4 [2.0–8.5]). The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

Urinary NGAL and LFABP have been reported to be major urinary biomarkers for the detection of acute kidney injury (AKI) in acute HF patients in European and Japanese cohorts.[19,20] Urinary LFABP may help prevent free fatty acid-induced tubulointerstitial damage and reflect various kinds of stressors that cause such damage. This makes LFABP a potentially useful clinical marker for the progression of kidney damage and monitoring renal tubulointerstitial damage,[21] with kidney injury assessed by direct measurement of protein levels.[22] Urinary LFABP concentrations may increase before the occurrence of tubular structural damage; therefore, LFABP may be a real-time indicator of tubulointerstitial damage and become a useful biomarker for the early detection of AKI. Under AKI conditions, various stressors increase NGAL concentrations in the circulation, which induces neutrophil activation and increases the amount of NGAL filtered through the glomeruli. Some NGAL molecules are reabsorbed by the damaged proximal tubules, whereas others are excreted. Therefore, increased urinary NGAL concentrations is primarily attributed to impaired renal absorption. Urinary NGAL is recognized as the gold-standard urinary biomarker for detecting AKI. Therefore, changes in urinary NGAL and LFABP do not take longer to manifest than other biomarkers after the development of tubular injury.

Urinary kidney injury molecule-1 (KIM-1) and NAG can also indicate renal tubular injury in patients with stable chronic HF.[23–25] Both KIM-1 and NAG are secreted from proximal tubular cells, and their superiority as markers of renal tubular injury over NGAL in stable chronic HF has been reported.[23,25] KIM-1 is thought to be excreted in the urine when tubular dysfunction develops, and urinary NAG is a deviant enzyme from the proximal tubule that has been recognized as a traditional urinary marker useful for detecting renal tubular injury. The response time of NAG is much slower than that of other markers.[26] Thus, urinary KIM-1 and NAG better reflect the severity of chronic tubular injury caused by chronic HF (cardiorenal

Table 4. Time-Dependent Changes in Urinary Biomarkers in the Subgroup Analysis According to the Presence of CKD

	No CKD (n=31)					
	Empagliflozin (n=13)		P value	Control (n=18)		P value
	Start date	6 months		Start date	6 months	
Urinary concentrations						
NGAL (ng/mL)	10.0 [10.0–28.3]	10.0 [10.0–11.0]	0.046	10.0 [10.0–13.8]	10.0 [10.0–28.0]	0.721
LFABP (ng/mL)	1.07 [0.77–1.23]	0.82 [0.50–1.71]	0.213	0.50 [0.50–0.82]	0.74 [0.51–2.89]	0.877
NAG (IU/L)	4.1 [2.4–9.8]	1.9 [1.3–3.4]	0.023	1.7 [0.9–3.1]	3.1 [1.5–5.6]	0.795

	CKD (n=27)					
	Empagliflozin (n=15)		P value	Control (n=12)		P value
	Start date	6 months		Start date	6 months	
Urinary concentrations						
NGAL (ng/mL)	16.5 [11.0–43.7]	19.8 [10.0–51.7]	0.594	14.8 [10.0–21.9]	21.0 [10.8–34.5]	0.534
LFABP (ng/mL)	5.89 [0.94–15.05]	5.28 [1.85–18.93]	0.173	1.57 [0.98–3.96]	5.80 [2.18–10.99]	0.508
NAG (IU/L)	5.6 [3.3–15.9]	4.4 [3.3–7.5]	0.081	5.0 [2.3–8.4]	8.1 [4.2–11.2]	0.374

Unless indicated otherwise, data are given as the median [interquartile range]. The significance of differences between the start date and the 6-month time point were determined using the Wilcoxon test. LFABP, liver-type fatty acid-binding protein. Other abbreviations as in Table 1.

syndrome) than urinary NGAL and LABP.[23] We were unable to evaluate urinary KIM-1 concentrations in the present study, so urinary NAG was deemed the most reasonable biomarker for the evaluation of chronic tubular injury in patients with compensated HF.

Effects of SGLT2 Inhibitors on Renal Tubular Injury

The renal benefits induced by SGLT2 inhibitors have been reported from various perspectives.[13,27,28] SGLT2 inhibitors slow the progression of kidney function decline, thereby reducing the risk of dialysis, transplantation, and death due to kidney disease.[13] However, although SGLT2 inhibitors are known to inhibit AKI, the mechanisms underlying their renal protective effects have not been completely elucidated, and they may be multifactorial.

We focused on a brief report describing the reduction in renal tubular cell injury in patients with diabetes.[29] We hypothesized that the renal protection induced by this mechanism may also be expected in patients with HF. Because the effects of SGLT2 inhibitors have not been demonstrated in a chronic or compensated HF cohort, we conducted a detailed investigation in the present study. Urinary NAG was significantly decreased at 6 months after the administration of empagliflozin, especially in the group in which the dose of loop diuretics was reduced. As noted above, urinary NAG is an appropriate biomarker for chronic tubular injury; therefore, the results of the present study suggest that renal tubular injury was alleviated by the administration of the SGLT2 inhibitor. Because urinary LFABP can indicate acute tubular injury, the reason for the increase in urinary LFABP in the non-empagliflozin control group remains unclear.

SGLT2 inhibitors reduce the reabsorption of filtered sodium and glucose in the proximal tubule and decrease the oxygen-consuming transport workload. Less oxygen stress may lead to improved tubular cell integrity.[30] Although we did not measure erythropoietin directly, another study reported that the erythropoietin concentration was increased after administration of an SGLT2 inhibitor, peaking around 2–4 weeks later. Simultaneously, there is a transient increase in reticulocyte count, followed by increases in hemoglobin and hematocrit levels.[31] Oxygen consumption by the proximal tubules is higher in patients with diabetes than in healthy people because of the increased workload due to excessive glucose reabsorption.[32] Sano et al hypothesized that the high oxygen requirement of the proximal tubules due to excessive glucose reabsorption induces tubulointerstitial hypoxia.[30] Transformation into dysfunctional fibroblasts, which fail to produce sufficient erythropoietin, leads to injury of the renal tubules. SGLT2 inhibitors reduce the workload of the proximal tubules, thereby allowing for restoration of tubulointerstitial function and increasing the production of erythropoietin by neural crest-derived fibroblasts.[9] In the present study, hemoglobin levels were significantly increased and urinary NAG excretion was significantly decreased 6 months after SGLT2 inhibitor administration. These results may be reasonable and consistent with the previous hypothesis suggested by Sano et al.[30]

Furthermore, hyperglycemia exerts proinflammatory effects on the renal tubules. Reducing hyperglycemia (e.g., through the appropriate treatment of diabetes) may reduce the generation of proinflammatory cytokines.[33] However, even though the present study was a multicenter study, the number of patients in each group was relatively small, which may have decreased our ability to detect significant effects. More large-scale, multicenter trials will be required to confirm our results.

Study Limitations

The present study has several limitations. First, although all the physicians involved in the outpatient clinic were cardiologists, definitive criteria used by the physicians in determining when to decrease the dose of diuretics were not suggested in the present study. If the HF status remained compensated based on certain evaluations (e.g., patient complaints, chest X-ray, general condition, laboratory findings [serum creatinine and BNP concentrations]), the dose of diuretics was changed at the discretion of the attending physician. Patient complaints and laboratory

findings (serum creatinine and BNP concentrations) were the most important factors in this decision, as indicated in **Table 3**. Second, the present study was an open-label study, which may have affected the clinical judgments made by the investigators. Finally, data (cardiac and urinary biomarker values) for 2 patients in the empagliflozin group were not collected due to human error.

Conclusions

The dose of loop diuretics was decreased and hemoglobin levels increased following the addition of SGLT2 inhibitor therapy in diabetic patients with compensated HF. Urinary NAG excretion was decreased in the empagliflozin-treated group at the 6-month follow-up. Renal tubular injury may be alleviated by the administration of SGLT2 inhibitors through a reduction in the dose of loop diuretics administered and the production of erythropoietin.

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Disclosures

The authors declare no conflicts of interest in association with the present study.

IRB Information

This study was approved by the Institutional Review Board of Nippon Medical School Chiba Hokusoh Hospital (Reference no. 53002).

Data Availability

The deidentified participant data will not be shared.

References

1. Shimokawa H, Miura M, Nochioka K, Sakata Y. Heart failure as a general pandemic in Asia. *Eur J Heart Fail* 2015; **17:** 884–892.
2. Sakata Y, Shimokawa H. Epidemiology of heart failure in Asia. *Circ J* 2013; **77:** 2209–2217.
3. Shirakabe A, Hata N, Yamamoto M, Kobayashi N, Shinada T, Tomita K, et al. Immediate administration of tolvaptan prevents the exacerbation of acute kidney injury and improves the mid-term prognosis of patients with severely decompensated acute heart failure. *Circ J* 2014; **78:** 911–921.
4. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015; **373:** 2117–2128.
5. Neal B, Perkovic V, Matthews DR. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 2017; **377:** 2097–2099 [Letter].
6. Byrne NJ, Parajuli N, Levasseur JL, Boisvenue J, Beker DL, Masson G, et al. Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. *JACC Basic Transl Sci* 2017; **2:** 347–354.
7. Packer M, Butler J, Filippatos GS, Jamal W, Salsali A, Schnee J, et al. Evaluation of the effect of sodium-glucose co-transporter 2 inhibition with empagliflozin on morbidity and mortality of patients with chronic heart failure and a reduced ejection fraction: Rationale for and design of the EMPEROR-Reduced trial. *Eur J Heart Fail* 2019; **21:** 1270–1278.
8. Jensen J, Omar M, Kistorp C, Poulsen MK, Tuxen C, Gustafsson I, et al. Empagliflozin in heart failure patients with reduced ejection fraction: A randomized clinical trial (Empire HF). *Trials* 2019; **20:** 374.
9. Seo Y, Yamamoto M, Machino-Ohtsuka T, Ishizu T, Aonuma K. Effects and safety of sodium glucose cotransporter 2 inhibitors in diabetes patients with drug-refractory advanced heart failure. *Circ J* 2018; **82:** 1959–1962.
10. Nunez J, Palau P, Dominguez E, Mollar A, Nunez E, Ramon JM, et al. Early effects of empagliflozin on exercise tolerance in patients with heart failure: A pilot study. *Clin Cardiol* 2018; **41:** 476–480.
11. Savarese G, Sattar N, Januzzi J, Verma S, Lund LH, Fitchett D, et al. Empagliflozin is associated with a lower risk of post-acute heart failure rehospitalization and mortality. *Circulation* 2019; **139:** 1458–1460.
12. Wojcik C, Warden BA. Mechanisms and evidence for heart failure benefits from SGLT2 inhibitors. *Curr Cardiol Rep* 2019; **21:** 130.
13. Neuen BL, Young T, Heerspink HJL, Neal B, Perkovic V, Billot L, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: A systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2019; **7:** 845–854.
14. Sano M, Goto S. Possible mechanism of hematocrit elevation by sodium glucose cotransporter 2 inhibitors and associated beneficial renal and cardiovascular effects. *Circulation* 2019; **139:** 1985–1987.
15. Tsutsui H, Isobe M, Ito H, Ito H, Okumura K, Ono M, et al; on behalf of the Japanese Circulation Society and the Japanese Heart Failure Society Joint Working Group. JCS 2017/JHFS 2017 guideline on diagnosis and treatment of acute and chronic heart failure: Digest version. *Circ J* 2019; **83:** 2084–2184.
16. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016; **18:** 891–975.
17. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: Cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation* 2016; **134:** 752–772.
18. Verma S, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: A state-of-the-art review. *Diabetologia* 2018; **61:** 2108–2117.
19. Maisel AS, Mueller C, Fitzgerald R, Brikhan R, Hiestand BC, Iqbal N, et al. Prognostic utility of plasma neutrophil gelatinase-associated lipocalin in patients with acute heart failure: The NGAL EvaLuation Along with B-type NaTriuretic Peptide in acutely decompensated heart failure (GALLANT) trial. *Eur J Heart Fail* 2011; **13:** 846–851.
20. Shirakabe A, Hata N, Kobayashi N, Okazaki H, Matsushita M, Shibata Y, et al. Clinical usefulness of urinary liver fatty acid-binding protein excretion for predicting acute kidney injury during the first 7 days and the short-term prognosis in acute heart failure patients with non-chronic kidney disease. *Cardiorenal Med* 2017; **7:** 301–315.
21. Yokoyama T, Kamijo-Ikemori A, Sugaya T, Hoshino S, Yasuda T, Kimura K. Urinary excretion of liver type fatty acid binding protein accurately reflects the degree of tubulointerstitial damage. *Am J Pathol* 2009; **174:** 2096–2106.
22. Veerkamp JH, Peeters RA, Maatman RG. Structural and functional features of different types of cytoplasmic fatty acid-binding proteins. *Biochim Biophys Acta* 1991; **1081:** 1–24.
23. Jungbauer CG, Birner C, Jung B, Buchner S, Lubnow M, von Bary C, et al. Kidney injury molecule-1 and *N*-acetyl-beta-D-glucosaminidase in chronic heart failure: Possible biomarkers of cardiorenal syndrome. *Eur J Heart Fail* 2011; **13:** 1104–1110.
24. Damman K, Masson S, Hillege HL, Voors AA, van Veldhuisen DJ, Rossignol P, et al. Tubular damage and worsening renal function in chronic heart failure. *JACC Heart Fail* 2013; **1:** 417–424.
25. Brankovic M, Akkerhuis KM, Hoorn EJ, van Boven N, van den Berge JC, Constantinescu A, et al. Renal tubular damage and worsening renal function in chronic heart failure: Clinical determinants and relation to prognosis (Bio-SHiFT study). *Clin Cardiol* 2020; **43:** 630–638.
26. Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P, et al. Urinary biomarkers in the early diagnosis of acute kidney injury. *Kidney Int* 2008; **73:** 863–869.

27. Heerspink HJ, Desai M, Jardine M, Balis D, Meininger G, Perkovic V. Canagliflozin slows progression of renal function decline independently of glycemic effects. *J Am Soc Nephrol* 2017; **28:** 368–375.
28. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med* 2016; **375:** 323–334.
29. Dekkers CCJ, Petrykiv S, Laverman GD, Cherney DZ, Gansevoort RT, Heerspink HJL. Effects of the SGLT-2 inhibitor dapagliflozin on glomerular and tubular injury markers. *Diabetes Obes Metab* 2018; **20:** 1988–1993.
30. Sano M, Takei M, Shiraishi Y, Suzuki Y. Increased hematocrit during sodium-glucose cotransporter 2 inhibitor therapy indicates recovery of tubulointerstitial function in diabetic kidneys. *J Clin Med Res* 2016; **8:** 844–847.
31. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes Obes Metab* 2013; **15:** 853–862.
32. O'Neill J, Fasching A, Pihl L, Patinha D, Franzen S, Palm F. Acute SGLT inhibition normalizes O_2 tension in the renal cortex but causes hypoxia in the renal medulla in anaesthetized control and diabetic rats. *Am J Physiol Renal Physiol* 2015; **309:** F227–F234.
33. Bonventre JV. Can we target tubular damage to prevent renal function decline in diabetes? *Semin Nephrol* 2012; **32:** 452–462.