


ORIGINAL INVESTIGATION

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# Effect of canagliflozin on left ventricular diastolic function in patients with type 2 diabetes

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## Abstract

**Background:** Type 2 diabetes mellitus (T2DM) greatly increases the risks of cardiovascular disease and heart failure. In particular, left ventricular diastolic dysfunction that develops from the early stages of T2DM is an important factor in the onset and exacerbation of heart failure. The effect of sodium-glucose cotransporter 2 inhibitors on left ventricular diastolic function has not been elucidated. We have performed the first prospective study on the effects of canagliflozin on left ventricular diastolic function in T2DM.

**Methods:** This study was performed to evaluate the effects of additional treatment with canagliflozin for 3 months on left ventricular diastolic function in patients with T2DM. A total of 38 patients with T2DM were consecutively recruited for this study. Left ventricular diastolic function was assessed by echocardiography. The primary study outcome was a change in the septal E/e' as a parameter of left ventricular diastolic function.

**Results:** A total of 37 patients (25 males and 12 females) were included in the analysis. Mean age of participants was  $64.2 \pm 8.1$  years (mean  $\pm$  SD), mean duration of diabetes was  $13.5 \pm 8.1$  years, and mean HbA1c was  $7.9 \pm 0.7\%$ . Of the participants, 86.5% had hypertension, 100% had dyslipidemia, and 32.4% had cardiovascular disease. Canagliflozin significantly improved left ventricular diastolic function (septal E/e' ratio  $13.7 \pm 3.5$ – $12.1 \pm 2.8$ ,  $p = 0.001$ ). Furthermore, among the various parameters that changed through the administration of canagliflozin, only changes in hemoglobin significantly correlated with changes in the septal E/e' ratio ( $p = 0.002$ ). In multiple regression analysis, changes in hemoglobin were also revealed to be an independent predictive factor for changes in the septal E/e' ratio.

**Conclusions:** This study showed for the first time that canagliflozin could improve left ventricular diastolic function within 3 months in patients with T2DM. The benefit was especially apparent in patients with substantially improved hemoglobin values.

*Trial registration* UMIN Clinical Trials Registry UMIN000028141

**Keywords:** Sodium-glucose cotransporter 2 inhibitors, Left ventricular diastolic function, Heart failure with preserved ejection fraction, Autonomic function, Type 2 diabetes mellitus

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## Background

Type 2 diabetes mellitus (T2DM) greatly increases the risks of cardiovascular disease (CVD) and heart failure [1–3]. Especially, left ventricular diastolic dysfunction, which develops early in T2DM, is an important factor in the onset and exacerbation of heart failure [4–6]. T2DM has been shown to cause left ventricular dysfunction independently of glycemic control, hypertension, and coronary artery disease [7, 8], and its pathogenic mechanism involves enhanced oxidative stress and chronic inflammation, which accompanies hyperglycemia [9, 10]. However, glycemic control in those using anti-diabetic drugs was not found to be effective in improving left ventricular diastolic function [11]. Small-scale studies showed that dipeptidyl peptidase (DPP)-4 inhibitors and thiazolidinediones (TZD) were effective in improving left ventricular diastolic function [12, 13], but larger clinical studies showed exacerbation of heart failure with the use of such agents [14, 15]. The combined effect of factors such as aging, plasma glucose values, hypertension, and obesity might be partially responsible for the lack of an effective therapy for left ventricular diastolic dysfunction [4, 5, 8].

Recently, empagliflozin and canagliflozin, which are sodium-glucose cotransporter 2 (SGLT2) inhibitors, were reported to reduce all-cause mortality, cardiovascular mortality, and hospitalization due to heart failure in T2DM [16–19]. However, it is not clear yet how SGLT2 inhibitors reduced hospitalizations due to heart failure, which was shown in the early period of the EMPA-REG and CANVAS trials [16, 18]. Furthermore, the effect of SGLT2 inhibitors on left ventricular diastolic function has not been elucidated.

We have performed the first prospective study of the effects of canagliflozin on left ventricular diastolic function in patients with T2DM.

## Methods

### Study participants

This prospective single-center pilot study was performed to evaluate the effects of additional treatment with canagliflozin on left ventricular function in T2DM. Between July 2017 and December 2017, 38 diabetic patients who had inadequately controlled T2DM were consecutively recruited from outpatients at Tsuruoka Kyoritsu Hospital, Yamagata, Japan. All participants were evaluated at baseline and at 3 months after beginning additional treatment with canagliflozin. During the 6 months before the start of this study and the 3 months after the start of this study, participants had not changed their use of antidiabetic drugs or any other drugs that could affect glucose metabolism such as renin–angiotensin–aldosterone system (RAAS) inhibitors (angiotensin-converting

enzyme inhibitors and/or angiotensin receptor blockers), beta blockers, diuretics, and statins. The inclusion criteria were as follows: (1) T2DM with HbA1c (NGSP) of  $\geq 7.0\%$ ,  $< 10.5\%$  and (2) experiencing no changes in antidiabetic drugs or any other drugs during the 6 months prior to the start of this study. Inclusion criteria also included (3) satisfaction of either (a) or (b) as follows: (a) age  $\geq 45$  and  $< 75$  years without a history of CVD and with  $\geq 2$  of the following risk factors determined at the screening visit: duration of T2DM  $\geq 10$  years, systolic blood pressure (SBP)  $\geq 140$  mmHg (average of 3 readings at screening visit), taking at least one anti-hypertensive agent, current daily cigarette smoker (Brinkmann index  $\geq 200$ ), microalbuminuria or macroalbuminuria (microalbuminuria: urinary albumin excretion from 30 to 300 mg/g Cr; macroalbuminuria: urinary albumin excretion of  $\geq 300$  mg/g Cr); dyslipidemia (abnormal values for  $\geq 1$  among high-density lipoprotein [HDL] cholesterol  $< 40$  mg/dL, low-density lipoprotein (LDL) cholesterol  $\geq 120$  mg/dL, and/or triglycerides (TG)  $\geq 150$  mg/dL); carotid intima media thickness  $\geq 1.1$  mm, and plaque positive; or (b) age  $\geq 35$  and  $< 75$  years with a history of  $\geq 1$  of the following indicating CVD: old cerebral infarction, old myocardial infarction, hospital admission for unstable angina, chronic heart failure, coronary artery bypass graft, percutaneous coronary intervention (with or without stenting), peripheral revascularization (angioplasty or surgery), symptomatic with documented hemodynamically significant carotid or peripheral vascular disease, or amputation secondary to vascular disease. Patients were excluded based on: (1) age  $< 35$  years or  $\geq 75$  years; (2) taking antipsychotics; (3) arrhythmia; (4) severe renal dysfunction (serum Cr  $\geq 2.5$  mg/dL); (5) severe liver dysfunction ( $3\times$  upper limit of normal); (6) diabetic ketosis or diabetic coma; (7) insulin-dependent diabetes mellitus; (8) malignancy, or (9) acute heart failure or acute myocardial infarction. All participants underwent blood tests, including those for fasting plasma glucose, HbA1c, estimated glomerular filtration rate (eGFR), HDL cholesterol, LDL cholesterol, TG, brain natriuretic peptide (BNP), hemoglobin (Hb), and hematocrit (Hct). Clinical data (duration of diabetes; body mass index [BMI]; SBP; diastolic blood pressure [DBP]; heart rate [HR]; history of CVD; insulin therapy; and use of oral anti-diabetic drugs, anti-hypertensive agents, and lipid-lowering agents) were obtained from medical records and a questionnaire.

Thirty-seven patients were included in the analysis after excluding 1 with arrhythmias.

The study protocol was approved by the ethics committee of Tsuruoka Kyoritsu Hospital (approval number: 2017-01; date: 7th June, 2017), and the study was conducted according to the principles of the Helsinki

**Declaration II.** This study was registered at the UMIN Clinical Trial Registry (UMIN000028141). All patients were informed of the purpose of the study after which consent was obtained.

### Analysis of echocardiographic findings

Standardized transthoracic and Doppler echocardiographic examinations were performed in all participants using commercially available equipment (iE33, Philips, Amsterdam, Netherlands). Left atrial diameter and end-diastolic and end-systolic left ventricular internal diameters were measured on a 2-dimensional guided M-mode recording. Left ventricular ejection fraction (EF) was assessed by the modified Simpson's Biplane Method. Using two-dimensional echocardiograms the left ventricular mass index was determined by the area-length method [20]. To assess diastolic function, the following mitral pulse wave Doppler and tissue Doppler parameters were measured: peak early (E) and late (A) diastolic filling velocities, E/A ratio, deceleration time of E wave (DT), septal early diastolic mitral annular tissue velocity (septal  $e'$ ), and lateral early diastolic mitral annular tissue velocity (lateral  $e'$ ). We also calculated the E/ $e'$  ratio by dividing the transmitral E peak by  $e'$  [21].

### Assessment of baroreflex sensitivity (BRS)

Using the spontaneous sequence method, the beat-to-beat BP was measured for 15 min after 15 min of supine rest as the slope of the relationship between spontaneous changes in SBP and the pulse interval. Measurement was made using the second and third fingers of the right hand by the vascular unloading technique (Task Force Monitor, CNSystems, Graz, Austria). For calculation of BRS, the relative changes in SBP (mmHg) and the R–R interval (msec), which is expressed as the distance between corresponding QRS complexes, were considered according to the sequence method with cut-off points of 1 mmHg and 3 ms, respectively [22].

### Assessment of heart rate variability (HRV)

A 3-lead ECG with a sampling frequency of 1000 Hz was recorded for 15 min after the participant had rested for 15 min. The HRV was calculated during the test and included all recorded R–R intervals. HRV was calculated by the frequency domain method using a power spectral analysis of rhythmic oscillations of the R–R interval. The relative contributions of the low frequency (LF) power (0.04–0.15 Hz), high frequency (HF) power (0.15–0.4 Hz), and very-low-frequency (VLF) power (0–0.04 Hz) to total power were calculated as follows: *LF normalized units (LFnu%) = LF absolute power (ms<sup>2</sup>)/total power (ms<sup>2</sup>) – VLF power (ms<sup>2</sup>) × 100 and HFnu% = HF absolute power (ms<sup>2</sup>)/total power*

*(ms<sup>2</sup>) – VLF (ms<sup>2</sup>) × 100*. The LFnu%/HFnu% ratio was considered to indicate sympathetic/vagal tone. All calculations were performed using the Task Force Monitor (CNSystems, Graz, Austria) [23].

### Study outcome

The primary study outcome was a change in the septal E/ $e'$  as a parameter of left ventricular diastolic function. Secondary endpoints included changes in the following variables at the end of the 12-week treatment relative to the baseline: (1) echocardiographic parameters: left atrial diameter, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, EF, left ventricular mass index, E, A, E/A, DcT, septal  $e'$ , lateral  $e'$ , and lateral E/ $e'$ ; (2) autonomic function: BRS and LF/HF; (3) glycaemic control variables: fasting plasma glucose and HbA1c level; (4) lipid metabolism variables: TG, LDL cholesterol, and HDL cholesterol; (5) BMI; (6) SBP and DBP, (7) HR; (8) BNP; (9) Hb and Hct; and (10) eGFR.

### Sample size and statistical analyses

Because there has been no report on the effect of SGLT-2 inhibitors to improve the septal E/ $e'$ , we used information from a previous report on the effect of another drug on the septal E/ $e'$  (approximately a 1.5 reduction) to estimate the required sample size [12]. Sample size estimates were based on the following: standard deviation, 3;  $\alpha$ -level, 0.05; and power, 80%. Sample size was estimated to be 33 people. Assuming a dropout rate of 10%, the target number of patients was therefore set at 38 patients.

Data analyses were performed using the Statistical Package for the Social Sciences 22.0 software (IBM, Armonk, NY, USA). Patients' characteristics and results are presented as mean  $\pm$  SD, mean  $\pm$  standard error, or median with interquartile range (IQR) as appropriate according to the data distribution. Comparison of variables between baseline and 3 months after treatment were made using the paired  $t$  test or Wilcoxon signed-rank test (Table 2). Pearson's correlation analysis or Spearman's rank correlation coefficient test was used for single correlations (Table 3). Multiple-linear regression was used to assess individual and cumulative effects of  $\Delta$ Hb, age, sex, SBP, eGFR, and HR on the  $\Delta$ septal E/ $e'$  ratio. Independent variables were selected among variables that were significantly correlated with the  $\Delta$ septal E/ $e'$  ratio (Table 4). The delta ( $\Delta$ ) values for BRS and LF/HF were calculated according to values at 3 months after treatment-value at baseline. All other delta values were calculated as [(value at 3 months after treatment-value at baseline)  $\times$  100]/value at baseline (%). As shown in Fig. 2 and Table 5, the baseline septal E/ $e'$  ratio was divided into tertiles (T1 < 12, T2  $\geq$  12- < 16, T3  $\geq$  16). The Jonckheere trend test was

used to test for linear trends in  $\Delta$ septal  $E/e'$  ratio in relation to baseline septal  $E/e'$  ratio tertiles. The analysis of variance (ANOVA) was used to compare  $\Delta$ septal  $E/e'$  ratios among participants with various baseline septal  $E/e'$  ratio tertiles. In ANOVA, the Tukey post hoc test was also used to compare  $\Delta$ septal  $E/e'$  ratios among different baseline septal  $E/e'$  ratio groups. As shown in Table 6, the effects of canagliflozin on  $\Delta$ septal  $E/e'$  ratios were analyzed for the following subgroups: sex, history of hypertension, presence of CVD, insulin use, sulfonylurea use, metformin use, DPP-4 inhibitor use, calcium channel blocker use, RAAS inhibitor use, beta blocker use, and diuretic use. In the subgroup analysis, mean  $\Delta$ septal  $E/e'$  was compared using Student's  $t$  test. Hypertension was defined as follows: SBP  $\geq 140$  mmHg, DBP  $\geq 90$  mmHg and/or the use of at least one anti-hypertensive agent. CVD was considered to be present if any of the following had occurred: cerebral infarction, myocardial infarction, hospital admission for unstable angina, heart failure, coronary artery bypass graft, percutaneous coronary intervention (with or without stenting), peripheral revascularization (angioplasty or surgery), symptoms consistent with documented hemodynamically significant carotid or peripheral vascular disease, and/or amputation secondary to vascular disease. As shown in Additional file 1: Table S1, participants were divided into a primary prevention group without a history of CVD, a secondary prevention group with a history of CVD, and an overall population group. In each group, comparison of variables between baseline and 3 months after treatment were made using the paired  $t$  test. The Student's  $t$  test was used to compare mean  $\Delta$ septal  $E/e'$  in the primary prevention group and secondary prevention group. A  $p$  value  $< 0.05$  was considered significant.

## Results

### Baseline characteristics of study participants

A total of 37 patients (25 males and 12 females) were included in the analysis. Table 1 shows the baseline clinical, anthropometric, and pharmacologic data on the study participants. Mean age of participants was  $64.2 \pm 8.1$  years (mean  $\pm$  SD), mean duration of diabetes was  $13.5 \pm 8.1$  years, and mean HbA1c was  $7.9 \pm 0.7\%$ . Of the participants, 86.5% had hypertension, 100% had dyslipidemia, and 32.4% had CVD. At baseline, 32.4% of patients were on insulin, 37.8% on sulfonylureas, 54.1% on metformin, 43.2% on DPP-4 inhibitors, 45.9% on calcium-channel blockers, 45.9% on RAAS inhibitors (angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers), 13.5% on beta blockers, 10.8% on diuretics, 48.6% on statins, and fibrates on 2.7%.

**Table 1 Baseline characteristics of the study population**

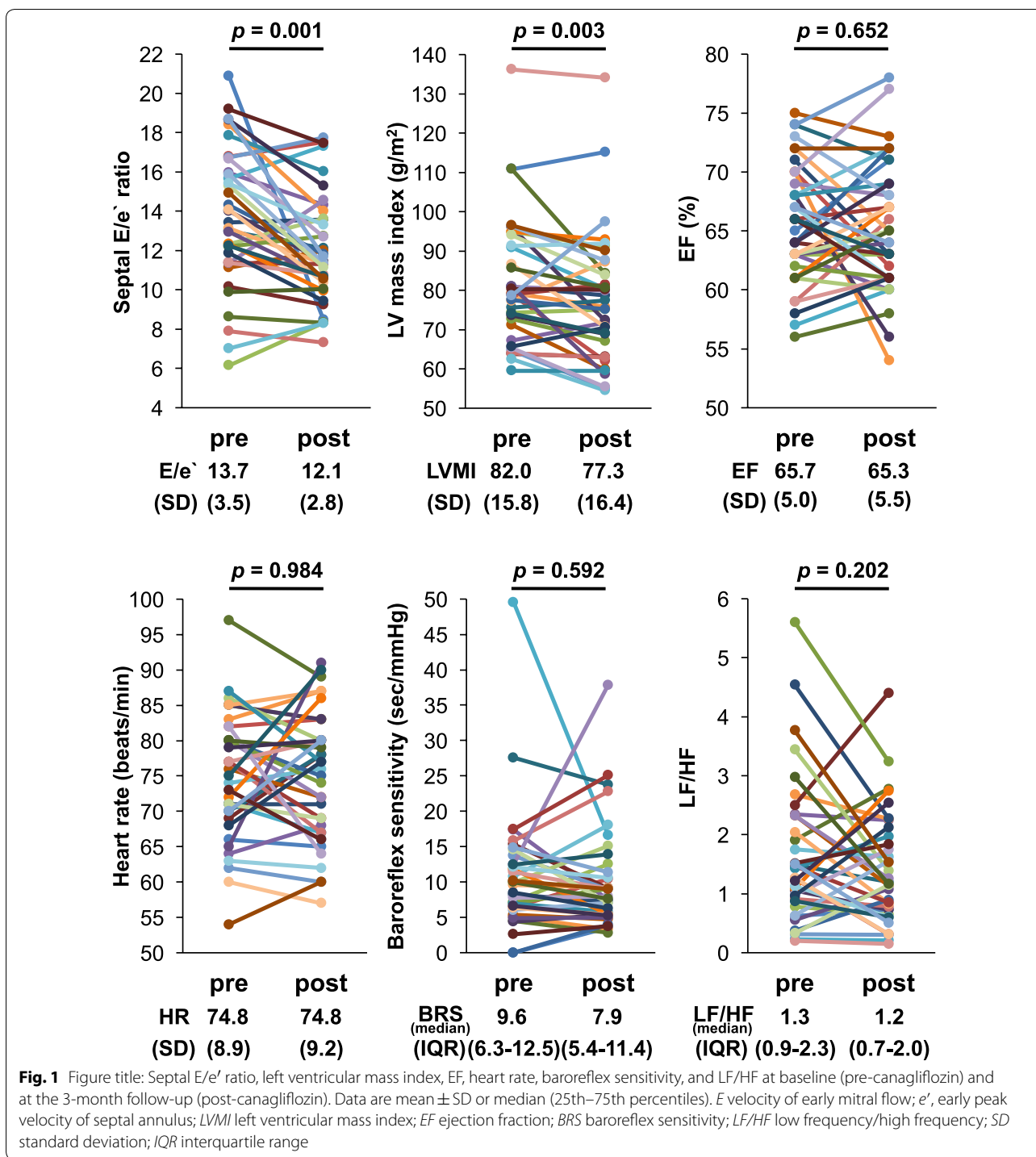
Age, (years)	64.2 $\pm$ 8.1
No. patients	37
Sex, male/female	25/12
Duration of diabetes, (years)	13.5 $\pm$ 8.1
BMI, (kg/m <sup>2</sup> )	27.1 $\pm$ 4.6
Fasting plasma glucose, (mg/dL)	146.3 $\pm$ 28.8
HbA1c, (%)	7.9 $\pm$ 0.7
Hypertension, n (%)	32 (86.5)
Dyslipidemia, n (%)	37 (100)
History of cardiovascular disease, n (%)	12 (32.4)
Oral anti-diabetic drugs, n (%)	34 (91.9)
Insulin, n (%)	12 (32.4)
Sulfonylureas, n (%)	14 (37.8)
Glinides, n (%)	1 (2.7)
Metformin, n (%)	20 (54.1)
Thiazolidinediones, n (%)	5 (13.5)
DPP-4 inhibitors, n (%)	16 (43.2)
GLP-1 receptor agonists, n (%)	2 (5.4)
$\alpha$ -glucosidase inhibitors, n (%)	10 (27)
Anti-hypertensive agents, n (%)	25 (67.6)
Calcium channel blockers, n (%)	17 (45.9)
RAAS inhibitors, n (%)	17 (45.9)
Beta blockers, n (%)	5 (13.5)
Diuretics, n (%)	4 (10.8)
Lipid-lowering agents, n (%)	18 (48.6)
Statins, n (%)	18 (48.6)
Fibrates, n (%)	1 (2.7)

Values are mean  $\pm$  SD or no. (%)

BMI body mass index; DPP dipeptidyl peptidase-4; GLP glucagon-like peptide; RAAS renin-angiotensin-aldosterone system

### Comparison of values from baseline to after 3 months

Compared to baseline levels, decreases were found at 3 months in mean fasting plasma glucose ( $146.3 \pm 28.8$  mg/dL to  $125.9 \pm 24.6$  mg/dL,  $p=0.001$ ), mean HbA1c ( $7.9 \pm 0.7\%$  to  $7.1 \pm 0.6\%$ ,  $p<0.001$ ), mean SBP ( $134.1 \pm 14.3$  mmHg to  $130.3 \pm 15.1$  mmHg,  $p=0.028$ ), mean left ventricular mass index ( $82.0 \pm 15.8$  g/m<sup>2</sup> to  $77.3 \pm 16.4$  g/m<sup>2</sup>,  $p=0.003$ ), mean septal  $E/e'$  ratio ( $13.7 \pm 3.5$  to  $12.1 \pm 2.8$ ,  $p=0.001$ ), and mean lateral  $E/e'$  ratio ( $9.7 \pm 2.9$ – $8.3 \pm 1.9$ ,  $p<0.001$ ). Increases after 3 months of treatment were identified in mean Hb ( $13.9 \pm 1.2$  g/dL to  $14.7 \pm 1.4$  g/dL,  $p<0.001$ ) and mean Hct ( $40.7 \pm 3.3\%$  to  $44.0 \pm 3.7\%$ ,  $p<0.001$ ). There were no differences from baseline values to the end of the 3-month treatment period in median BNP ( $11.4$  [IQR 9.1–20.6] pg/mL to  $10.6$  [IQR 6.3–15.8] pg/dL,  $p=0.061$ ), mean EF ( $65.7 \pm 5.0\%$  to  $65.3 \pm 5.5\%$ ,  $p=0.652$ ), median BRS ( $9.6$  [IQR 6.3–12.5] ms/mmHg to  $7.9$  [IQR 5.4–11.4] ms/mmHg,  $p=0.592$ ), and median



LF/HF (1.3 [IQR 0.9–2.3] to 1.2 [IQR 0.7–2.0],  $p=0.202$ ) (Fig. 1, Table 2).

**Univariate correlates of changes in the septal E/e' ratio**

Correlation analysis showed that the  $\Delta$ septal E/e' ratio was correlated with the baseline septal E/e' ratio

( $r = -0.618$ ,  $p = 0.000$ ), age ( $r = -0.464$ ,  $p = 0.004$ ), baseline SBP ( $r = -0.335$ ,  $p = 0.042$ ), baseline HR ( $r = 0.359$ ,  $p = 0.029$ ), baseline eGFR ( $r = 0.355$ ,  $p = 0.031$ ), and  $\Delta$ Hb ( $r = -0.496$ ,  $p = 0.002$ ) (Table 3).



**Table 2 Comparison of variables between baseline and 3 months after treatment**

Variables	Pre canagliflozin	Post canagliflozin	p value
Fasting blood glucose (mg/dL)	146.3 ± 28.8	125.9 ± 24.6	0.001
HbA1c (%)	7.9 ± 0.7	7.1 ± 0.6	< 0.001
BMI (kg/m <sup>2</sup> )	27.1 ± 4.6	26.5 ± 4.3	< 0.001
Blood pressure (mmHg)			
Systolic	134.1 ± 14.3	130.3 ± 15.1	0.028
Diastolic	80.8 ± 9.9	81.4 ± 9.5	0.627
Heart rate (beats/min)	74.8 ± 8.9	74.8 ± 9.2	0.984
Lipid profile (mg/dL)			
Triglycerides	146 (101–190)	114 (85–148)	< 0.001
LDL cholesterol	111.2 ± 32.7	113.9 ± 31.0	0.516
HDL cholesterol	47.9 ± 10.5	53.9 ± 11.9	< 0.001
eGFR (mL/min/1.73 m <sup>2</sup> )	76.1 ± 19.5	71.4 ± 16.9	0.001
BNP (pg/mL)	11.4 (9.1–20.6)	10.6 (6.3–15.8)	0.061
Hemoglobin (g/dL)	13.9 ± 1.2	14.7 ± 1.4	< 0.001
Hematocrit (%)	40.7 ± 3.3	44.0 ± 3.7	< 0.001
Echocardiographics			
Left atrial diameter (mm)	36.4 ± 5.3	36.5 ± 5.2	0.789
LV end-diastolic diameter (mm)	47.8 ± 5.4	47.2 ± 4.8	0.253
LV end-systolic diameter (mm)	31.2 ± 5.1	30.5 ± 4.0	0.226
EF (%)	65.7 ± 5.0	65.3 ± 5.5	0.652
LV mass index (g/m <sup>2</sup> )	82.0 ± 15.8	77.3 ± 16.4	0.003
E (m/s)	0.7 ± 0.1	0.6 ± 0.1	0.013
A (m/s)	0.9 ± 0.2	0.2 ± 0.2	0.053
E/A	0.8 ± 0.2	0.8 ± 0.2	0.446
DcT (ms)	265.5 ± 53.4	276.2 ± 58.0	0.298
Septal e' (cm/s)	5.3 ± 1.2	5.4 ± 1.2	0.294
Lateral e' (cm/s)	7.5 ± 1.6	7.8 ± 1.6	0.097
Septal E/e' ratio	13.7 ± 3.5	12.1 ± 2.8	0.001
Lateral E/e' ratio	9.7 ± 2.9	8.3 ± 1.9	< 0.001
Autonomic function			
BRS (s/mmHg)	9.6 (6.3–12.5)	7.9 (5.4–11.4)	0.592
LF/HF	1.3 (0.9–2.3)	1.2 (0.7–2.0)	0.202

Data are mean ± SD or median (25th–75th percentiles)

*BMI* body mass index; *LDL* low density lipoprotein; *HDL* high density lipoprotein; *eGFR* estimated glomerular filtration rate; *BNP* brain natriuretic peptide; *LV* left ventricular; *EF* ejection fraction; *E* velocity of early mitral flow; *A* velocity of late mitral flow; *DcT* deceleration time of early mitral inflow; *e'* early peak velocity of annulus; *BRS* baroreflex sensitivity; *LF/HF* low frequency/high frequency

### Multivariate analysis of changes in the septal E/e' ratio

Multiple regression analysis showed that  $\Delta$ Hb were inversely related to the  $\Delta$ septal E/e' ratio. These findings remained after adjusting the  $\Delta$ septal E/e' ratio for age, sex, SBP, eGFR, and HR (Table 4).

### Comparison of changes in the septal E/e' ratio with the baseline septal E/e' ratio

Figure 2 and Table 5 show the comparisons of  $\Delta$ septal E/e' ratio among participants with various baseline septal E/e' ratios according to tertiles based on ANOVA. There was a significant difference in  $\Delta$ septal E/e' ratios among

these three groups. The results were then analyzed by the Tukey post hoc test. The T2 ( $p=0.03$ ) and T3 ( $p=0.009$ ) groups had decreased septal E/e' ratios in comparison with the T1 group. This observation was confirmed by the Jonckheere trend test:  $\Delta$ septal E/e' ratio ( $p=0.008$ ) was significantly correlated with tertiles of baseline septal E/e' ratios.

### Effects of canagliflozin on septal E/e' ratio in subgroups of the study population

The septal E/e' ratio was significantly improved in patients using sulfonylurea ( $p=0.006$ ) or a DPP-4

inhibitor ( $p=0.014$ ) at baseline. However, there was no significant difference in patients grouped according to sex, history of hypertension, presence of CVD, insulin

use, metformin use, calcium channel blocker use, RAAS inhibitor use, beta blocker use, and diuretic use (Table 6).

**Table 3 Univariate correlates of change in septal E/e' ratio vs. baseline and change in variables**

Variables	$\Delta$ septal E/e' vs. baseline variables		$\Delta$ septal E/e' vs. $\Delta$ variables	
	r	p	r	p
Age (years)	-0.464	0.004	-	-
Duration of diabetes (years)	0.021	0.902	-	-
Septal E/e' ratio	-0.618	<0.001	-	-
LV mass index (g/m <sup>2</sup> )	-0.252	0.133	-0.158	0.351
BMI (kg/m <sup>2</sup> )	-0.054	0.753	0.034	0.840
Fasting plasma glucose (mg/dL)	-0.009	0.960	-0.277	0.097
HbA1c (%)	-0.009	0.957	-0.060	0.725
SBP (mmHg)	-0.335	0.042	0.066	0.699
DBP (mmHg)	-0.022	0.899	-0.092	0.588
Heart rate (beats/min)	0.359	0.029	-0.223	0.184
BNP (pg/mL)	0.074	0.665	-0.084	0.623
Hemoglobin (g/dL)	0.092	0.588	-0.496	0.002
Hematocrit (%)	0.095	0.575	-0.265	0.112
eGFR (mL/min/1.73 m <sup>2</sup> )	0.355	0.031	0.016	0.924
Triglycerides (mg/dL)	-0.115	0.497	-0.159	0.346
LDL cholesterol (mg/dL)	0.036	0.833	-0.084	0.621
HDL cholesterol (mg/dL)	0.124	0.463	0.039	0.819
BRS (s/mmHg)	0.232	0.167	0.266	0.111
LF/HF	0.211	0.211	-0.322	0.052

The delta ( $\Delta$ ) values for baroreflex sensitivity, and LF/HF were calculated as values at 3 months after treatment-value at baseline. All other delta values were calculated as [(value at 3 months after treatment - value at baseline)  $\times$  100]/value at baseline (%)

E velocity of early mitral flow; e' early peak velocity of annulus; LV left ventricular; BMI body mass index; SBP systolic blood pressure; DBP diastolic blood pressure; BNP brain natriuretic peptide; eGFR estimated glomerular filtration rate; LDL low density lipoprotein; HDL high density lipoprotein; BRS baroreflex sensitivity; LF/HF low frequency/high frequency

**Discussion**

This is the first prospective study of the effects of canagliflozin on left ventricular diastolic function in patients with T2DM who were at high risk of a cardiovascular event. Furthermore, among various parameters that changed through the administration of canagliflozin, only  $\Delta$ Hb significantly correlated with the  $\Delta$ septal E/e' ratio. Multiple regression analysis also revealed that  $\Delta$ Hb was an independent predictive factor for the  $\Delta$ septal E/e' ratio. On the other hand, canagliflozin caused no exacerbation of autonomic function as assessed by BRS and frequency domain analysis of HRV.

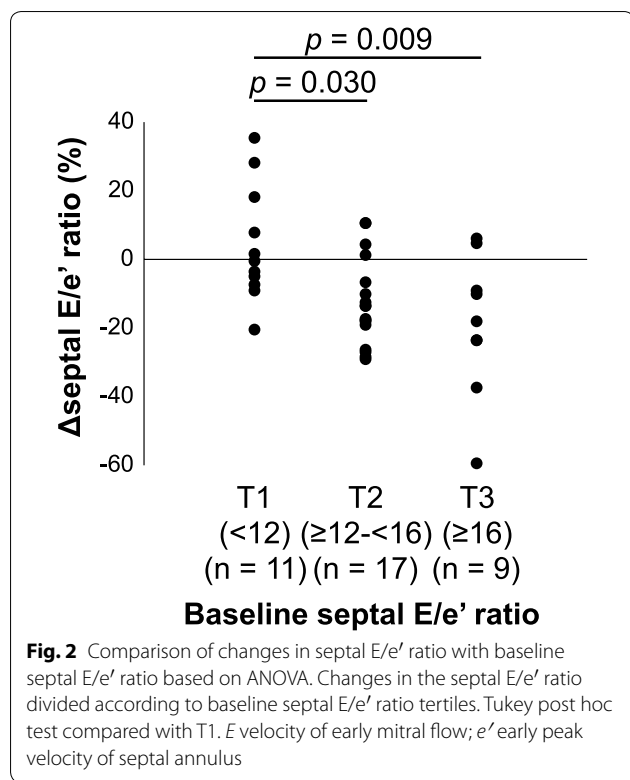
SGLT2 inhibitors were reported to increase Hb, and the involvement of erythropoietin in the mechanism for this increase was reported [24–26]. Inhibited reabsorption of glucose from the proximal tubule was suggested to reduce oxygen consumption in proximal tubule cells, accelerate the recovery of erythropoietin production from interstitial fibroblasts, and thus cause increases in Hb [25, 27]. Reports showed that erythropoietin had cardioprotective effects [28–31] and that left ventricular diastolic function was improved in patients whose Hb was increased by an erythropoiesis-stimulating agent [32, 33]. In addition, increases in Hb improve the oxygen supply to tissues, which also results in improved left ventricular diastolic function [34, 35]. Another important mechanism for the increased Hb values is a reduction in plasma volume due to osmotic diuresis and the natriuretic effect [26, 36]. Chronic expanded plasma volume is known to increase the burden on the myocardium and trigger cardiac disorders. In patients having heart or renal failure, including those with concomitant diabetes, increases in Hb through decreased plasma volume were reported to correlate with improved mortality [37, 38].

**Table 4 Multiple regression analysis of changes in the septal E/e' ratio**

Independent variables	Model 1		Model 2		Model 3	
	$\beta$	p	$\beta$	p	$\beta$	p
$\Delta$ Hemoglobin (g/dL)	-0.357	0.024	-0.374	0.017	-0.424	0.005
Age (years)	-0.266	0.086	-0.192	0.238	-0.174	0.244
Sex (male/female)	-0.195	0.186	-0.265	0.063	-0.266	0.049
SBP (mmHg)	-0.186	0.206				
eGFR (mL/min/1.73 m <sup>2</sup> )			0.188	0.213		
Heart rate (beats/min)					0.311	0.024

Dependent variable was  $\Delta$  septal E/e', and the independent variables were Model 1, Model 2, and Model 3. Model 1:  $\Delta$ hemoglobin, age, sex, and SBP; Model 2:  $\Delta$ hemoglobin, age, sex, and eGFR; Model 3:  $\Delta$ hemoglobin, age, sex, and heart rate. Model 1: R-squared 0.420, adjusted R-squared 0.348; Model 2: R-squared 0.419, adjusted R-squared 0.347; Model 3: R-squared 0.481, adjusted R-squared 0.416

E velocity of early mitral flow; e' early peak velocity of septal annulus; SBP systolic blood pressure; eGFR estimated glomerular filtration rate



As SGLT2 inhibitors have been considered to reduce the cardiac workload by decreasing plasma volume [39, 40], in the present study, decreased plasma volume may have reduced the preload and afterload, resulting in improved left ventricular diastolic function. Such improvement might also be attributed to the simultaneous effects of decreased BP [41], reduced body weight, less visceral fat [42, 43], and improvements in endothelial dysfunction [44, 45], systemic micro-inflammation [43], cardiac

injury [46], left ventricular hypertrophy, etc. Further investigation is needed to elucidate the mechanism for the improved left ventricular diastolic function resulting from the administration of an SGLT2 inhibitor.

Reductions in BP and plasma volume by an SGLT2 inhibitor diminish the cardiac load, but these agents could possibly have the adverse effect of enhancing sympathetic nervous system activity. Increased sympathetic nervous system activity has important roles in the feedback mechanism of lowered BP and reduced plasma volume; but over the long term, it is known to cause increases in heart load due to an elevated HR and fluid retention and thus becomes a factor in left ventricular diastolic dysfunction. Loop diuretics used for heart failure patients are known to increase sympathetic nervous system activity by reducing plasma volume and thus to adversely affect the long-term outcome [47]. In this study, canagliflozin did not exacerbate the autonomic function assessed by BRS and frequency domain analysis of HRV. As an SGLT2 inhibitor was reported to improve BRS in an animal study [48], research on the effects of long-term administration of SGLT2 inhibitors on autonomic function are awaited.

In our subgroup analysis, the improvement in left ventricular diastolic function was significantly greater in those who took sulfonylurea before the start of the study and continued thereafter than in those not given this agent. As sulfonylurea was reported to be involved in left ventricular diastolic dysfunction [11] and has attenuated cardioprotective effects [49], it is possible that canagliflozin compensated for the dysfunction caused by the administration of sulfonylurea while reducing the load on the heart. Furthermore, our study patients who were provided with a DPP-4 inhibitor had a significantly greater improvement in their left ventricular diastolic function

**Table 5 Comparison of Δseptal E/e' ratios with baseline septal E/e' ratios based on ANOVA**

Baseline septal E/e' ratio tertiles	T1 (<12) (n=11)	T2 (≥12-<16) (n=17)	T3 (≥16) (n=9)	ANOVA p value	Test for trend p value
Δseptal E/e' ratio (%)	4.1 ± 0.3	-12.6 ± 0.4	-19.0 ± 0.4	0.007	0.008
p value		0.030	0.009		
Age (years)	58.6 ± 7.7	64.4 ± 7.5	70.6 ± 4.2		
Duration of diabetes (years)	10.5 ± 5.7	13.1 ± 8.6	17.9 ± 8.7		
SBP (mmHg)	127.9 ± 10.7	132.3 ± 13.4	145.1 ± 14.8		
Heart rate (beats/min)	78.2 ± 7.9	72.1 ± 9.2	76.0 ± 8.6		
eGFR (mL/min/1.73 m <sup>2</sup> )	84.6 ± 23.3	76.3 ± 18.9	65.1 ± 9.4		
Δhemoglobin (%)	5.0 ± 3.5	6.1 ± 5.1	6.4 ± 4.6		

Δseptal E/e' ratios are mean ± SE. All other values are mean ± SD. Changes in the septal E/e' ratio were divided according to baseline septal E/e' ratio tertiles. Results of the Tukey post hoc test compared with T1

E velocity of early mitral flow; e' early peak velocity of septal annulus; SBP systolic blood pressure; eGFR estimated glomerular filtration rate



**Table 6 Effects of canagliflozin on the septal E/e' ratio in subgroups of study patients**

Subgroup	No. (%)	$\Delta$ septal E/e' ratio (%)	p value for interaction
Sex			
Male	25 (68)	$-5.6 \pm 3.1$	0.076
Female	12 (32)	$-16.9 \pm 6.2$	
Hypertension			
Yes	32 (86)	$-9.4 \pm 3.3$	0.883
No	5 (14)	$-8.1 \pm 7.5$	
History of cardiovascular disease			
Yes	12 (32)	$-14.0 \pm 3.4$	0.277
No	25 (68)	$-7.0 \pm 3.4$	
Insulin use			
Yes	12 (32)	$-2.8 \pm 5.7$	0.141
No	25 (68)	$-12.3 \pm 3.4$	
Sulfonylurea use			
Yes	14 (38)	$-19.4 \pm 4.7$	0.006
No	23 (62)	$-3.1 \pm 3.3$	
Metformin use			
Yes	20 (54)	$-10.4 \pm 4.4$	0.688
No	17 (46)	$-7.9 \pm 4.0$	
DPP-4 inhibitor use			
Yes	16 (43)	$-17.5 \pm 4.3$	0.014
No	21 (57)	$-3.0 \pm 3.6$	
Calcium channel blocker use			
Yes	17 (46)	$-13.2 \pm 4.4$	0.230
No	20 (54)	$-5.9 \pm 4.0$	
RAAS inhibitor use			
Yes	17 (46)	$-13.8 \pm 3.3$	0.157
No	20 (54)	$-5.3 \pm 4.7$	
Beta blocker use			
Yes	5 (14)	$-21.9 \pm 4.5$	0.092
No	32 (86)	$-7.2 \pm 3.3$	
Diuretic use			
Yes	4 (11)	$-25.7 \pm 4.4$	0.053
No	33 (89)	$-7.2 \pm 3.1$	

Values are mean  $\pm$  SE or no. (%)

DPP dipeptidyl peptidase-4; RAAS renin-angiotensin-aldosterone system

than those not provided with this drug. A DPP-4 inhibitor is an oral anti-hyperglycemic agent that has many advantages, such as an antiarteriosclerotic effect [50] and vascular endothelial function-improving effect [51], but increased hospitalizations due to heart failure have been noted [15]. We found that combination therapy of a DPP-4 inhibitor with the SGLT2 inhibitor improved left ventricular diastolic function possibly because the SGLT2 inhibitor compensated for some of the concerns

regarding DPP-4 inhibitors. In this study, a significant correlation ( $r = -0.464$ ,  $p = 0.004$ ) was observed between age and improvement in left ventricular diastolic function. Patients administered sulfonylurea ( $p = 0.025$ ) or a DPP-4 inhibitor ( $p = 0.004$ ) were significantly older than those with whom they were compared; thus the results possibly reflect these differences.

Recently, although empagliflozin was reported to have improved left ventricular diastolic function in patients with T2DM with a history of CVD [52], this is the first report showing the possibility of canagliflozin improving left ventricular diastolic function in patients with T2DM who were mainly grouped according to primary prevention. As the sub-analysis in the CANVAS trial showed a similar inhibitory effect on heart failure-associated hospitalizations between the primary and secondary prevention participants [19] and canagliflozin significantly suppressed such hospitalizations compared to a DPP-4 inhibitor and a glucagon-like peptide-1 receptor agonist [53], it is possible that improvement in left ventricular diastolic function was involved in those results. Also, a recent sub-analysis in the EMPA-REG trial indicated that Hb increases were involved in suppression of cardiovascular events independently of lowered plasma glucose and decreased BP, suggesting the involvement of improved left ventricular diastolic function via rises in Hb [54].

The incidence of heart failure, particularly heart failure with preserved ejection fraction (HFpEF) for which diastolic dysfunction is a major cause, is rapidly increasing globally with aging of the population [55, 56]. Thus establishment of therapy for such conditions is imperative. However, the outcome of HFpEF cannot be improved even with an angiotensin-converting-enzyme inhibitor, angiotensin receptor blocker, beta blocker, or aldosterone antagonist [57–60], all of which are effective for improving the outcome in heart failure with a reduced ejection fraction (HFrEF) [61]. A possible reason may be that HFpEF develops due to multiple factors, such as diabetes, hypertension, obesity, and renal dysfunction; optimal treatment of these diseases is likely important in improving left ventricular diastolic function and outcome in HFpEF patients. An SGLT2 inhibitor is likely to be a promising drug in the prevention of HFpEF and improving the outcome of HFpEF by improving left ventricular diastolic function in patients with T2DM. In addition, our results suggested that increases in Hb are involved in improved left ventricular diastolic function and can be used as a predictive marker of such improvement.

This study has three limitations. First, although this was a prospective study, the number of study patients

was small and this was uncontrolled observational study. Therefore, larger randomized controlled trials are needed. Second, we could not verify whether the improvement in diastolic function was solely attributable to canagliflozin. Finally, the relationship between the administration of canagliflozin and cardiovascular events could not be verified due to the short research period.

## Conclusions

This study showed for the first time that canagliflozin could improve left ventricular diastolic function in patients with T2DM. The benefit was especially apparent in patients with substantial improvement in Hb values. This result suggests that canagliflozin may have a role in preventing cardiovascular events and heart failure.

## Additional file

**Additional file 1: Table S1.** Comparison of echocardiographic findings between baseline and at the end of the 3-months study period in the total population, the primary, and secondary prevention.

## Abbreviations

T2DM: type 2 diabetes mellitus; SGLT: sodium-glucose cotransporter; CVD: cardiovascular disease; BRS: baroreflex sensitivity; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction.

## Authors' contributions

DM and MS contributed to the study design, data acquisition, and data analysis and wrote the manuscript. YK, NT, RH, and KU reviewed and edited the intellectual content. The funder had no role in study design, analysis, interpretation of data, writing of the manuscript, and the decision to submit the manuscript for publication. All authors read and approved the final manuscript.

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## Competing interests

The authors of this manuscript have the following competing interests: M.S. has participated in speaker's bureaus/advisory panels for Sanofi, Daiichi-Sankyo, Astellas, and Tanabe-Mitsubishi. N.T. has received a research grant and support from Daiichi-Sankyo and Otsuka Pharmaceutical and has participated in speaker's bureaus organized by Daiichi-Sankyo and MSD. K.U. has received research support from Terumo, Kowa, Taisho, Arkray, Kyowa Kirin, MSD, Astellas, Boehringer Ingelheim, Ono, Novo Nordisk, Kissei and Tanabe-Mitsubishi and has participated in speaker's bureau/advisory panels for Astellas, Astra Zeneca, Kowa, MSD, Eli Lilly, Taisho, Novo Nordisk and Sanofi. The other authors have no competing interests to declare.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

The study protocol was approved by the local ethics committee, and the study was conducted according to the principles of the Helsinki Declaration II. All patients were informed of the purpose of the study after which consent was obtained.

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