


ORIGINAL RESEARCH

Different Responses of Muscle Sympathetic Nerve Activity to Dapagliflozin Between Patients With Type 2 Diabetes With and Without Heart Failure

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BACKGROUND: Sodium-glucose cotransporter 2 inhibitors improve cardiovascular outcomes in patients with diabetes with and without heart failure (HF). However, their influence on sympathetic nerve activity (SNA) remains unclear. The purpose of this study was to evaluate the effect of sodium-glucose cotransporter 2 inhibitors on SNA and compare the responses of SNA to sodium-glucose cotransporter 2 inhibitors in patients with type 2 diabetes with and without HF.

METHODS AND RESULTS: Eighteen patients with type 2 diabetes, 10 with HF (65.4±3.68 years) and 8 without HF (63.3±3.62 years), were included. Muscle SNA (MSNA), heart rate, and blood pressure were recorded before and 12 weeks after administration of dapagliflozin (5 mg/day). Sympathetic and cardiovagal baroreflex sensitivity were simultaneously calculated. Brain natriuretic peptide level increased significantly at baseline in patients with HF than those without HF, while MSNA, blood pressure, and hemoglobin A1c did not differ between the 2 groups. Fasting blood glucose and homeostatic model assessment of insulin resistance did not change in either group after administering dapagliflozin. MSNA decreased significantly in both groups. However, the reduction in MSNA was significantly higher in patients with HF than patients with non-HF (−20.2±3.46 versus −9.38±3.65 bursts/100 heartbeats; $P=0.049$), which was concordant with the decrease in brain natriuretic peptide.

CONCLUSIONS: Dapagliflozin significantly decreased MSNA in patients with type 2 diabetes regardless of its blood glucose-lowering effect. Moreover, the reduction in MSNA was more prominent in patients with HF than in patients with non-HF. These results indicate that the cardioprotective effects of sodium-glucose cotransporter 2 inhibitors may, in part, be attributed to improved SNA.

Key Words: baroreflex sensitivity ■ diabetes mellitus ■ heart failure ■ muscle sympathetic nerve activity

Augmented sympathetic nerve activity (SNA) is related to worsening prognosis in patients with heart failure (HF).¹ Type 2 diabetes is also reported to increase SNA.^{2,3} Diabetes increases the incidence of cardiovascular complications, including coronary artery disease and HF.⁴ Further augmented SNA in patients with type 2 diabetes with HF presumably results in adverse outcomes. The goals of diabetes treatments

are to prevent these complications and improve cardiovascular mortality. Sodium-glucose cotransporter 2 inhibitors (SGLT2i) reduce the mortality rate from cardiovascular complications in patients with type 2 diabetes.^{5–7} Moreover, SGLT2i reduces the rate of worsening HF and death in patients with HF regardless of the presence or absence of diabetes⁸; hence, these results do not account for solely a glucose-lowering

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CLINICAL PERSPECTIVE

What Is New?

- Twelve weeks of a sodium-glucose cotransporter 2 inhibitor (dapagliflozin 5 mg/day) administration significantly decreased muscle sympathetic nerve activity in patients with type 2 diabetes.
- The decrease in muscle sympathetic nerve activity in patients with diabetes with heart failure was more pronounced than in patients with diabetes without heart failure.
- The decrease in muscle sympathetic nerve activity was correlated with the decrease in brain natriuretic peptide but not with diabetic profiles.

What Are the Clinical Implications?

- Dapagliflozin showed a significant sympatho-inhibition which could be a part of cardioprotective effects.
- Our finding supports the current evidence that sodium-glucose cotransporter 2 inhibitors are effective in patients with heart failure regardless of the presence or absence of diabetes and might help to expand the indications for other sympatho-exited diseases in the future.

Nonstandard Abbreviations and Acronyms

BF	burst frequency
BI	burst incidence
BRS	baroreflex sensitivity
MSNA	muscle sympathetic nerve activity
SGLT2i	sodium-glucose cotransporter 2 inhibitor
SNA	sympathetic nerve activity

effect.⁹ Osmotic diuresis is a favorable mechanism in which SGLT2i reduces blood pressure and improves HF,¹⁰ but SGLT2i is reported to lower blood pressure (BP) without increasing heart rate (HR) which cannot be explained by the diuretic effect.¹¹

Several studies have demonstrated that SGLT2i reduces SNA.^{12,13} SGLT2i-reduced SNA is attributed to improved baroreflex sensitivity (BRS) in the streptozotocin-induced diabetic rat model.¹⁴ In humans, muscle SNA (MSNA) recordings are recognized as the gold standard method for assessing SNA outflow to peripheral vascular beds.¹⁵ We previously demonstrated that pioglitazone decreases MSNA associated with improved insulin resistance.¹⁶ However, it is still not clearly understood whether SGLT2i reduces SNA in patients with

type 2 diabetes, and efficacy in patients with and without HF is unclear.

In this study, we examined the effect of an SGLT2i on MSNA in patients with type 2 diabetes and evaluated whether the responses are related to insulin resistance and/or BRS. We also investigated whether the response of MSNA to SGLT2i differed between patients with type 2 diabetes with and without HF.

METHODS

Subjects

Patients with type 2 diabetes whose hemoglobin A1c (HbA1c) was >7.0% and were aged 20 to 80 years with at least 2 atherosclerotic risk factors were included in this study. Patients with an estimated glomerular filtration rate <45 mL/min per 1.73 m², decompensating HF, or HbA1c >10% were excluded from this study. The diabetes diagnosis was confirmed as recommended by the Japan Diabetic Society.¹⁷ Briefly, diabetes was diagnosed when the patient's HbA1c was >6.5% and fasting blood glucose (FBG) was ≥126 mg/dL (or casual blood glucose ≥200 mg/dL). A re-examination was performed within 1 month if only HbA1c or blood glucose was within the diabetic range.¹⁷ Patients were divided into 2 groups by the presence (HF group) or absence (non-HF group) of HF. HF was diagnosed according to the guideline of the Japanese Circulation Society.¹⁸ HF was diagnosed when patients had New York Heart Association class II or III symptoms and/or their brain natriuretic peptide (BNP) was ≥100 pg/mL, and cardiac dysfunction was confirmed by echocardiography (eg, reduced left ventricular ejection fraction, significant valvular disease, diastolic dysfunction, or signs of elevated right and/or left ventricular pressure). This study protocol was approved by the Research Ethics Board of Kanazawa University (Kanazawa, Japan). This study was registered at the University Hospital Medical Information Network Center (UMIN, Tokyo, Japan) Clinical Trials Registration System (UMIN000026335). All participants provided written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Protocol

Baseline MSNA were recorded after anthropometric and laboratory data were collected, and 5 mg/day dapagliflozin was prescribed. Follow-up measurements were performed 12 weeks after drug administration began. No restrictions were placed on the patients' medications before beginning the study; however, changes in drugs used to treat HF and diabetes were prohibited during the study except when the disease condition worsened.

Measurements

All experiments were performed in a quiet, electrically shielded room in the morning with the subject in the supine position. All participants were asked to abstain from alcohol, caffeine, and strenuous exercise for 24 hours. HR was determined from a continuous ECG. beat-to-beat BP was recorded continuously from the radial artery using a non-invasive tonometry monitoring system (JENTOW-7700; Nihon Colin, Komaki, Japan). Postganglionic MSNA was recorded from the left peroneal nerve, as described previously.^{19–21} Briefly, with the subject in a comfortable supine position, the common peroneal nerve was located by palpation and stimulated electrically via the skin surface. A tungsten microelectrode was inserted percutaneously into a motor fascicle of the peroneal nerve. The electrode was adjusted until spontaneous pulse-synchronous sympathetic burst activities could be recorded. The electrodes were connected to a preamplifier at a gain of 1000 and an amplifier at a gain of 70. The signal was fed through a band-pass filter (500–3000 kHz) and a resistance-capacitance integrated circuit with a time constant of 0.1 seconds to produce a mean voltage neurogram using a PowerLab recording system (Model ML 785/85P; ADInstruments, Bella Vista, Australia). The raw nerve signal was obtained at 12 000 Hz; other signals were obtained at 1000 Hz. Multi-unit MSNA was identified offline based on its relationship to cardiac activity in the integrated nerve recording in a blinded fashion by an experienced investigator. Burst activity was identified as a >3 signal to noise ratio on mean voltage neurograms. For each subject, multi-unit MSNA was expressed as the number per minute (burst frequency [BF]) (bursts/minute) and the number per 100 heartbeats (burst incidence [BI]) (bursts/100 heartbeats). Data were acquired for at least 10 minutes. Multi-unit MSNA has high reproducibility.^{21,22}

Echocardiography (Aplio; Toshiba, Tokyo, Japan) was performed on the same day or within 1 week before the MSNA measurement. Left ventricular ejection fraction was measured using Simpson method. E/e' was calculated by the E wave derived from transmitral flow per e' measured by tissue Doppler imaging of the left ventricular basal septal wall. FBG, immunoreactive insulin, HbA1c, BNP, and lipids were measured on the same day. The homeostasis model assessment of the insulin resistance index was calculated as $\text{HOMA-IR} = \text{fasting glucose (mg/dL)} \times \text{fasting insulin } (\mu\text{U/mL}) / 405$.²³

BRS Analysis

Spontaneous arterial baroreflex modulation of HR (cardiovascular BRS [CBRS]) was estimated using the sequence method.²⁴ The beat-to-beat time series of systolic BP (SBP) and the RR interval were analyzed

using HemoLab software (Harald Stauss Scientific, Iowa City, IA) as reported previously.^{25,26} Briefly, sequences of ≥ 3 beats, during which SBP and the RR interval of the following beat changed in parallel (ie, positive correlation), were detected and divided into 2 groups (up sequences, rising SBP; down sequences, decreasing SBP). The mean value of the slope calculated from the relationship between the change in SBP and RR interval (ms/mm Hg) was regarded as CBRS. The mean slope of all sequences (ie, a combination of the up and down sequences) was calculated as the CBRS slope. A least-square linear regression analysis was applied to each sequence and only sequences in which $R^2 > 0.80$ were accepted. Arterial baroreflex modulation of MSNA (sympathetic BRS [SBRS]) was assessed by examining the relationship between the occurrence of MSNA and diastolic BP (DBP).^{20,27,28} Over a 5-minute resting period, the DBP of individual heartbeats was grouped into 2 mm Hg interval bins, and the percentage of diastolic cycles associated with a sympathetic burst for each interval was plotted against the mean pressure interval. The reflex gain was defined as the slope of the regression line and expressed as bursts/100 heartbeats/mm Hg. If the regression line possessed an $R \geq 0.5$, the slope of the line was taken as the SBRS slope.

Statistical Analysis

Values are expressed as mean \pm SE. The statistical analysis was performed using SPSS software (version 25.0, IBM Corp., Armonk, NY). The paired t test was used to compare differences in the variables in all patients between baseline and 12 weeks after dapagliflozin administration. The unpaired t test was used to compare differences between the HF and non-HF groups. Welch t test was applied if the variance was heteroscedastic. The Chi-square test was adopted to compare differences in the population rate between groups. Linear regression analyses were performed to determine the relationship between change in MSNA and other variables (HbA1c, FBG, HOMA-IR, insulin, HR, BMI, estimated glomerular filtration rate, CBRS, and SBRS). Linear regression analyses were also performed between changes in BNP and others (MSNA, HR, SBP, DBP, BMI, HbA1c, age, and baseline medications). A $P < 0.05$ (2-sided) was considered significant.

RESULTS

Twenty-two patients were submitted to this study between March 2017 and March 2020. Among them, the baseline MSNA of 3 patients could not be adequately recorded, and they rejected attending the remainder of the study. One patient withdrew his agreement, and rejected follow-up measurements; however, baseline

Table 1. Baseline Characteristics in Each Group

	Non-HF (n=8)	HF (n=10)	P value
Age, y	65.4±3.68	63.3±3.62	0.696
Men, n (%)	6 (75.0)	8 (80.0)	0.800
Body weight, kg	72.1±3.92	68.8±4.94	0.633
BMI, kg/m ²	26.3±1.07	24.9±0.99	0.361
Heart rate, /min	76.9±4.64	76.5±2.14	0.895
SBP, mm Hg	119±4.84	121±3.73	0.841
DBP, mm Hg	72.1±3.82	73.5±3.20	0.785
Hypertension, n (%)	7 (87.5)	10 (100)	0.250
Dyslipidemia, n (%)	8 (100)	10 (100)	1.000
Blood test			
LDL, mg/dL	72.3±6.54	82.7±9.44	0.389
FBG, mg/dL	137±7.57	163±15.9	0.159
HbA1c, %	7.49±0.09	7.98±0.21	0.057
Insulin, μU/mL	12.7±3.53	16.2±4.30	0.552
HOMA-IR	4.14±1.27	6.68±2.45	0.176
eGFR, mL/min per 1.73 m ²	61.4±7.68	60.6±6.18	0.940
BNP, pg/mL	23.9±9.37	161±58.6	0.045
UCG			
LVEF, %	65.3±2.18	43.7±3.95	<0.001
LVDd, mm	45.9±1.18	54.2±5.11	0.145
LVDs, mm	29.6±1.25	42.4±4.98	0.033
LAD, mm	39.6±1.47	39.0±4.44	0.890
E/e'	13.7±1.14	12.6±2.82	0.728
Sympathetic nerve activity			
BF, bursts/min	44.5±2.35	50.1±1.88	0.078
BI (bursts/100 heartbeats)	61.2±4.31	69.0±1.77	0.127
CBRS, ms/mm Hg	6.06±0.93	11.2±4.21	0.318
SBRS (bursts·100 heartbeats ⁻¹ mm Hg ⁻¹)	-1.24±0.33	-2.17±1.06	0.427
Medication, n (%)			
β-blocker	3 (37.5)	9 (90)	0.006
Calcium channel blocker	5 (62.5)	3 (30)	0.168
Statin	8 (100)	10 (100)	1.000
ARB or ACE-I	6 (75.0)	10 (100)	0.094
Diuretics	2 (25.0)	7 (70.0)	0.058
Sulfonyl urea	1 (12.5)	1 (10.0)	0.866
DPP-4 inhibitor	2 (25.0)	4 (40.0)	0.502
Glinide	0 (0)	2 (20.0)	0.180
α-GI	1 (12.5)	2 (20.0)	0.671
GLP-1 agonist	2 (25.0)	2 (20.0)	0.800
Biguanide	3 (37.5)	1 (10.0)	0.163
Thiazolidine	1 (12.5)	0 (0.0)	0.250
Insulin	2 (25.0)	4 (40.0)	0.502

Unpaired-t test was performed to compare differences in each parameter between groups. Welch t test was applied if the variance was heteroscedastic. The Chi-square test was adopted to compare differences in the population rate of diseases and medications between groups. Insulin and HOMA-IR were compared among patients without insulin treatment (All, N=12; non-CHF, n=6; CHF, n=6). ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BF, burst frequency; BI, burst incidence; BMI, body mass index; BNP, brain natriuretic peptide; CBRS, cardiovagal baroreflex sensitivity; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; GLP-1, glucagon like peptide; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis assessment of insulin resistance; LAD, left atrial dimension; LDL, low-density lipoprotein; LVDd, left ventricular end diastolic diameter; LVDs, left ventricular end systolic diameter; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; SBRS, sympathetic baroreflex sensitivity; UCG, ultrasound cardiography; and α-GI, α-glucosidase inhibitor.

MSNA was recorded. Finally, 18 patients with type 2 diabetes (10 patients, in the HF group; and 8 patients, in the non-HF group) were included in the study and completed the follow-up. No acute worsening of HF or diabetes requiring an urgent visit and/or hospitalization was observed, and all subjects continued the fixed dose of dapagliflozin during the study.

The baseline characteristics of the participants categorized by the HF group are presented in Table 1. No differences in the physical findings were observed between the non-HF and HF groups (all, $P>0.05$). BNP was significantly higher in the HF group than the non-HF group (161±58.6 versus 23.9±9.37 pg/mL, $P=0.045$), but other blood results including the diabetic parameters were not different between the groups (all, $P>0.05$). As expected, ejection fraction was significantly lower in the HF group than the non-HF group (43.7±3.95% versus 65.3±2.18%, $P<0.001$). The mean values of the MSNA parameters (BF and BI) in both groups increased but tended to be higher in the HF group than the non-HF group (50.1±1.88 versus 44.5±2.35 bursts/min, $P=0.078$). The rate of β-blocker use was significantly higher in the HF group than the non-HF group (90% versus 37.5%, $P=0.006$). The use of diuretics tended to be higher in the HF group than the non-HF group (25% versus 70%, $P=0.058$). No differences were observed in the rates of use of other antihypertensive drugs or hypoglycemic agents between the groups (all, $P>0.05$).

Effects of SGLT2i in Patients With Diabetes With and Without HF

Typical MSNA recordings of 1 patient with type 2 diabetes and HF are shown in Figure 1. MSNA bursts decreased after dapagliflozin was administered. To investigate the difference in the effect of dapagliflozin between patients with type 2 diabetes with and without HF, its responses were compared between groups (Table 2). MSNA (BF and BI) decreased significantly in the HF group compared with baseline (BF, -15.2±2.73 bursts/min, $P<0.001$; BI, -20.2±3.36 bursts/100 heartbeats, $P<0.001$), while BMI was unchanged (-0.02±0.20 kg/m², $P=0.910$). BNP and HR also significantly decreased in the HF group (BNP, -47.7±20.7, $P=0.047$; HR, -4.60±1.24 beats/min, $P=0.005$). In the non-HF group, BMI, HbA1c, and MSNA (BI) decreased significantly compared with baseline (BMI, -0.87±0.22 kg/m², $P=0.008$; HbA1c, -0.56±0.19%, $P=0.021$; BI, -9.38±3.65 bursts/100 heartbeats, $P=0.037$). The reduction in BMI was significantly lower in the non-HF group than in the HF group ($P=0.014$). No differences in other parameters, including BP, diabetic profile, renal function, cardiac function, or BRS were observed between the groups (all, $P>0.05$). SBRS and CBRS were not different between baseline and

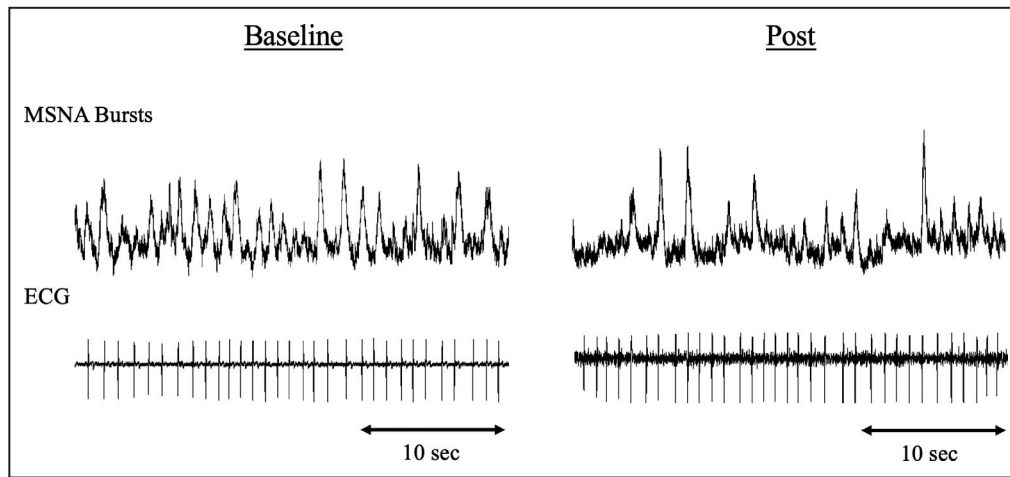


Figure 1. Representative recording of muscle sympathetic nerve activity in a patient with type 2 diabetes with heart failure.

Muscle sympathetic nerve activity was high at baseline and apparently decreased by 12 weeks administration of dapagliflozin. MSNA indicates muscle sympathetic nerve activity.

12 weeks administration in both groups (SBRS of HF, -2.58 ± 2.42 , $P=0.459$; SBRS of non-HF, 0.27 ± 0.35 , $P=0.334$; CBRS of HF, 1.44 ± 2.33 , $P=0.245$; CBRS of non-HF, 2.18 ± 1.72 , $P=0.58$).

As shown in Figure 2, the reductions in MSNA were significantly greater in the HF group than the non-HF group (both in absolute value and percentage reduction) ($\Delta\text{BF}\%$, -29.4 ± 4.46 versus $-9.24 \pm 7.96\%$, $P=0.034$; $\Delta\text{BI}\%$, -28.5 ± 4.37 versus $-12.6 \pm 6.37\%$, $P=0.049$).

Relationship Between MSNA and Other Parameters in Patients With Type 2 Diabetes Treated With an SGLT2i

A linear regression analysis was performed to evaluate the relationship between the change in MSNA and other parameters (Table 3, Figure 3) among all subjects. The amount of change in MSNA (ΔBF) was significantly correlated with that of BNP (ΔBF , $R=0.50$, $P=0.034$; ΔBI , $R=0.54$, $P=0.022$) (Figure 3). However, no relationship was detected between reduced MSNA and the other parameters, including the diabetes parameters (ΔHbA1c , $R=-0.27$, $P=0.313$; ΔFBG , $R=0.09$, $P=0.780$; $\Delta\text{HOMA-IR}$, $R=0.36$, $P=0.481$; $\Delta\text{Insulin}$, $R=-0.53$, $P=0.280$), HR ($R=0.22$, $P=0.389$) and BRS (CBRS, $R=-0.29$, $P=0.384$; SBRS, $R=0.16$, $P=0.593$) (Table 3). To scrutinize the effect of MSNA reduction on BNP, univariate and multivariate linear regression analyses were performed (Table 4). In addition to MSNA change (ΔBF), ΔHR , ΔSBP , ΔDBP were selected as independent variables. HbA1c was also selected because HbA1c was significantly decreased by SGLT2i in the non-HF group (Table 2). Baseline medications that were commonly used for HF treatment and may

affect BNP (β -blocker, ARB/ACE-I, diuretics)¹⁸ were also included in the analysis. In univariate analysis, no variables other than ΔBF were significantly correlated with ΔBNP (all, $P>0.05$) (Table 4). However statistically not significant, ΔHR and ΔBP were positively correlated with ΔBF (ΔHR , $R=0.22$, $P=0.389$; ΔSBP , $R=0.13$, $P=0.605$; ΔDBP , 0.16 , $P=0.517$) and favorable effect of HR and BP reduction on cardiac workload/BNP are clinically reasonable (the decrease in HR and BP can improve myocardial stress via decreasing myocardial oxygen consumption).^{29,30} Moreover, average HR was significantly decreased by SGLT2i in HF group (Table 2). So, multivariate analysis was performed by using these factors as independent variables (Table 4). As a result, ΔBF was significantly correlated with ΔBNP (model 1, standardized coefficients [β]=0.57, $P=0.018$; model 2, $\beta=0.52$, $P=0.045$; model 3, $\beta=0.62$, $P=0.037$) independent of HR ($\beta=-0.31$, $P=0.174$) and BP change (ΔSBP , $\beta=-0.06$, $P=0.809$; ΔDBP , $\beta=-0.20$, $P=0.466$).

DISCUSSION

The novel findings of the present study are as follows: 12 weeks of 5 mg/day dapagliflozin significantly decreased MSNA in patients with type 2 diabetes with and without HF; dapagliflozin reduced MSNA more markedly in patients with HF than in patients without HF; and the reduction in MSNA was significantly correlated with the change in BNP but not with the change in BRS or the diabetic profiles, such as insulin resistance. These results indicate that the cardioprotective effects of SGLT2i are, in part, attributed to improved SNA.

Table 2. Changes in Parameters by Dapagliflozin in Each Group

	Non-HF	HF	P value
ΔSBP, mm Hg	1.75±5.34	-8.30±5.05	0.193
ΔDBP, mm Hg	1.63±4.44	-2.60±2.91	0.422
ΔHeart rate, /min	-1.63±2.88	-4.60±1.24*	0.322
ΔBody mass index, kg/m ²	-0.87±0.22*	-0.02±0.20	0.014
Blood test			
ΔHbA1c, %	-0.56±0.19*	-0.17±0.14	0.113
ΔFBG, mg/dL	-11.5±7.34	-23.9±14.8	0.474
ΔHOMA-IR	0.04±0.37	0.22±0.09	0.668
ΔInsulin, μU/mL	-1.83±1.71	0.53±0.37	0.235
ΔLDL, mg/dL	-10.2±6.21	-1.13±5.59	0.300
ΔBNP, pg/mL	4.5±3.67	-47.7±20.7*	0.033
ΔeGFR, mL/min per 1.73 m ²	-1.47±2.66	-2.75±2.97	0.753
UCG			
ΔEF, %	0.67±2.83	2.00±2.88	0.753
ΔLAD, mm	-3.28±1.94	-1.65±1.38	0.496
ΔLVDd, mm	-3.50±2.62	0.13±1.46	0.221
ΔLVDs, mm	-2.5±1.88	0.00±1.18	0.259
ΔE/e'	-1.15±1.16	0.68±0.89	0.227
Sympathetic nerve activity			
ΔBF, bursts/min	-4.95±3.10	-15.2±2.73*	0.025
ΔBI (bursts/100 heartbeats)	-9.38±3.65*	-20.2±3.46*	0.049
ΔCBRS, ms/mm Hg	2.18±1.72	1.44±2.33	0.806
ΔSBRS (bursts·100 heartbeats ⁻¹ mm Hg ⁻¹)	0.27±0.35	-2.58±2.42	0.295

Paired-t test was performed to compare within group differences in each parameter between before and 12 weeks after treatment. Unpaired-t test was performed to compare differences in each parameter change between groups. Welch t test was applied if the variance was heteroscedastic. BF indicates burst frequency; BI, burst incidence; BNP, brain natriuretic peptide; CBRS, cardiovagal baroreflex sensitivity; DBP, diastolic blood pressure; EF, ejection fraction; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis assessment of insulin resistance; LAD, left atrial dimension; LDL, low-density lipoprotein; LVDd, left ventricular end diastolic diameter; LVDs, left ventricular end systolic diameter; P value, P value between non-CHF group and CHF group; SBP, systolic blood pressure; SBRS, sympathetic baroreflex sensitivity; and UCG, ultrasound cardiography.

*P<0.05 compared with the baseline of the same group.

Benefits of SGLT2i Beyond the Glucose-Lowering Effect

A variety of favorable effects of SGLT2i have been reported regardless of the drug type.³¹ SGLT2i reduce body weight³² and improve non-alcoholic fatty liver disease³³ and renal function.^{34,35} Furthermore, SGLT2i successfully improve cardiovascular outcomes regardless of the presence or absence of diabetes.^{8,9} Increased glycosuria and natriuresis attributable to the inhibitory effect of SGLT2 at the renal proximal tubule

is regarded as the main pathway of SGLT2i to lower glucose.³⁶ Natriuresis reduces plasma volume and blood pressure; hence, both cardiac preload and afterload decrease.³⁷ In the present study, BMI was significantly decreased in non-HF group, which is consistent with previous reports. The reduction in plasma volume unloads the cardiopulmonary baroreceptor,³⁸ and lowering BP unloads the arterial baroreceptor.³⁹ Unloading of these baroreceptors decreases vagal nerve activity and increases SNA, resulting in a higher HR.⁴⁰ However, in previous studies, SGLT2i did not increase HR despite a significant reduction in BP.⁵⁻⁷ Moreover, in some studies including the present study, HR decreased in response to SGLT2i.⁴¹ An effect of SGLT2i on SNA is suggested from these results, in addition to the evidence of improving cardiovascular outcomes.^{12,13}

An osmotic diuretic effect is a key mechanism of how SGLT2 beneficially affects patients with type 2 diabetes with and without HF.³⁷ In our study, the reduction in BMI was larger in the non-HF group than the HF group and the change was only significant in the non-HF group. The baseline diuretics usage rate tended to be higher in the HF group than the non-HF group. Although not compared in this study, the amount of the baseline diuretic dose is expected to be larger in the HF group than the non-HF group, which could attenuate the diuretic effect of SGLT2i. This difference in the volume change by SGLT2i between groups might affect the difference in the BMI and HbA1c change. The reduction in HbA1c was significant in the non-HF group but not in the HF group, and although statistically not significant, the change in low-density lipoprotein in the non-HF group seems higher than in the HF group. Same with BMI change, these might be explained by the difference in the diuretic effect between groups since the hypoglycemic effect of SGLT2i depends on urination.³⁷ We did not measure urine volume change in this study, so we could not confirm the difference between groups. Further studies are needed to determine the differences in diuretic and hypoglycemic effects of SGLT2i in the presence and absence of HF. On the contrary, the changes in MSNA and BNP in the HF group were significantly greater than in the non-HF group. These results indicate that the beneficial effects of SGLT2i on patients with HF were not caused only by the diuretic effect, but other mechanisms also played a crucial role.

Possible Mechanisms for the Reduction in MSNA and the Difference Between Groups

An animal study using a nondiabetic rat model of chronic kidney disease revealed that SGLT2i reduce the low-frequency component of heart rate

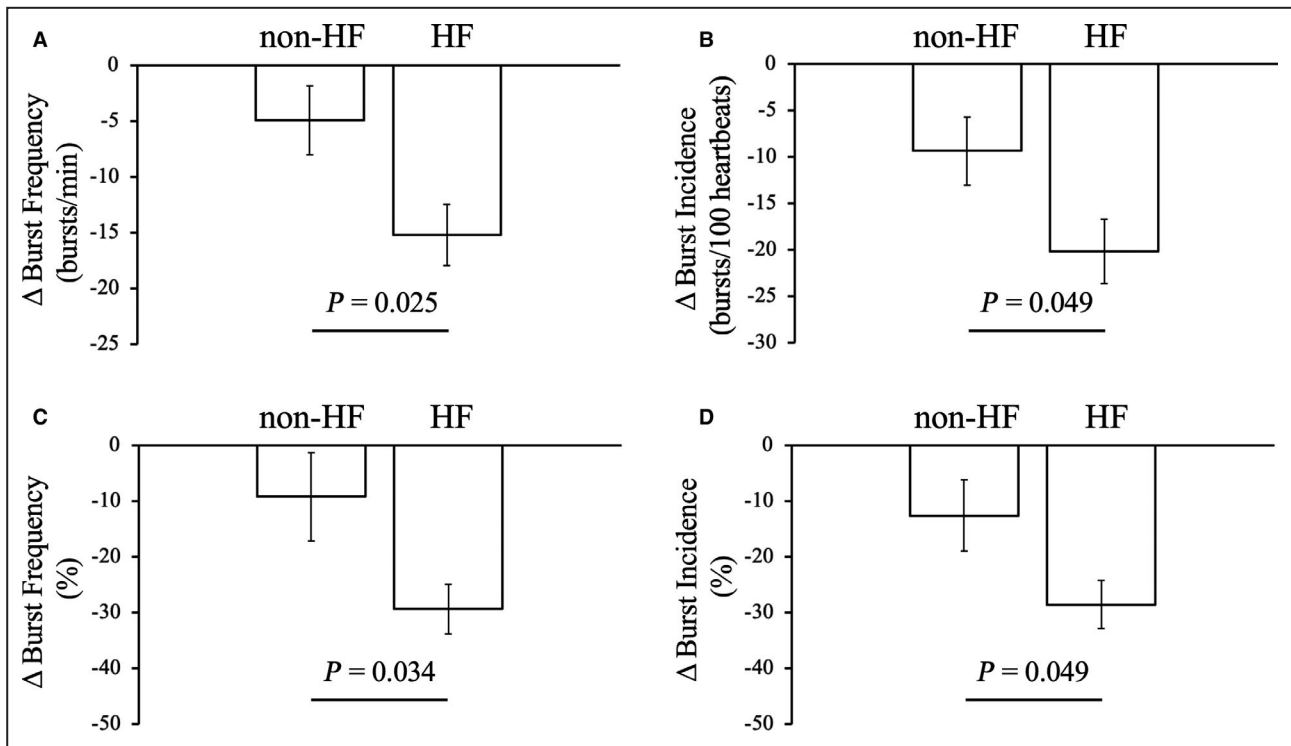


Figure 2. Difference in muscle sympathetic nerve activity (MSNA) response to sodium glucose cotransporter 2 inhibitors between patients with diabetes with and without heart failure.

Change in MSNA (burst frequency and burst incidence) absolute value and percentage reduction by 12 weeks administration of dapagliflozin was significantly higher in patients with diabetes and heart failure than patients with diabetes without heart failure. **A**, Changes in absolute MSNA (burst frequency) value by dapagliflozin. **B**, Changes in absolute MSNA (burst incidence) value by dapagliflozin. **C**, Percentage reduction in MSNA (burst frequency) after 12 weeks administration of dapagliflozin. **D**, Percentage reduction in MSNA (burst incidence) after 12 weeks administration of dapagliflozin. HF indicates heart failure. Unpaired-t test were performed to compare differences between groups.

variability, indicating reduced SNA.⁴² Another study using streptozotocin-induced diabetic rats showed that a non-depressor dose of SGLT2i ameliorates arterial pressure lability associated with sympatho-inhibition and improves the BRS.¹⁴ A recent study demonstrated that MSNA does not increase in response to SGLT2i despite a numerical increase in urine volume and significant reductions in BP and body weight.⁴³ By contrast, in our study, MSNA decreased significantly in response to dapagliflozin. A possible reason for this dissociation is thought to be the timing of measurements. In the previous study, MSNA was measured 4 days after administration had begun. The increase in urine volume reaches its peak during the first 24 hours of SGLT2i administration and gradually decreases after a few days.^{44,45} Thus, 4 days after administration was near the peak of volume loss. Therefore, the reduction of SNA may have been buffered by significant volume loss and lowering of blood pressure that can cause the arterial baroreflex and cardiopulmonary baroreceptor unloading, as the authors reported.⁴³ In our study, MSNA was measured 12 weeks after administration, which was far from the peak of volume loss.

A recent study reported that 24 weeks of administration of SGLT2i significantly reduces the ratio of the low frequency (LF) component to the high frequency (HF) component (LF/HF ratio) of heart rate variability in patients with diabetes with acute myocardial infarction.⁴⁶ The LF/HF ratio is an indirect parameter of SNA. This result is consistent with our results on long-term treatment with SGLT2i.

We measured insulin resistance, SBRS, and CBRS to clarify the potential mechanism that SGLT2i reduces MSNA. However, contrary to our hypothesis, the reduction in MSNA was not related to BRS or the diabetic profile. We could not clarify the underlying mechanism for the reduction of MSNA in this cohort; however, our results indicate that other potential effects of SGLT2i contributed to reducing MSNA. One plausible mechanism is that an improvement of renal afferent SNA has been suggested as a pathway to reduce SNA.^{13,47} Another mechanism is a direct effect of SGLT2i on the central nervous system. SGLT2i can cross the blood-brain barrier and has been the focus as a new treatment method for brain diseases, including seizures⁴⁸ and Alzheimer disease.⁴⁹ Therefore, it

Table 3. Relationship Between Changes in MSNA and Changes in Other Parameters

Δ BF (bursts/min)	<i>R</i>	<i>P</i> value
Δ HbA1c, %	-0.27	0.313
Δ FBG, mg/dL	0.09	0.780
Δ HOMA-IR	0.36	0.481
Δ Insulin, μ U/mL	-0.53	0.280
Δ Heart rate, /min	0.22	0.389
Δ BMI, kg/m ²	-0.32	0.207
Δ eGFR, mL/min per 1.73 m ²	0.26	0.322
Δ CBRS, ms/mm Hg	-0.29	0.384
Δ SBRS (bursts·100 heartbeats ⁻¹ mm Hg ⁻¹)	0.16	0.593

Univariate linear regression analysis between Δ BF and change in other parameters were performed. BMI indicates body mass index; CBRS, cardiovascular baroreflex sensitivity; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis assessment of insulin resistance; HR, heart rate; and SBRS, sympathetic baroreflex sensitivity.

might be possible that SGLT2i reduces SNA by directly reducing the central discharge of SNA. To verify these issues, future studies assessing the effect of SGLT2i, particularly on renal SNA and/or central commands, are expected.

Δ BNP was significantly correlated with Δ MSNA in the regression analysis. The univariate linear regression analyses showed that no baseline factors and parameters change by SGLT2i other than Δ MSNA associated with Δ BNP, and the multivariate analysis showed that Δ MSNA significantly correlated to Δ BNP independent of SBP, DBP, and HR change. It has been reported

that BNP is significantly related to MSNA and plasma norepinephrine concentrations in patients with HF.⁵⁰ It is likely that improvements in MSNA reduced cardiac stress, which contributed to the BNP reduction in our study. This result supports the hypothesis that SGLT2i improves HF by reducing SNA. Despite that MSNA decreased significantly in response to SGLT2i, most ultrasound cardiography parameters remained unchanged. Studies applying a longer and more detailed follow-up are needed to evaluate the change in cardiac function by SGLT2i. However, it is conceivable that unloading of cardiac stress, which could be caused by reducing SNA, was more beneficial for impaired hearts than normal hearts, resulting in improved MSNA and BNP more prominently in patients with diabetes with HF than those without HF.

Clinical Implications for Adding SGLT2i to Conventional HF Treatment and/or Other Cardiovascular Diseases

β -blocker administration is a well-established treatment for inhibiting SNA and improving the prognosis in patients with HF.⁵¹ β -blockers diminish the SNA effect by inhibiting β adrenoreceptors at the effector organs (eg, heart), but not by directly reducing central efferent firing of SNA itself.⁵² As a result, withdrawal of a β -blocker can cause significant sympatho-excitation and worsen cardiovascular outcomes in concert with the upregulation of β adrenoreceptors.^{53–55} In our study, SGLT2i reduced MSNA in patients with HF treated with a β -blocker (90%), which reflects the reduction of central sympathetic efferent neuronal discharge to skeletal muscle. This character of SGLT2i also provides an additional sympatho-inhibitory effect on HF,

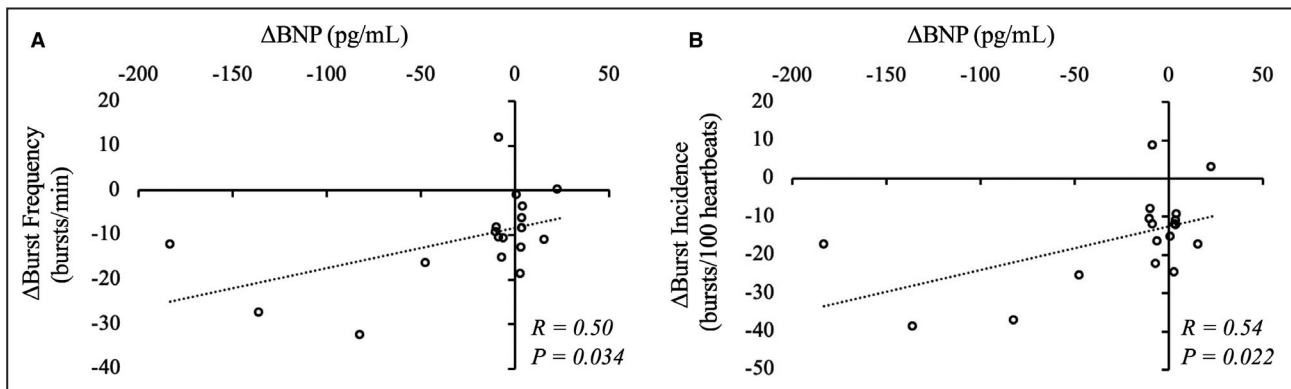


Figure 3. Relationship between changes in muscle sympathetic nerve activity and changes in brain natriuretic peptide by 12 weeks administration of dapagliflozin.

The changes in muscle sympathetic nerve activity (burst frequency and burst incidence) were significantly related to the change in brain natriuretic peptide. **A**, A linear relationship between the change in burst frequency and the change in brain natriuretic peptide after 12 weeks administration of dapagliflozin; **B**) a linear relationship between the change in burst incidence and the change in brain natriuretic peptide after 12 weeks administration of dapagliflozin. BNP indicates brain natriuretic peptide; and *R*, correlation coefficient by univariate linear regression analysis.

Table 4. Relationship Between Changes in BNP and Changes in Other Parameters

Δ BNP (pg/mL)	Univariate analysis		Multivariate analysis		
	<i>R</i>	<i>P</i> value	β	<i>P</i> value	VIF
	Δ BF (bursts/min) (model 1)	0.50	0.034	0.57	0.018
Δ BF (bursts/min) (model 2)			0.52	0.045	1.15
Δ BF (bursts/min) (model 3)			0.62	0.037	1.54
Δ Heart rate, /min	0.22	0.389	-0.31	0.174	1.05
Δ SBP, mm Hg	0.13	0.605	-0.06	0.809	1.15
Δ DBP, mm Hg	0.16	0.517	-0.20	0.466	1.54
Δ BMI, kg/m ²	-0.20	0.453			
Δ HbA1c, %	-0.24	0.380			
Age	0.07	0.787			
β -blocker	-0.39	0.107			
ARB or ACE-I	-0.15	0.564			
Diuretics	-0.13	0.612			

Univariate linear regression analysis was performed between Δ BNP and other parameters. Multivariate linear regression analysis was performed using 3 models below. Model 1: Dependent variable, Δ BNP; independent variables, Δ BF and Δ HR. Model 2: dependent variable, Δ BNP; independent variables, Δ BF and Δ SBP. Model 3: dependent variable, Δ BNP; Independent variables, Δ BF and Δ DBP. ACE-I indicates angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BF, burst frequency; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; MSNA, muscle sympathetic nerve activity; *R*, correlation coefficient value in univariate analysis; SBP, systolic blood pressure; VIF, variance inflation factor; and β , standardized coefficients value in multivariate analysis.

which contributes to the improvement in cardiovascular mortality in conventionally treated patients with HF. Increased SNA is associated with many cardiovascular diseases including cardiac ischemia,^{56,57} resistant hypertension,⁵⁸ and pulmonary hypertension.⁵⁹ While some diseases as obstructive sleep apnea were reported to develop cardiovascular diseases via sympatho-excitation.^{60,61} Therefore, the sympatho-inhibitory effect of SGLT2i observed in our study might be applicable to prevent/treat these diseases regardless of the presence or absence of diabetes. Future studies are expected to verify the effect of SGLT2i on patients with various cardiovascular diseases.

Limitations

This study had some limitations. First, the result that average BP was unchanged in all groups and HR decreased in HF group are inconsistent with the results of previous randomized trials in which BP decreased significantly and HR was unchanged.⁵⁻⁷ In this study, baseline treatments for HF and HT were already

adopted. Although it is difficult to compare statistically, baseline average BP in our study was well controlled, and tended to be lower than that of previous trials.⁵⁻⁷ This difference might affect the dissociation in the BP change by SGLT2i between our study and previous trials. It has been shown that HR in patients with resting HR >70 bpm decreased, but resting HR in patients with resting HR \leq 70 remained unchanged by SGLT2i. This result indicates that SGLT2i may reduce HR only in patients with augmented SNA.⁴¹ In our study, baseline average HR was >70 bpm in both groups and MSNA tended to be higher in HF group than non-HF group. Therefore, the result that HR was significantly decreased in HF group is consistent with the previous report.⁴¹ Diabetic profiles were also unchanged except HbA1c in the non-HF group even though SGLT2i significantly decreased HbA1c/FBG and improved insulin sensitivity in previous studies.⁶² In the previous studies, baseline HbA1c before SGLT2i treatment was prone to be higher than our study (>8%).^{5,7} In our study, the subjects had already received the treatment for diabetes, and the baseline value of HbA1c was <8% in both HF and non-HF groups. So, the impact of SGLT2i on glycemic control might have been smaller than in previous studies. Also, a previous study, which was consistent with our results, demonstrated that HbA1c was not significantly decreased by SGLT2i in patients with type 2 diabetes with well-controlled HbA1c.⁴⁶ Because our primary outcome was the change in MSNA by SGLT2i, we can't exclude that the results for the glycemic control were statistically underpowered given the relatively small sample size. Noteworthy, dapagliflozin significantly reduced MSNA in our study without significant improvement in the diabetic profiles. Second, in other studies, SGLT2i significantly decreased other parameters including serum uric acid and erythropoietin,³⁷ but these were not measured in our study. These parameters may have improved in our study, yet our main purpose was to evaluate the effect of SGLT2i on SNA. Measuring these parameters was unnecessary for our primary outcome. Third, we measured HOMA-IR from serum FBG and insulin as parameters of insulin resistance. To analyze the actual insulin resistance of each organ, other detailed methods, such as a glucose clamp, might be better.⁶³ However, HOMA-IR, calculated from FBG and insulin, is a reliable marker of systemic insulin resistance and is an independent predictor of cardiovascular disease in patients with type 2 diabetes.²³ We believe the result that the significant decrease of MSNA regardless of the HOMA-IR value supports a recent randomized clinical trial showing a favorable effect of SGLT2i on cardiovascular outcome regardless of the presence or absence of diabetes.⁸ Fourth, the sample size was relatively small and only Asians were included, also

most subjects were men (78%). So, it is not clear that these results can apply to a larger population of diverse races. Finally, no control group was used in this study. A control group is important to confirm a cause-and-effect relationship in a study. Since there is no control group in our study, the influence of other modifying factors, including the placebo effect, on the results cannot be completely ruled out. To strengthen our findings, future studies designed to compare the differences in the effects on SNA between SGLT2i and placebo or other drugs with larger numbers and more diverse races are needed.

CONCLUSIONS

Twelve weeks of dapagliflozin 5 mg/day administration significantly decreased MSNA regardless of the diabetic profile or BRS change in patients with type 2 diabetes. The change of MSNA in patients with HF was more remarkable than that of patients with non-HF. These results indicate that SGLT2i reduced SNA, which could be beneficial in patients with diabetes complicated by HF.

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Disclosures

None.

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