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Complete List of Authors:	Iwakura, Katsuomi; Sakurabashi Watanabe Hospital, Division of Cardiology Ito, Hiroshi; Okayama University, Department of Cardiovascular Medicine Ishii, Katsuhisa; Kansai Electric Power Hospital, Department of Cardiology Date, Motoo; Sakurabashi Watanabe Hospital, Division of Cardiology Nakamura, Fumiaki; Ibaraki Iseikai Hospital, Ibaraki, Division of Medicine Nagano, Toshihiko; Iwasa Dai-ichi Hospital, Internal Medicine Takiuchi, Shin; Higashi-Takarazuka Satoh Hospital, Department of Cardiology
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Effect of azelnidipine, a novel calcium channel blocker, on left ventricular relaxation in hypertensive patients with diabetes.

Katsuomi Iwakura[1], Hiroshi Ito[2], Katsuhisa Ishii[3], Motoo Date[1], Fumiaki Nakamura[4], Toshihiko Nagano[5] and Shin Takiuchi[6] for the Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial investigators[7]

[1]Department of Cardiology, Sakurabashi Watanabe Hospital, Osaka, Japan
[2]Department of Cardiovascular Medicine, Okayama University, Graduate School of Medicine, Okayama, Japan
[3]Division of Cardiology, Kansai Electric Power Hospital, Osaka, Japan
[4]Division of Medicine, Ibaraki Iseikai Hospital, Ibaraki, Japan
[5]Internal Medicine, Iwasa Dai-ichi Hospital, Gifu, Japan
[6]Department of Cardiology, Higashi-Takarazuka Satoh Hospital, Takarazuka, Japan
[7]CALVLOC trial investigators list is provided in the Appendix.

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Corresponding Author:
Katsuomi Iwakura, MD
Division of Cardiology, Sakurabashi Watanabe Hospital
2-4-32, Umeda, Kita-ku
Osaka 5300001 Japan
Tel: 81-6-6341-8651 Fax:81-6-6341-0785
E-mail:iwakura@mac.com

Abstract

Objectives: We previously demonstrated that a calcium channel blocker, azelnidipine, improves left ventricular relaxation in patients with hypertension and diastolic dysfunction in a multicenter, CALVLOC trial. The objectives of the present subanalysis were to investigate (1) the impact of diabetes on diastolic function in hypertensive patients, and (2) the efficacy of azelnidipine on diastolic function in diabetic- and non-diabetic patients.

Design: Subanalysis of a prospective single-arm multicenter study.

Participants: 228 hypertensive patients with normal ejection fraction and impaired left ventricular relaxation (septal e' velocity< 8 cm/s on echocardiography) enrolled for CALVLOC trial. They were divided into two groups based on presence or absence of diabetes.

Interventions: Administration of 16mg of azelnidipine for 8 months (range: 6-10 month).

Main outcome measures: Septal e' velocity before and at the end of the study.

Results: Whereas diabetic patients (n=53, 23.2%) had lower systolic blood pressure (BP) than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03), they had lower e' velocity (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04) at baseline. Azelnidipine decreased BP and heart rate, and increased e' velocity similarly in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and non-diabetic patients (6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Increase in e' velocity was not influenced by presence of diabetes, and diabetic patients still had lower e' velocity after treatment (p=0.006). There was

a significant correlation between increase in e' velocity and decrease in systolic BP (R=0.25, p=0.0001), which was not influenced by diabetes.

Conclusions: Comorbid diabetes could impair left ventricular relaxation independently in patients with hypertension, which might not be improved solely by BP lowering.

Key Words: calcium channel blockers, diabetic heart disease, tissue Doppler

Article Summary

Strengths and limitations of this study

- Based on a prospective, multicenter trial, the impact of diabetes on left ventricular (LV) relaxation in hypertensive patients was investigated.
- Azelnidipine, a unique calcium channel blocker which lowers blood pressure without increasing heart rate, was used as an intervention to improve LV relaxation.
- LV relaxation was more impaired in hypertensive patients with diabetes than in those without diabetes, and azelnidipine improved LV relaxation in both groups to the same degree.
- The persistence of diastolic dysfunction in diabetic patients after azelnidipine treatment implied that hypertension and diabetes might impair diastolic function through different mechanisms.
- This was a subanalysis of a one-arm, open label study including only 228 patients, and therefore, the results might be inconclusive about the impact of diabetes on diastolic function.

Hypertension and diabetes are two major risk factors for heart failure with preserved ejection fraction, and both of them are highly associated with left ventricular (LV) diastolic dysfunction[1-3]. These two diseases frequently coexist and often share comorbidities and conditions that can affect LV function, such as obesity and LV hypertrophy. Therefore, it is not easy to clarify how hypertension and diabetes are interacted in the development of LV diastolic dysfunction. Appropriate blood pressure (BP) control is the most important treatment in patients with heart failure with preserved reduction[2,3]. Calcium channel blockers (CCBs) are not recommended for routine treatment in patients with heart failure with reduced ejection fraction[2], because they might reduce the myocardial contractility. However, their effects on LV diastolic function are still not fully elucidated. Combination of CCB and an angiotensin receptor blocker could improve LV relaxation effectively in hypertensive patients[4]. On the other hands, dihydropyridine CCBs might have unfavorable effects on diastolic function due to reflex tachycardia. Azelnidipine is a unique dihydropyridine CCB which lowers BP as well as amlodipine without increasing, or even slightly decreasing, heart rate[5]. We previously demonstrated that azelnidipine improved LV relaxation in hypertensive patients with LV diastolic dysfunction in the prospective multicenter, Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial[6].

In this ad-hoc analysis of the CALVLOC study, we investigated (1) how comorbidity of

diabetes affected LV diastolic function in patients with hypertension, and (2) whether azelnidipine could improve diastolic function in hypertensive patients with diabetes as well as in those without diabetes.

METHODS

Study design: The CALVLOC trial was a multi-center, prospective single-arm trial to evaluate the effects of azelnidipine treatment on LV relaxation in hypertensive patients. The study design and main results was reported elsewhere[6]. We enrolled patients with stage 1 or 2 essential hypertension (mean systolic BP >140mmHg or diastolic BP >90mmHg) who had impaired LV relaxation, defined as septal mitral annular relaxation velocity (e') <8cm/s on echocardiography, irrespective of history of antihypertensive treatment. The exclusion criteria were LV ejection fraction of <50%, atrial fibrillation, and the administration of CCBs other than amlodipine. The study patients were enrolled between January 2006 and October 2007 in 11 participating institutes with in Osaka, Hyogo, Aichi and Gifu prefectures, Japan.

Azelnidipine (16mg/day) was administered to patients who had not received CCBs. If patients had been on amlodipine at the time of enrollment, amlodipine was substituted with 16mg of azelnidipine. No other medications were changed throughout the study period. Patients were assessed at 4–8 week intervals at least for 24weeks, and BP and heart rate were measured

at each study visit. Blood and urine tests were performed at baseline and at the end of the study, including measurement of fasting blood glucose, glycosylated hemoglobin A1c (HbA1c), brain natriuretic peptide, high sensitivity C-reactive protein and urine albumin. Echocardiography was recorded before enrollment and at the end of the study. The primary endpoints were changes in septal e' velocity and the ratio of transmitral E wave velocity to the e' (E/e' ratio) from the baseline to the follow-up. Secondary endpoints included changes in BP, heart rate, LV wall thickness, LV mass index and left atrial volume index on echocardiography. The CALVLOC trial was conducted in accordance with the Declaration of Helsinki and with the approval of the institutional ethics committees in each participating institutions. Written informed consent was obtained from each patient enrolled in the study.

The present study was conducted as an ad-hoc analysis of the CALVLOC trial. We divided the study patients into two groups based on the presence or absence of diabetes, which was diagnosed according to the guidelines by Japan Diabetes Society[7]. We compared the differences in the primary and secondary endpoints described above between two groups.

Analysis of echocardiography: We performed standard echocardiography examination in all patients. Doppler echocardiographic assessment included the peak velocities of transmitral E and A wave, and deceleration time of the E wave. We recorded tissue Doppler images from the apical 4-chamber view, and measured septal e' velocity on the pulse-wave Doppler spectrum.

LV mass was calculated as 0.80 x (1.04 x [{septal wall thickness in diastole + LV end-diastolic dimension + posterior wall thickness in diastole}^{3} - LV end-diastolic dimension^3])+0.6 (grams) and indexed to body surface area as LV mass index. Relative wall thickness was calculated as 2 x (posterior wall thickness in diastole)/LV end-diastolic dimension. Left atrial volume (mL) was determined by the prolate ellipse method at ventricular end systole, and it was indexed to body surface area as left atrial volume index. All echocardiography data were measured and determined by two independent doctors or sonographers blinded to the patients' clinical data.

Statistics. All continuous variables were expressed as mean ± standard deviation and were compared by one-way analysis of variance (ANOVA). Significance of difference was calculated with Tukey's HSD test for factor analysis. Categorical variables were compared with Fisher's exact test. The influence of age and body mass index on e' velocity was adjusted using analysis of covariance (ANCOVA). The correlations between e' and fasting blood glucose or HbA1c were analyzed using linear correlation analysis. The changes in BP, heart rate and e' velocity during treatment were compared between diabetic- and non-diabetic patients using two-way repeated measure ANOVA. The influence of diabetes on the relation between decrease in BP and increase in e' velocity was analyzed using ANCOVA. StatView 5.0 (SAS Institute Inc.) was used for statistical analysis.

Results

Patients Characteristics: The original CALVLOC trial enrolled 253 patients, and 21 patients were excluded because of failure to follow-up (15 patients) and of protocol violation (6 patients). For the present analysis, four more patients were excluded because of insufficient data about diabetic status. Thus, the final study group for the present analysis was consisted of 228 patients. Their mean age was 66±11 (range; 31 – 95) year old, and 120 (52.6%) of them were male. Diabetes was diagnosed in 53 patients (23.2%), and all of them were diagnosed with Type 2 diabetes. Diabetic patients showed higher fasting blood glucose (139±37 vs. 99±11 mg/dL, $p<0.0001$) and higher HbA1c (6.9±0.7 vs. 5.7±0.3 %, $p<0.0001$) than non-diabetic patients. Table 1 demonstrates the baseline characteristics. There were no differences in age, gender, body size, the prevalence of ischemic heart disease or stroke, and renal function between diabetic- and non-diabetic patients. No differences were observed in antihypertensive drugs including amlodipine administered before enrollment between two groups. Statins were more frequently administered (45.3% vs. 28.0%, $p=0.03$) in diabetic patients. High density lipoprotein cholesterol was significantly lower in diabetic patients (50±13 vs. 56±16 mg/dL, $p=0.01$) while no differences were observed in other lipid profile.

Effects of azelnidipine on hemodynamics. Table 2 demonstrated BP and heart rate on enrollment (baseline) and at the end of study. The mean interval between baseline and follow

up study was 8 months (range; 6–10 months). Diabetic patients had lower systolic BP at baseline than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03). No differences were observed in diastolic BP and heart rate at baseline between two groups.

Azelnidipine treatment significantly decreased systolic- and diastolic BP and heart rate in diabetic- and non-diabetic patients. There were no differences in systolic- and diastolic BP and in heart rate after azelnidipine treatment between two groups. Two-way repeated measure ANOVA was conducted to compare changes of parameters between two groups before and after treatment. The test for the interaction between systolic BP change and diabetes was significant (F=4.49, p=0.04), while the interactions between diabetes and diastolic pressure reduction or heart rate change were not significant (F=0.53, p=0.47 and F=0.48, p=0.49, respectively). These results indicated that azelnidipine lowered systolic BP, but not diastolic BP or heart rate, more effectively in non-diabetic patients than diabetic patients.

Diabetes and echocardiography parameters. The echocardiography parameters at baseline and at follow-up study were demonstrated in Table 3. There were no significant differences in LV dimensions and ejection fraction at baseline between diabetic- and non-diabetic patients. Also there were no differences in wall thickness and LV mass index between two groups. Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Diabetic patients still had lower e' velocity after adjustment with age and body mass

index (p=0.04 by ANCOVA). Diabetic patients had significantly lower E/A ratio (0.72±0.18 vs. 0.86±0.24 cm/s, p=0.0003). Left atrial volume index was tended to be larger in diabetic patients (p=0.07), but no difference was observed in E/e' ratio between two groups. HbA1c was weakly but significantly correlated with e' velocity at baseline (R= 0.21, p=0.002), while there was no correlation between fasting blood glucose and e' velocity (p=0.37).

Azelnidipine treatment significantly increased e' velocity both in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and in non-diabetic patients (6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Two-way repeated measure ANOVA demonstrated that the interaction between change of e' velocity and diabetes was not significant (F=0.48, p=0.48), indicating that increase in e' velocity was not influenced by diabetes (Figure 1). The difference in e' velocity between two groups still remained after azelnidipine treatment (p=0.006). The increase in e' velocity was significantly correlated with decrease in systolic BP during treatment (R=0.25, p=0.0001). This relation was not interacted with presence or absence of diabetes (F=0.27, p=0.60, by ANCOVA). No significant correlation was observed between changes in e' velocity and those in heart rate (R=0.13, p=0.06).

The increase in e' velocity was also weakly but significantly correlated with changes in HbA1c (R=0.16, p=0.03). There were no significant differences in HbA1c during treatment both in diabetic- (6.9±0.7 to 6.8±0.6%, p=0.29) and in non-diabetic patients (5.7±0.3 to

5.7±0.3%, p=0.34), therefore, the contribution of changes in HbA1c would be very small even if present.

E/e' was significantly decreased in non-diabetic patients (11.4±3.4 to 10.1±2.9, p<0.0001) but not in diabetic patients (11.5±4.2 to 10.8±3.6, p=0.11). Left atrial volume index was decreased only in diabetic patients (20.2±8.9 to 19.6±8.3 mL/m2, p=0.004). E/A ratio were increased during treatment in diabetic patients (0.72±0.18 to 0.86±0.24, p=0.02) while the change did not reach statistical significance in non-diabetic patients (0.86±0.24 to 0.89±0.23, p=0.06). The difference in E/A ratio between two groups was not observed after treatment (p=0.50). No significant changes in LV diameters, ejection fraction, wall thickness and LV mass index were observed after azelnidipine treatment in two groups.

Discussion.

We investigated the influence of diabetes on LV relaxation in 228 hypertensive patients who received azelnidipine treatment. Patients with diabetes had significantly lower e' velocity and lower E/A ratio at baseline than those without it, while no difference was observed in E/e' ratio. Azelnidipine treatment for a mean of 8 months significantly lowered heart rate, systolic- and diastolic BP both in diabetic- and non-diabetic patients, and diabetic patients showed larger systolic BP reduction than non-diabetic patients. Azelnidipine increased e' velocity in both

groups similarly, and diabetic patients still had lower e' velocity after treatment. The changes in e' velocity were almost parallel between diabetic- and non-diabetic patients (Figure 1). The increase in e' velocity was correlated with the decrease in systolic BP by azelnidipine, and this correlation was not affected by presence or absence of diabetes. These results demonstrated that LV relaxation was more impaired in diabetic patients than in non-diabetic one among the hypertensive patients, and that the improvement of e' velocity by azelnidipine was little affected by presence or absence of diabetes. The latter suggested that hypertension and diabetes might impair LV relaxation through different mechanisms, and that the impairment associated with diabetes might not be improved by adequate BP control.

Prior studies had demonstrated that patients with both hypertension and diabetes had lower LV diastolic function than those with hypertension or diabetes alone[8-10]. Hypertension and diabetes impaired left atrial performance, which could reflect diastolic function, in an additive fashion[11], suggesting that diabetes and hypertension would impair LV diastolic function through different mechanisms. Hypertension is associated with increased collagen deposition, increased interstitial fibrosis, and disturbance of calcium homeostasis in the myocardium[12], all of which may contribute to deteriorating diastolic function. Diabetes may increase LV mass independently of arterial blood pressure[13]. Collagen deposition around intramural vessels and between myofibers is increased, and collagen type III is accumulated in diabetic

patient, which could mechanically impair diastolic function[14]. It is unclear how the changes associated with diabetes and those with hypertension are overlapped or interacted in the development of diastolic dysfunction.

The present study was a relatively small one and not conducted as a prespecified subgroup analysis, and therefore, the results were not fully conclusive. Improvement of diastolic function in hypertensive patients is correlated with the degree of systolic BP reduction regardless of antihypertensive agents[4]. However, it is unclear whether the changes in e' velocity was caused by BP lowering or by a unique action of azelnidipine. The follow up period might not be long enough to detect the clinical outcomes[15]. We did not determine the variability of e' velocity measurement among the institutions, although e' velocity could be a relatively robust parameter. We measured only septal e' velocity for the original CALVLOC study[14]. Although septal e' velocity might be sufficient for the evaluation of LV relaxation in most cases, wall motion abnormality within septum might affect the septal velocity. We did not assess myocardial ischemia directly, and subclinical coronary artery disease might be dismissed. We did not analyze the duration of diabetes and the effects of anti-diabetic treatment or those of antihypertensive drugs concomitantly used.

Despite of limitations described above, the present study provided an important insight into the mechanisms of LV diastolic dysfunction in hypertension and diabetes. Standard BP

lowering might not be enough for improvement of diastolic function in hypertensive patients with diabetes. It is unclear whether diabetic control has an additive or synergic effect with BP lowering on diastolic function. The correlation between HbA1c and e' velocity in the present study was very weak, and intensive glycemic control might not be as effective as BP lowering for LV diastolic dysfunction, as suggested in the large-scale trials[16-18].

APPENDIX

CALVLOC trial investigators;

Atsuhito Otuska, MD (Ibaraki Iseikai Hospital, Ibaraki, Japan); Kou Fujisawa, MD (Iwasa Dai-ichi Hospital, Gifu, Japan); Yorihiko Higashino, MD (Higashi-Takarazuka Sato Hospital, Takarazuka, Japan); Kei Tawarahara, MD (Hamamatsu Red-cross Hospital, Hamamatsu, Japan); Mikio Mukai, MD (Kinki Chuo Hospital, Itami, Japan); Masanori Shinoda, MD (Kouseiren Kamo General Hospital, Toyota, Japan); Taro Minagawa, MD (Minagawa Clinic, Gifu, Japan); and Naoki Goto, MD (Goto Clinic, Gifu, Japan).

Contributorship statement

Katsuomi Iwakura played the leading role in this work. Hiroshi Ito provided the concept and study design of original CALVLOC study and its subanalysis, review of data and revision of the manuscript. Katsuhisa Ishii, Motoo Date, Fumiaki Nakamura, Toshihiko Nagano and Shin Takiuchi assisted in analysis and interpretation of original CALVLOC data.

Competing interests

There are no competing interests regarding the present study.

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Data sharing

Extra data is available by emailing iwakura@mac.com.

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Figure Legend

Figure 1. Changes in e' velocity during azelnidipine treatment.

Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Azelnidipine treatment significantly increased e' velocity both in diabetic patients (p=0.0003) and in non-diabetic patients (p<0.0001). The changes in e' velocity were parallel between two groups, implying that the effects of azelnidipine were similar between two groups. Diabetic patients had lower e' velocity than non-diabetic patients even after treatment (6.3±1.5 vs. 6.9±1.4 cm/s, p=0.006). *: p=0.04, †:p=0.006 vs. non-diabetic patients.

Table 1. Patients Characteristics

	Diabetes	Non-Diabetes	p value
Number of patients, n (%)	53 (23.2%)	175 (76.8%)	
Age, year	68±10	65±12	0.09
Gender, male/female	31/22	89/86	0.35
Height, cm	158.1±9.5	159.5±9.7	0.35
Weight, kg	62.9±12.8	62.1±11.3	0.64
Body mass index	25.0±3.3	24.3±3.3	0.20
Dyslipidemia, n (%)	30 (56.6)	76 (43.4)	0.12
Smoker, n (%)	13 (24.5)	61 (34.9)	0.18
History of myocardial infarction, n (%)	2 (3.8)	7 (4.0)	0.99
Angina pectoris, n (%)	7 (13.2)	27 (15.4)	0.83
Myocardial infarction + angina, n (%)	8 (15.1)	31 (17.7)	0.84
History of stroke, n(%)	3 (5.7)	10 (5.7)	0.99
Medications			
amlodipine	14 (26.4)	58 (33.1)	0.40
renin-angiotensin-aldosterone system inhibitors, n (%)	28 (52.8)	78 (44.6)	0.35
β-blockers, n (%)	8 (15.6)	26 (14.7)	0.99
diuretics, n (%)	4 (7.5)	10 (5.7)	0.74
statins, n (%)	24 (45.3)	49 (28.0)	0.03
Fasting blood glucose, mg/dL	139±37	99±11	<0.0001

Hemoglobin A1c, %	6.9±0.7	5.7±0.3	<0.0001
Total cholesterol, mg/dL	198±35	207±31	0.08
Low density lipoprotein cholesterol, mg/dL	115±35	120±30	0.38
High density lipoprotein cholesterol, mg/dL	50±13	56±16	0.01
Triglyceride, mg/dL	163±87	158±109	0.76
Serum creatinine, mg/dL	0.90±0.48	0.88±0.63	0.88
Estimated glomerular filtration rate, mL/min/1.73m^2	61.4±15.5	63.2±16.3	0.50
Brain natriuretic peptide, pg/dL	33.4±40.5	39.3±66.7	0.56
High sensitive C-reactive protein, mg/dL	1.60±2.60	1.71±2.48	0.79

Each value depicts mean ± standard deviation or number of the patients (%).

Table 2. Hemodynamic Parameters

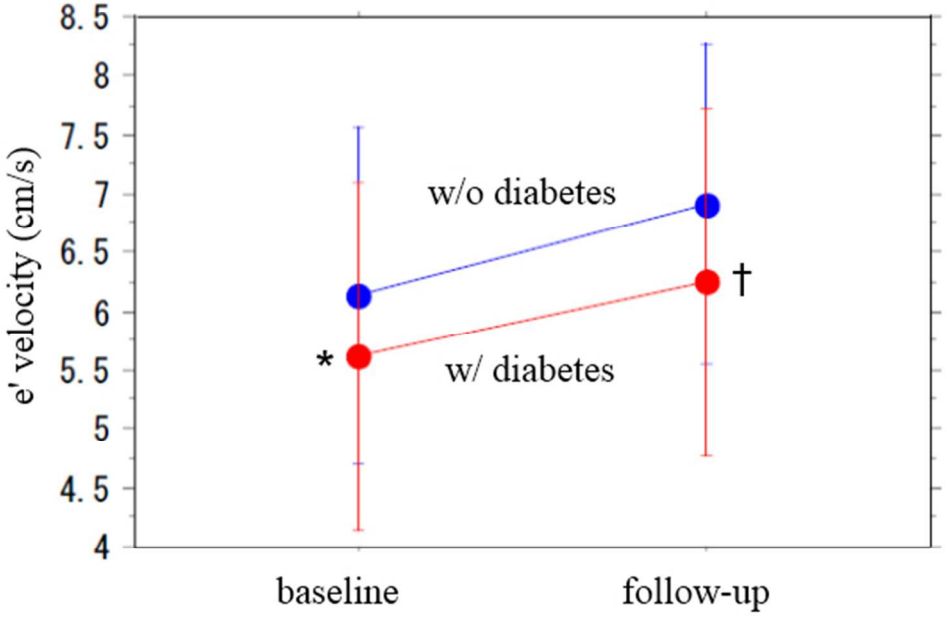
	baseline			Follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
Systolic blood pressure, mmHg	155±17	161±16	0.03	138±12*	139±11*	0.86
Diastolic blood pressure, mmHg	85±13	88±13	0.11	77±10*	79±9*	0.16
Heart rate, bpm	73±10	73±10	0.99	69±10 †	69±9*	0.58

Each value depicts mean ± standard deviation. *, $p<0.0001$; †, $p<0.001$ vs. baseline.

Table 3. Echocardiography Parameters

	baseline study			follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
LV end-diastolic dimension, cm	4.7±0.5	4.7±0.5	0.55	4.6±0.5	4.6±0.4	0.98
LV end-systolic dimension, cm	2.9±0.5	2.8±0.4	0.12	2.9±0.5 §	2.8±0.4	0.11
LV ejection fraction (%)	68±7	69±8	0.10	69±9	70±7	0.37
Septal wall thickness, cm	1.0±0.2	1.0±0.2	0.14	1.0±0.2	1.0±0.2	0.14
Posterior wall thickness, cm	1.0±0.2	1.0±0.2	0.64	1.0±0.2	1.0±0.1	0.09
Relative wall thickness	0.43±0.09	0.43±0.08	0.98	0.44±0.08	0.42±0.08	0.20
LV mass index, g/m^2	99.9±42.3	92.5±36.1	0.21	91.3±40.8 §	90.4±37.2	0.88
Left atrial volume index, mL/m^2	22.9±9.4	20.2±8.9	0.07	22.6±8.2	19.6±8.3 ‡	0.02
Peak E velocity, cm/s	60.8±14.1	66.8±15.4	0.01	64.8±16.0	67.4±15.9	0.31
Peak A velocity, cm/s	84.4±15.0	81.4±17.0	0.25	84.4±15.0	81.4±17.0 †	0.25
E/A	0.72±0.18	0.86±0.24	0.0003	0.86±0.42 §	0.89±0.23	0.50
Deceleration time of E wave, msec	234±57	230±56	0.68	218±50 §	222±46	0.64
e', cm/s	5.7±1.5	6.1±1.4	0.04	6.3±1.5 †	6.9±1.4*	0.006
E/e'	11.5±4.2	11.4±3.4	0.78	10.8±3.6	10.1±2.9*	0.19

Each value depicts mean ± standard. LV depicts left ventricle. *, p<0.0001; †, p<0.0005; ‡, p<0.005; §, p<0.05 vs. baseline study.



Changes in e' velocity during azelnidipine treatment.

Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Azelnidipine treatment significantly increased e' velocity both in diabetic patients (p=0.0003) and in non-diabetic patients (p<0.0001). The changes in e' velocity were parallel between two groups, implying that the effects of azelnidipine were similar between two groups. Diabetic patients had lower e' velocity than non-diabetic patients even after treatment (6.3±1.5 vs. 6.9±1.4 cm/s, p=0.006). *: p=0.04, †:p=0.006 vs. non-diabetic patients.
108x81mm (150 x 150 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
j Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5,6
Methods			
Study design	4	Present key elements of study design early in the paper	6,7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	6
		(*b*) For matched studies, give matching criteria and the number of controls per case	Not for this study.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7,8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how matching of cases and controls was addressed	Not for this study.
		(*e*) Describe any sensitivity analyses	Not for this study.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Not for this study.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic,	9

		clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	Not for this study.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	Not for this study.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not for this study.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12,13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14,15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Changes in left ventricular relaxation after azelnidipine treatment in hypertensive patients with diabetes: subanalysis of a prospective single-arm multicenter study

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Changes in left ventricular relaxation after azelnidipine treatment in hypertensive patients with diabetes: subanalysis of a prospective single-arm multicenter study

Katsuomi Iwakura[1], Hiroshi Ito[2], Katsuhisa Ishii[3], Motoo Date[1], Fumiaki Nakamura[4], Toshihiko Nagano[5] and Shin Takiuchi[6] for the Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial investigators[7]

[1]Department of Cardiology, Sakurabashi Watanabe Hospital, Osaka, Japan
[2]Department of Cardiovascular Medicine, Okayama University, Graduate School of Medicine, Okayama, Japan
[3]Division of Cardiology, Kansai Electric Power Hospital, Osaka, Japan
[4]Division of Medicine, Ibaraki Iseikai Hospital, Ibaraki, Japan
[5]Internal Medicine, Iwasa Dai-ichi Hospital, Gifu, Japan
[6]Department of Cardiology, Higashi-Takarazuka Satoh Hospital, Takarazuka, Japan
[7]CALVLOC trial investigators list is provided in the Appendix.

Running title: Influence of diabetes on LV relaxation

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Corresponding Author:
Katsuomi Iwakura, MD
Division of Cardiology, Sakurabashi Watanabe Hospital
2-4-32, Umeda, Kita-ku
Osaka 5300001 Japan
Tel: 81-6-6341-8651 Fax:81-6-6341-0785
E-mail:iwakura@mac.com

Key Words: calcium channel blockers, diabetic heart disease, tissue Doppler

Abstract

Objectives: We previously demonstrated that a calcium channel blocker, azelnidipine, improves left ventricular relaxation in patients with hypertension and diastolic dysfunction in a multicenter, CALVLOC trial. The objectives of the present subanalysis were to investigate the differences in diastolic function in hypertensive patients with and without diabetes, and the efficacy of azelnidipine on diastolic function among them.

Design: Subanalysis of a prospective single-arm multicenter study.

Participants: 228 hypertensive patients with normal ejection fraction and impaired left ventricular relaxation (septal e' velocity< 8 cm/s on echocardiography) enrolled for CALVLOC trial. They were divided into two groups based on presence or absence of diabetes.

Interventions: Administration of 16mg of azelnidipine for 8 months (range: 6-10 month).

Main outcome measures: Septal e' velocity before and at the end of the study.

Results: Whereas diabetic patients (n=53, 23.2%) had lower systolic blood pressure (BP) than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03), they had lower e' velocity (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04) at baseline. Azelnidipine decreased BP and heart rate, and increased e' velocity similarly in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and non-diabetic patients (6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Increase in e' velocity was not influenced by presence of diabetes, and diabetic patients still had lower e' velocity after treatment (p=0.006). There was

a significant correlation between increase in e' velocity and decrease in systolic BP (R=0.25, p=0.0001), which was not influenced by diabetes.

Conclusions: Comorbid diabetes could impair left ventricular relaxation independently in patients with hypertension, which might not be improved solely by BP lowering.

Article Summary

Strengths and limitations of this study

- Based on a prospective, multicenter trial, the difference in left ventricular (LV) relaxation in hypertensive patients with or without diabetes was investigated.
- Azelnidipine, a unique calcium channel blocker which lowers blood pressure without increasing heart rate, was used as an intervention to improve LV relaxation.
- LV relaxation was more impaired in hypertensive patients with diabetes than in those without diabetes, and azelnidipine improved LV relaxation in both groups to the same degree.
- The persistence of diastolic dysfunction in diabetic patients after azelnidipine treatment implied that hypertension and diabetes might impair diastolic function through different mechanisms.
- This was a subanalysis of a one-arm, open label study including only 228 patients, and therefore, the results might be inconclusive about the impact of diabetes on diastolic function.

Hypertension and diabetes are two major risk factors for heart failure with preserved ejection fraction, and both of them are highly associated with left ventricular (LV) diastolic dysfunction[1-3]. These two diseases frequently coexist and often share comorbidities and conditions that can affect LV function, such as obesity and LV hypertrophy. Therefore, it is not easy to clarify how hypertension and diabetes are interacted in the development of LV diastolic dysfunction. Appropriate blood pressure (BP) control is the most important treatment in patients with heart failure with preserved reduction[2,3]. Calcium channel blockers (CCBs) are not recommended for routine treatment in patients with heart failure with reduced ejection fraction[2], because they might reduce the myocardial contractility. However, their effects on LV diastolic function are still not fully elucidated. Combination of CCB and an angiotensin receptor blocker could improve LV relaxation effectively in hypertensive patients[4]. On the other hands, dihydropyridine CCBs might have unfavorable effects on diastolic function due to reflex tachycardia. Azelnidipine is a unique dihydropyridine CCB which lowers BP as well as amlodipine without increasing, or even slightly decreasing, heart rate[5]. We previously demonstrated that azelnidipine improved LV relaxation in hypertensive patients with LV diastolic dysfunction in the prospective multicenter, Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial[6].

In this post-hoc analysis of the CALVLOC study, we investigated (1) whether there was a

difference in LV diastolic function between hypertensive patients with and without diabetes, and (2) whether azelnidipine could improve diastolic function in diabetic patients as well as in non-diabetic patients.

METHODS

Study design: The CALVLOC trial was a multi-center, prospective single-arm trial to evaluate the effects of azelnidipine treatment on LV relaxation in hypertensive patients. The study design and main results was reported elsewhere[6]. We enrolled patients with stage 1 or 2 essential hypertension (mean systolic BP >140mmHg or diastolic BP >90mmHg) who had impaired LV relaxation, defined as septal mitral annular relaxation velocity (e') <8cm/s on echocardiography, irrespective of history of antihypertensive treatment. The exclusion criteria were LV ejection fraction of <50%, atrial fibrillation, and the administration of CCBs other than amlodipine. The study patients were enrolled between January 2006 and October 2007 in 11 participating institutes with in Osaka, Hyogo, Aichi and Gifu prefectures, Japan.

Azelnidipine (16mg/day) was administered to patients who had not received CCBs. If patients had been on amlodipine at the time of enrollment, amlodipine was substituted with 16mg of azelnidipine. No other medications were changed throughout the study period. Patients were assessed at 4–8 week intervals at least for 24weeks, and BP and heart rate were measured

at each study visit. Blood and urine tests were performed at baseline and at the end of the study, including measurement of fasting blood glucose, glycosylated hemoglobin A1c (HbA1c), brain natriuretic peptide, high sensitivity C-reactive protein and urine albumin. Echocardiography was recorded before enrollment and at the end of the study. The primary endpoints were changes in septal e' velocity and the ratio of transmitral E wave velocity to the e' (E/e' ratio) from the baseline to the follow-up. Secondary endpoints included changes in BP, heart rate, LV wall thickness, LV mass index and left atrial volume index on echocardiography. The CALVLOC trial was conducted in accordance with the Declaration of Helsinki and with the approval of the institutional ethics committees in each participating institutions. Written informed consent was obtained from each patient enrolled in the study.

The present study was conducted as an post-hoc analysis of the CALVLOC trial. We divided the study patients into two groups based on the presence or absence of diabetes, which was diagnosed according to the guidelines by Japan Diabetes Society[7]. We compared the differences in the primary and secondary endpoints described above between two groups.

Analysis of echocardiography: We performed standard echocardiography examination in all patients. Doppler echocardiographic assessment included the peak velocities of transmitral E and A wave, and deceleration time of the E wave. We recorded tissue Doppler images from the apical 4-chamber view, and measured septal e' velocity on the pulse-wave Doppler spectrum.

LV mass was calculated as 0.80 x (1.04 x [{septal wall thickness in diastole + LV end-diastolic dimension + posterior wall thickness in diastole}^{3} - LV end-diastolic dimension^3])+0.6 (grams) and indexed to body surface area as LV mass index. Relative wall thickness was calculated as 2 x (posterior wall thickness in diastole)/LV end-diastolic dimension. Left atrial volume (mL) was determined by the prolate ellipse method at ventricular end systole, and it was indexed to body surface area as left atrial volume index. All echocardiography data were measured and determined by two independent doctors or sonographers blinded to the patients' clinical data. All parameters were measured once except E and e' velocities, which were measured as an average of three consecutive cardiac cycles.

Statistics. All continuous variables were expressed as mean ± standard deviation and were compared by one-way analysis of variance (ANOVA). Significance of difference was calculated with Tukey's HSD test for factor analysis. Categorical variables were compared with Fisher's exact test. The influence of age and body mass index on e' velocity was adjusted using analysis of covariance (ANCOVA). The correlations between e' and fasting blood glucose or HbA1c were analyzed using linear correlation analysis. The changes in BP, heart rate and e' velocity during treatment were compared between diabetic- and non-diabetic patients using two-way repeated measure ANOVA. The influence of diabetes on the relation between decrease in BP and increase in e' velocity was analyzed using ANCOVA. StatView 5.0 (SAS

Institute Inc.) was used for statistical analysis.

Results

Patients Characteristics: The original CALVLOC trial enrolled 253 patients, and 21 patients were excluded because of failure to follow-up (15 patients) and of protocol violation (6 patients). For the present analysis, four more patients were excluded because of insufficient data about diabetic status. Thus, the final study group for the present analysis was consisted of 228 patients. Their mean age was 66±11 (range; 31 – 95) year old, and 120 (52.6%) of them were male. Diabetes was diagnosed in 53 patients (23.2%), and all of them were diagnosed with Type 2 diabetes. Diabetic patients showed higher fasting blood glucose (139±37 vs. 99±11 mg/dL, $p<0.0001$) and higher HbA1c (6.9±0.7 vs. 5.7±0.3 %, $p<0.0001$) than non-diabetic patients. Table 1 demonstrates the baseline characteristics. There were no differences in age, gender, body size, the prevalence of ischemic heart disease or stroke, and renal function between diabetic- and non-diabetic patients. No differences were observed in antihypertensive drugs including amlodipine administered before enrollment between two groups. Statins were more frequently administered (45.3% vs. 28.0%, $p=0.03$) in diabetic patients. High density lipoprotein cholesterol was significantly lower in diabetic patients (50±13 vs. 56±16 mg/dL, $p=0.01$) while no differences were observed in other lipid profile.

Effects of azelnidipine on hemodynamics. Table 2 demonstrated BP and heart rate on enrollment (baseline) and at the end of study. The mean interval between baseline and follow up study was 8 months (range; 6–10 months). Diabetic patients had lower systolic BP at baseline than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03). No differences were observed in diastolic BP and heart rate at baseline between two groups.

Azelnidipine treatment significantly decreased systolic- and diastolic BP and heart rate in diabetic- and non-diabetic patients. There were no differences in systolic- and diastolic BP and in heart rate after azelnidipine treatment between two groups. Two-way repeated measure ANOVA was conducted to compare changes of parameters between two groups before and after treatment. The test for the interaction between systolic BP change and diabetes was significant (F=4.49, p=0.04), while the interactions between diabetes and diastolic pressure reduction or heart rate change were not significant (F=0.53, p=0.47 and F=0.48, p=0.49, respectively). These results indicated that azelnidipine lowered systolic BP, but not diastolic BP or heart rate, more effectively in non-diabetic patients than diabetic patients.

Diabetes and echocardiography parameters. The echocardiography parameters at baseline and at follow-up study were demonstrated in Table 3. There were no significant differences in LV dimensions and ejection fraction at baseline between diabetic- and non-diabetic patients. Also there were no differences in wall thickness and LV mass index between two groups.

Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Diabetic patients still had lower e' velocity after adjustment with age and body mass index (p=0.04 by ANCOVA). Diabetic patients had significantly lower E/A ratio (0.72±0.18 vs. 0.86±0.24 cm/s, p=0.0003). Left atrial volume index was tended to be larger in diabetic patients (p=0.07), but no difference was observed in E/e' ratio between two groups. HbA1c was weakly but significantly correlated with e' velocity at baseline (R= 0.21, p=0.002), while there was no correlation between fasting blood glucose and e' velocity (p=0.37).

Azelnidipine treatment significantly increased e' velocity both in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and in non-diabetic patients (6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Two-way repeated measure ANOVA demonstrated that the interaction between change of e' velocity and diabetes was not significant (F=0.48, p=0.48), indicating that increase in e' velocity was not influenced by diabetes (Figure 1). The difference in e' velocity between two groups still remained after azelnidipine treatment (p=0.006). The increase in e' velocity was significantly correlated with decrease in systolic BP during treatment (R=0.25, p=0.0001). This relation was not interacted with presence or absence of diabetes (F=0.27, p=0.60, by ANCOVA). No significant correlation was observed between changes in e' velocity and those in heart rate (R=0.13, p=0.06).

The increase in e' velocity was also weakly but significantly correlated with changes in

HbA1c (R=0.16, p=0.03). There were no significant differences in HbA1c during treatment both in diabetic- (6.9±0.7 to 6.8±0.6%, p=0.29) and in non-diabetic patients (5.7±0.3 to 5.7±0.3%, p=0.34), therefore, the contribution of changes in HbA1c would be very small even if present.

E/e' was significantly decreased in non-diabetic patients (11.4±3.4 to 10.1±2.9, p<0.0001) but not in diabetic patients (11.5±4.2 to 10.8±3.6, p=0.11). Left atrial volume index was decreased only in diabetic patients (20.2±8.9 to 19.6±8.3 mL/m2, p=0.004). E/A ratio were increased during treatment in diabetic patients (0.72±0.18 to 0.86±0.24, p=0.02) while the change did not reach statistical significance in non-diabetic patients (0.86±0.24 to 0.89±0.23, p=0.06). The difference in E/A ratio between two groups was not observed after treatment (p=0.50). No significant changes in LV diameters, ejection fraction, wall thickness and LV mass index were observed after azelnidipine treatment in two groups.

Discussion.

We investigated the relation between diabetes and LV relaxation in 228 hypertensive patients who received azelnidipine treatment. Patients with diabetes had significantly lower e' velocity and lower E/A ratio at baseline than those without it, while no difference was observed in E/e' ratio. Azelnidipine treatment for a mean of 8 months significantly lowered heart rate, systolic-

and diastolic BP both in diabetic- and non-diabetic patients, and diabetic patients showed larger systolic BP reduction than non-diabetic patients. Azelnidipine increased e' velocity in both groups similarly, and diabetic patients still had lower e' velocity after treatment. The changes in e' velocity were almost parallel between diabetic- and non-diabetic patients (Figure 1). The increase in e' velocity was correlated with the decrease in systolic BP by azelnidipine, and this correlation was not affected by presence or absence of diabetes. These results demonstrated that LV relaxation was more impaired in diabetic patients than in non-diabetic one among the hypertensive patients, and that the improvement of e' velocity by azelnidipine was little affected by presence or absence of diabetes. The latter suggested that hypertension and diabetes might impair LV relaxation through different mechanisms, and that the impairment associated with diabetes might not be improved by adequate BP control.

Prior studies had demonstrated that patients with both hypertension and diabetes had lower LV diastolic function than those with hypertension or diabetes alone[8-10]. Hypertension and diabetes impaired left atrial performance, which could reflect diastolic function, in an additive fashion[11], suggesting that diabetes and hypertension would impair LV diastolic function through different mechanisms. Hypertension is associated with increased collagen deposition, increased interstitial fibrosis, and disturbance of calcium homeostasis in the myocardium[12], all of which may contribute to deteriorating diastolic function. Diabetes may increase LV

mass independently of arterial blood pressure[13]. Collagen deposition around intramural vessels and between myofibers is increased, and collagen type III is accumulated in diabetic patient, which could mechanically impair diastolic function[14]. It is unclear how the changes associated with diabetes and those with hypertension are overlapped or interacted in the development of diastolic dysfunction.

The present study was a relatively small one and not conducted as a prespecified subgroup analysis, and therefore, the results were not fully conclusive. Improvement of diastolic function in hypertensive patients is correlated with the degree of systolic BP reduction regardless of antihypertensive agents[4]. However, it is unclear whether the changes in e' velocity was caused by BP lowering or by a unique action of azelnidipine. The follow up period might not be long enough to detect the clinical outcomes[15]. We did not determine the variability of e' velocity measurement among the institutions, although e' velocity could be a relatively robust parameter. We measured only septal e' velocity for the original CALVLOC study[14]. Although septal e' velocity might be sufficient for the evaluation of LV relaxation in most cases, wall motion abnormality within septum might affect the septal velocity. We did not assess myocardial ischemia directly, and subclinical coronary artery disease might be dismissed. We did not analyze the duration of diabetes and the effects of anti-diabetic treatment or those of antihypertensive drugs concomitantly used.

Despite of limitations described above, the present study provided an important insight into the mechanisms of LV diastolic dysfunction in hypertension and diabetes. Standard BP lowering might not be enough for improvement of diastolic function in hypertensive patients with diabetes. It is unclear whether diabetic control has an additive or synergic effect with BP lowering on diastolic function. The correlation between HbA1c and e' velocity in the present study was very weak, and intensive glycemic control might not be as effective as BP lowering for LV diastolic dysfunction, as suggested in the large-scale trials[16-18].

APPENDIX

CALVLOC trial investigators;

Atsuhito Otuska, MD (Ibaraki Iseikai Hospital, Ibaraki, Japan); Kou Fujisawa, MD (Iwasa Dai-ichi Hospital, Gifu, Japan); Yorihiko Higashino, MD (Higashi-Takarazuka Sato Hospital, Takarazuka, Japan); Kei Tawarahara, MD (Hamamatsu Red-cross Hospital, Hamamatsu, Japan); Mikio Mukai, MD (Kinki Chuo Hospital, Itami, Japan); Masanori Shinoda, MD (Kouseiren Kamo General Hospital, Toyota, Japan); Taro Minagawa, MD (Minagawa Clinic, Gifu, Japan); and Naoki Goto, MD (Goto Clinic, Gifu, Japan).

Contributorship statement

Katsuomi Iwakura provided the study design of the present subanalysis along with Dr. Ito, performed statistical analysis, interpreted the results, and wrote the draft of the manuscript. Hiroshi Ito provided the concept and study design of original CALVLOC study and its subanalysis, review of data and revision of the manuscript. Katsuhisa Ishii, Motoo Date, Fumiaki Nakamura, Toshihiko Nagano and Shin Takiuchi assisted in analysis and interpretation of original CALVLOC data. All authors carefully read and approved the manuscript.

Competing interests

There are no competing interests regarding the present study.

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Data sharing

No additional data available.

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Figure Legend

Figure 1. Changes in e' velocity during azelnidipine treatment.

Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Azelnidipine treatment significantly increased e' velocity both in diabetic patients (p=0.0003) and in non-diabetic patients (p<0.0001). The changes in e' velocity were parallel between two groups, implying that the effects of azelnidipine were similar between two groups. Diabetic patients had lower e' velocity than non-diabetic patients even after treatment (6.3±1.5 vs. 6.9±1.4 cm/s, p=0.006). *: p=0.04, †:p=0.006 vs. non-diabetic patients.

Table 1. Patients Characteristics

	Diabetes	Non-Diabetes	p value
Number of patients, n (%)	53 (23.2%)	175 (76.8%)	
Age, year	68±10	65±12	0.09
Gender, male/female	31/22	89/86	0.35
Height, cm	158.1±9.5	159.5±9.7	0.35
Weight, kg	62.9±12.8	62.1±11.3	0.64
Body mass index	25.0±3.3	24.3±3.3	0.20
Dyslipidemia, n (%)	30 (56.6)	76 (43.4)	0.12
Smoker, n (%)	13 (24.5)	61 (34.9)	0.18
History of myocardial infarction, n (%)	2 (3.8)	7 (4.0)	0.99
Angina pectoris, n (%)	7 (13.2)	27 (15.4)	0.83
Myocardial infarction + angina, n (%)	8 (15.1)	31 (17.7)	0.84
History of stroke, n(%)	3 (5.7)	10 (5.7)	0.99
Medications			
amlodipine	14 (26.4)	58 (33.1)	0.40
renin-angiotensin-aldosterone system inhibitors, n (%)	28 (52.8)	78 (44.6)	0.35
β-blockers, n (%)	8 (15.6)	26 (14.7)	0.99
diuretics, n (%)	4 (7.5)	10 (5.7)	0.74
statins, n (%)	24 (45.3)	49 (28.0)	0.03
Fasting blood glucose, mg/dL	139±37	99±11	<0.0001

Hemoglobin A1c, %	6.9±0.7	5.7±0.3	<0.0001
Total cholesterol, mg/dL	198±35	207±31	0.08
Low density lipoprotein cholesterol, mg/dL	115±35	120±30	0.38
High density lipoprotein cholesterol, mg/dL	50±13	56±16	0.01
Triglyceride, mg/dL	163±87	158±109	0.76
Serum creatinine, mg/dL	0.90±0.48	0.88±0.63	0.88
Estimated glomerular filtration rate, mL/min/1.73m^2	61.4±15.5	63.2±16.3	0.50
Brain natriuretic peptide, pg/dL	33.4±40.5	39.3±66.7	0.56
High sensitive C-reactive protein, mg/dL	1.60±2.60	1.71±2.48	0.79

Each value depicts mean ± standard deviation or number of the patients (%).

Table 2. Hemodynamic Parameters

	baseline			Follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
Systolic blood pressure, mmHg	155±17	161±16	0.03	138±12*	139±11*	0.86
Diastolic blood pressure, mmHg	85±13	88±13	0.11	77±10*	79±9*	0.16
Heart rate, bpm	73±10	73±10	0.99	69±10 †	69±9*	0.58

Each value depicts mean ± standard deviation. *, $p<0.0001$; †, $p<0.001$ vs. baseline.

Table 3. Echocardiography Parameters

	baseline study			follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
LV end-diastolic dimension, cm	4.7±0.5	4.7±0.5	0.55	4.6±0.5	4.6±0.4	0.98
LV end-systolic dimension, cm	2.9±0.5	2.8±0.4	0.12	2.9±0.5 §	2.8±0.4	0.11
LV ejection fraction (%)	68±7	69±8	0.10	69±9	70±7	0.37
Septal wall thickness, cm	1.0±0.2	1.0±0.2	0.14	1.0±0.2	1.0±0.2	0.14
Posterior wall thickness, cm	1.0±0.2	1.0±0.2	0.64	1.0±0.2	1.0±0.1	0.09
Relative wall thickness	0.43±0.09	0.43±0.08	0.98	0.44±0.08	0.42±0.08	0.20
LV mass index, g/m^2	99.9±42.3	92.5±36.1	0.21	91.3±40.8 §	90.4±37.2	0.88
Left atrial volume index, mL/m^2	22.9±9.4	20.2±8.9	0.07	22.6±8.2	19.6±8.3 ‡	0.02
Peak E velocity, cm/s	60.8±14.1	66.8±15.4	0.01	64.8±16.0	67.4±15.9	0.31
Peak A velocity, cm/s	84.4±15.0	81.4±17.0	0.25	84.4±15.0	81.4±17.0 †	0.25
E/A	0.72±0.18	0.86±0.24	0.0003	0.86±0.42 §	0.89±0.23	0.50
Deceleration time of E wave, msec	234±57	230±56	0.68	218±50 §	222±46	0.64
e', cm/s	5.7±1.5	6.1±1.4	0.04	6.3±1.5 †	6.9±1.4*	0.006
E/e'	11.5±4.2	11.4±3.4	0.78	10.8±3.6	10.1±2.9*	0.19

Each value depicts mean ± standard. LV depicts left ventricle. *, p<0.0001; †, p<0.0005; ‡, p<0.005; §, p<0.05 vs. baseline study.

Effect of azelnidipine, a novel calcium channel blocker, on left ventricular relaxation in hypertensive patients with diabetes.

Katsuomi Iwakura[1], Hiroshi Ito[2], Katsuhisa Ishii[3], Motoo Date[1], Fumiaki Nakamura[4], Toshihiko Nagano[5] and Shin Takiuchi[6] for the Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial investigators[7]

[1]Department of Cardiology, Sakurabashi Watanabe Hospital, Osaka, Japan

[2]Department of Cardiovascular Medicine, Okayama University, Graduate School of Medicine, Okayama, Japan

[3]Division of Cardiology, Kansai Electric Power Hospital, Osaka, Japan

[4]Division of Medicine, Ibaraki Iseikai Hospital, Ibaraki, Japan

[5]Internal Medicine, Iwasa Dai-ichi Hospital, Gifu, Japan

[6]Department of Cardiology, Higashi-Takarazuka Satoh Hospital, Takarazuka, Japan

[7]CALVLOC trial investigators list is provided in the Appendix.

Running title: Influence of diabetes on LV relaxation

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Corresponding Author:
Katsuomi Iwakura, MD
Division of Cardiology, Sakurabashi Watanabe Hospital
2-4-32, Umeda, Kita-ku
Osaka 5300001 Japan
Tel: 81-6-6341-8651 Fax:81-6-6341-0785
E-mail:iwakura@mac.com

Abstract

Objectives: We previously demonstrated that a calcium channel blocker, azelnidipine, improves left ventricular relaxation in patients with hypertension and diastolic dysfunction in a multicenter, CALVLOC trial. The objectives of the present subanalysis were to investigate the differences in diastolic function in hypertensive patients with and without diabetes, and the efficacy of azelnidipine on diastolic function among them. ~~(1) the impact of diabetes on diastolic function in hypertensive patients, and (2) the efficacy of azelnidipine on diastolic function in diabetic- and non-diabetic patients.~~

Design: Subanalysis of a prospective single-arm multicenter study.

Participants: 228 hypertensive patients with normal ejection fraction and impaired left ventricular relaxation (septal e' velocity< 8 cm/s on echocardiography) enrolled for CALVLOC trial. They were divided into two groups based on presence or absence of diabetes.

Interventions: Administration of 16mg of azelnidipine for 8 months (range: 6-10 month).

Main outcome measures: Septal e' velocity before and at the end of the study.

Results: Whereas diabetic patients (n=53, 23.2%) had lower systolic blood pressure (BP) than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03), they had lower e' velocity (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04) at baseline. Azelnidipine decreased BP and heart rate, and increased e' velocity similarly in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and non-diabetic patients

(6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Increase in e' velocity was not influenced by presence of diabetes, and diabetic patients still had lower e' velocity after treatment (p=0.006). There was a significant correlation between increase in e' velocity and decrease in systolic BP (R=0.25, p=0.0001), which was not influenced by diabetes.

Conclusions: Comorbid diabetes could impair left ventricular relaxation independently in patients with hypertension, which might not be improved solely by BP lowering.

Key Words: calcium channel blockers, diabetic heart disease, tissue Doppler

Article Summary

Strengths and limitations of this study

- Based on a prospective, multicenter trial, the difference in ~~impact of diabetes on~~ left ventricular (LV) relaxation in hypertensive patients with or without diabetes was investigated.
- Azelnidipine, a unique calcium channel blocker which lowers blood pressure without increasing heart rate, was used as an intervention to improve LV relaxation.
- LV relaxation was more impaired in hypertensive patients with diabetes than in those without diabetes, and azelnidipine improved LV relaxation in both groups to the same degree.
- The persistence of diastolic dysfunction in diabetic patients after azelnidipine treatment implied that hypertension and diabetes might impair diastolic function through different mechanisms.
- This was a subanalysis of a one-arm, open label study including only 228 patients, and therefore, the results might be inconclusive about the impact of diabetes on diastolic function.

Hypertension and diabetes are two major risk factors for heart failure with preserved ejection fraction, and both of them are highly associated with left ventricular (LV) diastolic dysfunction[1-3]. These two diseases frequently coexist and often share comorbidities and conditions that can affect LV function, such as obesity and LV hypertrophy. Therefore, it is not easy to clarify how hypertension and diabetes are interacted in the development of LV diastolic dysfunction. Appropriate blood pressure (BP) control is the most important treatment in patients with heart failure with preserved reduction[2,3]. Calcium channel blockers (CCBs) are not recommended for routine treatment in patients with heart failure with reduced ejection fraction[2], because they might reduce the myocardial contractility. However, their effects on LV diastolic function are still not fully elucidated. Combination of CCB and an angiotensin receptor blocker could improve LV relaxation effectively in hypertensive patients[4]. On the other hands, dihydropyridine CCBs might have unfavorable effects on diastolic function due to reflex tachycardia. Azelnidipine is a unique dihydropyridine CCB which lowers BP as well as amlodipine without increasing, or even slightly decreasing, heart rate[5]. We previously demonstrated that azelnidipine improved LV relaxation in hypertensive patients with LV diastolic dysfunction in the prospective multicenter, Clinical impact of Azelnidipine on Left VentricuLar diastolic function and OutComes in patients with hypertension (CALVLOC) trial[6].

In this post~~ad~~-hoc analysis of the CALVLOC study, we investigated (1) ~~how~~ whether

~~comorbidity of diabetes affected~~ there was a difference in LV diastolic function between ~~in~~ hypertensive patients with and without ~~hypertension~~diabetes, and (2) whether azelnidipine could improve diastolic function in ~~hypertensive~~ diabetic patients ~~with diabetes~~ as well as in ~~those without diabetes~~non-diabetic patients.

METHODS

Study design: The CALVLOC trial was a multi-center, prospective single-arm trial to evaluate the effects of azelnidipine treatment on LV relaxation in hypertensive patients. The study design and main results was reported elsewhere[6]. We enrolled patients with stage 1 or 2 essential hypertension (mean systolic BP >140mmHg or diastolic BP >90mmHg) who had impaired LV relaxation, defined as septal mitral annular relaxation velocity (e') <8cm/s on echocardiography, irrespective of history of antihypertensive treatment. The exclusion criteria were LV ejection fraction of <50%, atrial fibrillation, and the administration of CCBs other than amlodipine. The study patients were enrolled between January 2006 and October 2007 in 11 participating institutes with in Osaka, Hyogo, Aichi and Gifu prefectures, Japan.

Azelnidipine (16mg/day) was administered to patients who had not received CCBs. If patients had been on amlodipine at the time of enrollment, amlodipine was substituted with 16mg of azelnidipine. No other medications were changed throughout the study period. Patients

were assessed at 4–8 week intervals at least for 24weeks, and BP and heart rate were measured at each study visit. Blood and urine tests were performed at baseline and at the end of the study, including measurement of fasting blood glucose, glycosylated hemoglobin A1c (HbA1c), brain natriuretic peptide, high sensitivity C-reactive protein and urine albumin. Echocardiography was recorded before enrollment and at the end of the study. The primary endpoints were changes in septal e' velocity and the ratio of transmitral E wave velocity to the e' (E/e' ratio) from the baseline to the follow-up. Secondary endpoints included changes in BP, heart rate, LV wall thickness, LV mass index and left atrial volume index on echocardiography. The CALVLOC trial was conducted in accordance with the Declaration of Helsinki and with the approval of the institutional ethics committees in each participating institutions. Written informed consent was obtained from each patient enrolled in the study.

The present study was conducted as an post~~ad~~-hoc analysis of the CALVLOC trial. We divided the study patients into two groups based on the presence or absence of diabetes, which was diagnosed according to the guidelines by Japan Diabetes Society[7]. We compared the differences in the primary and secondary endpoints described above between two groups.

Analysis of echocardiography: We performed standard echocardiography examination in all patients. Doppler echocardiographic assessment included the peak velocities of transmitral E and A wave, and deceleration time of the E wave. We recorded tissue Doppler images from the

apical 4-chamber view, and measured septal e' velocity on the pulse-wave Doppler spectrum. LV mass was calculated as 0.80 x (1.04 x [{septal wall thickness in diastole + LV end-diastolic dimension + posterior wall thickness in diastole}^{3} - LV end-diastolic dimension^3])+0.6 (grams) and indexed to body surface area as LV mass index. Relative wall thickness was calculated as 2 x (posterior wall thickness in diastole)/LV end-diastolic dimension. Left atrial volume (mL) was determined by the prolate ellipse method at ventricular end systole, and it was indexed to body surface area as left atrial volume index. All echocardiography data were measured and determined by two independent doctors or sonographers blinded to the patients' clinical data. All parameters were measured once except E and e' velocities, which were measured as an average of three consecutive cardiac cycles.

Statistics. All continuous variables were expressed as mean ± standard deviation and were compared by one-way analysis of variance (ANOVA). Significance of difference was calculated with Tukey's HSD test for factor analysis. Categorical variables were compared with Fisher's exact test. The influence of age and body mass index on e' velocity was adjusted using analysis of covariance (ANCOVA). The correlations between e' and fasting blood glucose or HbA1c were analyzed using linear correlation analysis. The changes in BP, heart rate and e' velocity during treatment were compared between diabetic- and non-diabetic patients using two-way repeated measure ANOVA. The influence of diabetes on the relation between

decrease in BP and increase in e' velocity was analyzed using ANCOVA. StatView 5.0 (SAS Institute Inc.) was used for statistical analysis.

Results

Patients Characteristics: The original CALVLOC trial enrolled 253 patients, and 21 patients were excluded because of failure to follow-up (15 patients) and of protocol violation (6 patients). For the present analysis, four more patients were excluded because of insufficient data about diabetic status. Thus, the final study group for the present analysis was consisted of 228 patients. Their mean age was 66±11 (range; 31 – 95) year old, and 120 (52.6%) of them were male. Diabetes was diagnosed in 53 patients (23.2%), and all of them were diagnosed with Type 2 diabetes. Diabetic patients showed higher fasting blood glucose (139±37 vs. 99±11 mg/dL, $p<0.0001$) and higher HbA1c (6.9±0.7 vs. 5.7±0.3 %, $p<0.0001$) than non-diabetic patients. Table 1 demonstrates the baseline characteristics. There were no differences in age, gender, body size, the prevalence of ischemic heart disease or stroke, and renal function between diabetic- and non-diabetic patients. No differences were observed in antihypertensive drugs including amlodipine administered before enrollment between two groups. Statins were more frequently administered (45.3% vs. 28.0%, $p=0.03$) in diabetic patients. High density lipoprotein cholesterol was significantly lower in diabetic patients (50±13 vs. 56±16 mg/dL,

p=0.01) while no differences were observed in other lipid profile.

Effects of azelnidipine on hemodynamics. Table 2 demonstrated BP and heart rate on enrollment (baseline) and at the end of study. The mean interval between baseline and follow up study was 8 months (range; 6–10 months). Diabetic patients had lower systolic BP at baseline than non-diabetic patients (155±17 vs. 161±16 mmHg, p=0.03). No differences were observed in diastolic BP and heart rate at baseline between two groups.

Azelnidipine treatment significantly decreased systolic- and diastolic BP and heart rate in diabetic- and non-diabetic patients. There were no differences in systolic- and diastolic BP and in heart rate after azelnidipine treatment between two groups. Two-way repeated measure ANOVA was conducted to compare changes of parameters between two groups before and after treatment. The test for the interaction between systolic BP change and diabetes was significant (F=4.49, p=0.04), while the interactions between diabetes and diastolic pressure reduction or heart rate change were not significant (F=0.53, p=0.47 and F=0.48, p=0.49, respectively). These results indicated that azelnidipine lowered systolic BP, but not diastolic BP or heart rate, more effectively in non-diabetic patients than diabetic patients.

Diabetes and echocardiography parameters. The echocardiography parameters at baseline and at follow-up study were demonstrated in Table 3. There were no significant differences in LV dimensions and ejection fraction at baseline between diabetic- and non-diabetic patients.

Also there were no differences in wall thickness and LV mass index between two groups. Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Diabetic patients still had lower e' velocity after adjustment with age and body mass index (p=0.04 by ANCOVA). Diabetic patients had significantly lower E/A ratio (0.72±0.18 vs. 0.86±0.24 cm/s, p=0.0003). Left atrial volume index was tended to be larger in diabetic patients (p=0.07), but no difference was observed in E/e' ratio between two groups. HbA1c was weakly but significantly correlated with e' velocity at baseline (R= 0.21, p=0.002), while there was no correlation between fasting blood glucose and e' velocity (p=0.37).

Azelnidipine treatment significantly increased e' velocity both in diabetic- (5.7±1.5 to 6.3±1.5 cm/s, p=0.0003) and in non-diabetic patients (6.1±1.4 to 6.9±1.4 cm/s, p<0.0001). Two-way repeated measure ANOVA demonstrated that the interaction between change of e' velocity and diabetes was not significant (F=0.48, p=0.48), indicating that increase in e' velocity was not influenced by diabetes (Figure 1). The difference in e' velocity between two groups still remained after azelnidipine treatment (p=0.006). The increase in e' velocity was significantly correlated with decrease in systolic BP during treatment (R=0.25, p=0.0001). This relation was not interacted with presence or absence of diabetes (F=0.27, p=0.60, by ANCOVA). No significant correlation was observed between changes in e' velocity and those in heart rate (R=0.13, p=0.06).

The increase in e' velocity was also weakly but significantly correlated with changes in HbA1c (R=0.16, p=0.03). There were no significant differences in HbA1c during treatment both in diabetic- (6.9±0.7 to 6.8±0.6%, p=0.29) and in non-diabetic patients (5.7±0.3 to 5.7±0.3%, p=0.34), therefore, the contribution of changes in HbA1c would be very small even if present.

E/e' was significantly decreased in non-diabetic patients (11.4±3.4 to 10.1±2.9, p<0.0001) but not in diabetic patients (11.5±4.2 to 10.8±3.6, p=0.11). Left atrial volume index was decreased only in diabetic patients (20.2±8.9 to 19.6±8.3 mL/m2, p=0.004). E/A ratio were increased during treatment in diabetic patients (0.72±0.18 to 0.86±0.24, p=0.02) while the change did not reach statistical significance in non-diabetic patients (0.86±0.24 to 0.89±0.23, p=0.06). The difference in E/A ratio between two groups was not observed after treatment (p=0.50). No significant changes in LV diameters, ejection fraction, wall thickness and LV mass index were observed after azelnidipine treatment in two groups.

Discussion.

We investigated the ~~influence of~~relation between diabetes ~~on~~ and LV relaxation in 228 hypertensive patients who received azelnidipine treatment. Patients with diabetes had significantly lower e' velocity and lower E/A ratio at baseline than those without it, while no

difference was observed in E/e' ratio. Azelnidipine treatment for a mean of 8 months significantly lowered heart rate, systolic- and diastolic BP both in diabetic- and non-diabetic patients, and diabetic patients showed larger systolic BP reduction than non-diabetic patients. Azelnidipine increased e' velocity in both groups similarly, and diabetic patients still had lower e' velocity after treatment. The changes in e' velocity were almost parallel between diabetic- and non-diabetic patients (Figure 1). The increase in e' velocity was correlated with the decrease in systolic BP by azelnidipine, and this correlation was not affected by presence or absence of diabetes. These results demonstrated that LV relaxation was more impaired in diabetic patients than in non-diabetic one among the hypertensive patients, and that the improvement of e' velocity by azelnidipine was little affected by presence or absence of diabetes. The latter suggested that hypertension and diabetes might impair LV relaxation through different mechanisms, and that the impairment associated with diabetes might not be improved by adequate BP control.

Prior studies had demonstrated that patients with both hypertension and diabetes had lower LV diastolic function than those with hypertension or diabetes alone[8-10]. Hypertension and diabetes impaired left atrial performance, which could reflect diastolic function, in an additive fashion[11], suggesting that diabetes and hypertension would impair LV diastolic function through different mechanisms. Hypertension is associated with increased collagen deposition,

increased interstitial fibrosis, and disturbance of calcium homeostasis in the myocardium[12], all of which may contribute to deteriorating diastolic function. Diabetes may increase LV mass independently of arterial blood pressure[13]. Collagen deposition around intramural vessels and between myofibers is increased, and collagen type III is accumulated in diabetic patient, which could mechanically impair diastolic function[14]. It is unclear how the changes associated with diabetes and those with hypertension are overlapped or interacted in the development of diastolic dysfunction.

The present study was a relatively small one and not conducted as a prespecified subgroup analysis, and therefore, the results were not fully conclusive. Improvement of diastolic function in hypertensive patients is correlated with the degree of systolic BP reduction regardless of antihypertensive agents[4]. However, it is unclear whether the changes in e' velocity was caused by BP lowering or by a unique action of azelnidipine. The follow up period might not be long enough to detect the clinical outcomes[15]. We did not determine the variability of e' velocity measurement among the institutions, although e' velocity could be a relatively robust parameter. We measured only septal e' velocity for the original CALVLOC study[14]. Although septal e' velocity might be sufficient for the evaluation of LV relaxation in most cases, wall motion abnormality within septum might affect the septal velocity. We did not assess myocardial ischemia directly, and subclinical coronary artery disease might be

dismissed. We did not analyze the duration of diabetes and the effects of anti-diabetic treatment or those of antihypertensive drugs concomitantly used.

Despite of limitations described above, the present study provided an important insight into the mechanisms of LV diastolic dysfunction in hypertension and diabetes. Standard BP lowering might not be enough for improvement of diastolic function in hypertensive patients with diabetes. It is unclear whether diabetic control has an additive or synergic effect with BP lowering on diastolic function. The correlation between HbA1c and e' velocity in the present study was very weak, and intensive glycemic control might not be as effective as BP lowering for LV diastolic dysfunction, as suggested in the large-scale trials[16-18].

APPENDIX

CALVLOC trial investigators;

Atsuhito Otuska, MD (Ibaraki Iseikai Hospital, Ibaraki, Japan); Kou Fujisawa, MD (Iwasa Dai-ichi Hospital, Gifu, Japan); Yorihiko Higashino, MD (Higashi-Takarazuka Sato Hospital, Takarazuka, Japan); Kei Tawarahara, MD (Hamamatsu Red-cross Hospital, Hamamatsu, Japan); Mikio Mukai, MD (Kinki Chuo Hospital, Itami, Japan); Masanori Shinoda, MD (Kouseiren Kamo General Hospital, Toyota, Japan); Taro Minagawa, MD (Minagawa Clinic, Gifu, Japan); and Naoki Goto, MD (Goto Clinic, Gifu, Japan).

Contributorship statement

Katsuomi Iwakura provided the study design of the present subanalysis along with Dr. Ito, performed statistical analysis, interpreted the results, and wrote the draft of the manuscript~~played the leading role in this work~~. Hiroshi Ito provided the concept and study design of original CALVLOC study and its subanalysis, review of data and revision of the manuscript. Katsuhisa Ishii, Motoo Date, Fumiaki Nakamura, Toshihiko Nagano and Shin Takiuchi assisted in analysis and interpretation of original CALVLOC data. All authors carefully read and approved the manuscript.

Competing interests

There are no competing interests regarding the present study.

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Data sharing

Extra data (a full dataset) is available by emailing Katsuomi Iwakura

(iwakura@mac.com).

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Figure Legend

Figure 1. Changes in e' velocity during azelnidipine treatment.

Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Azelnidipine treatment significantly increased e' velocity both in diabetic patients (p=0.0003) and in non-diabetic patients (p<0.0001). The changes in e' velocity were parallel between two groups, implying that the effects of azelnidipine were similar between two groups. Diabetic patients had lower e' velocity than non-diabetic patients even after treatment (6.3±1.5 vs. 6.9±1.4 cm/s, p=0.006). *: p=0.04, †:p=0.006 vs. non-diabetic patients.

Table 1. Patients Characteristics

	Diabetes	Non-Diabetes	p value
Number of patients, n (%)	53 (23.2%)	175 (76.8%)	
Age, year	68±10	65±12	0.09
Gender, male/female	31/22	89/86	0.35
Height, cm	158.1±9.5	159.5±9.7	0.35
Weight, kg	62.9±12.8	62.1±11.3	0.64
Body mass index	25.0±3.3	24.3±3.3	0.20
Dyslipidemia, n (%)	30 (56.6)	76 (43.4)	0.12
Smoker, n (%)	13 (24.5)	61 (34.9)	0.18
History of myocardial infarction, n (%)	2 (3.8)	7 (4.0)	0.99
Angina pectoris, n (%)	7 (13.2)	27 (15.4)	0.83
Myocardial infarction + angina, n (%)	8 (15.1)	31 (17.7)	0.84
History of stroke, n(%)	3 (5.7)	10 (5.7)	0.99
Medications			
amlodipine	14 (26.4)	58 (33.1)	0.40
renin-angiotensin-aldosterone system inhibitors, n (%)	28 (52.8)	78 (44.6)	0.35
β-blockers, n (%)	8 (15.6)	26 (14.7)	0.99
diuretics, n (%)	4 (7.5)	10 (5.7)	0.74
statins, n (%)	24 (45.3)	49 (28.0)	0.03
Fasting blood glucose, mg/dL	139±37	99±11	<0.0001

Hemoglobin A1c, %	6.9±0.7	5.7±0.3	<0.0001
Total cholesterol, mg/dL	198±35	207±31	0.08
Low density lipoprotein cholesterol, mg/dL	115±35	120±30	0.38
High density lipoprotein cholesterol, mg/dL	50±13	56±16	0.01
Triglyceride, mg/dL	163±87	158±109	0.76
Serum creatinine, mg/dL	0.90±0.48	0.88±0.63	0.88
Estimated glomerular filtration rate, mL/min/1.73m^2	61.4±15.5	63.2±16.3	0.50
Brain natriuretic peptide, pg/dL	33.4±40.5	39.3±66.7	0.56
High sensitive C-reactive protein, mg/dL	1.60±2.60	1.71±2.48	0.79

Each value depicts mean ± standard deviation or number of the patients (%).

Table 2. Hemodynamic Parameters

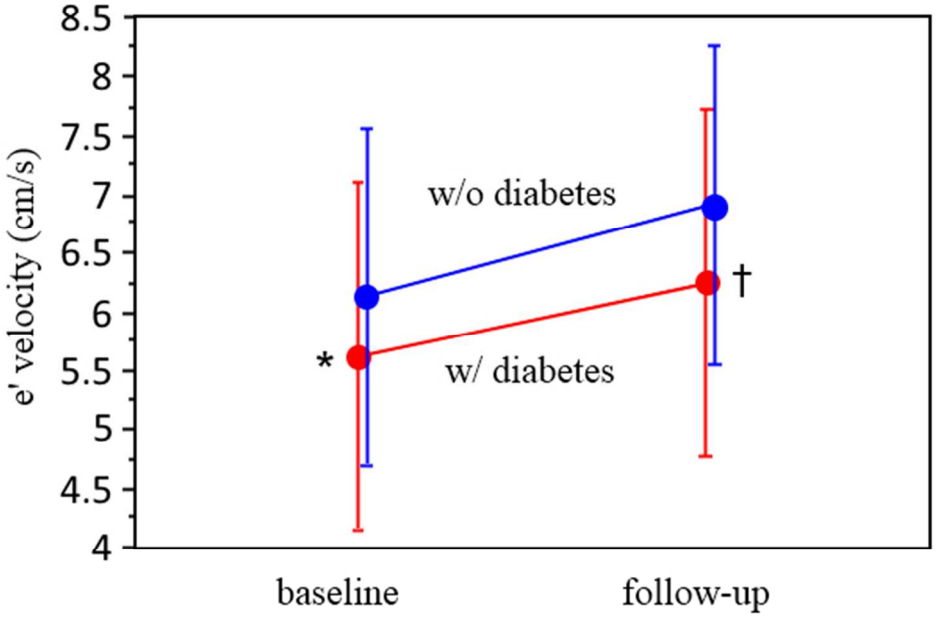
	baseline			Follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
Systolic blood pressure, mmHg	155±17	161±16	0.03	138±12*	139±11*	0.86
Diastolic blood pressure, mmHg	85±13	88±13	0.11	77±10*	79±9*	0.16
Heart rate, bpm	73±10	73±10	0.99	69±10 †	69±9*	0.58

Each value depicts mean ± standard deviation. *, p<0.0001; †, p<0.001 vs. baseline.

Table 3. Echocardiography Parameters

	baseline study			follow-up study		
	Diabetes	Non-Diabetes	p value	Diabetes	Non-Diabetes	p value
LV end-diastolic dimension, cm	4.7±0.5	4.7±0.5	0.55	4.6±0.5	4.6±0.4	0.98
LV end-systolic dimension, cm	2.9±0.5	2.8±0.4	0.12	2.9±0.5 §	2.8±0.4	0.11
LV ejection fraction (%)	68±7	69±8	0.10	69±9	70±7	0.37
Septal wall thickness, cm	1.0±0.2	1.0±0.2	0.14	1.0±0.2	1.0±0.2	0.14
Posterior wall thickness, cm	1.0±0.2	1.0±0.2	0.64	1.0±0.2	1.0±0.1	0.09
Relative wall thickness	0.43±0.09	0.43±0.08	0.98	0.44±0.08	0.42±0.08	0.20
LV mass index, g/m^2	99.9±42.3	92.5±36.1	0.21	91.3±40.8 §	90.4±37.2	0.88
Left atrial volume index, mL/m^2	22.9±9.4	20.2±8.9	0.07	22.6±8.2	19.6±8.3 ‡	0.02
Peak E velocity, cm/s	60.8±14.1	66.8±15.4	0.01	64.8±16.0	67.4±15.9	0.31
Peak A velocity, cm/s	84.4±15.0	81.4±17.0	0.25	84.4±15.0	81.4±17.0 †	0.25
E/A	0.72±0.18	0.86±0.24	0.0003	0.86±0.42 §	0.89±0.23	0.50
Deceleration time of E wave, msec	234±57	230±56	0.68	218±50 §	222±46	0.64
e', cm/s	5.7±1.5	6.1±1.4	0.04	6.3±1.5 †	6.9±1.4*	0.006
E/e'	11.5±4.2	11.4±3.4	0.78	10.8±3.6	10.1±2.9*	0.19

Each value depicts mean ± standard. LV depicts left ventricle. *, p<0.0001; †, p<0.0005; ‡, p<0.005; §, p<0.05 vs. baseline study.



Changes in e' velocity during azelnidipine treatment.
Diabetic patients showed lower e' velocity than non-diabetic patients (5.7±1.5 vs. 6.1±1.4 cm/s, p=0.04). Azelnidipine treatment significantly increased e' velocity both in diabetic patients (p=0.0003) and in non-diabetic patients (p<0.0001). The changes in e' velocity were parallel between two groups, implying that the effects of azelnidipine were similar between two groups. Diabetic patients had lower e' velocity than non-diabetic patients even after treatment (6.3±1.5 vs. 6.9±1.4 cm/s, p=0.006). *: p=0.04, †:p=0.006 vs. non-diabetic patients.
54x40mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
j Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5,6
Methods			
Study design	4	Present key elements of study design early in the paper	6,7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	6
		(*b*) For matched studies, give matching criteria and the number of controls per case	Not for this study.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7,8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how matching of cases and controls was addressed	Not for this study.
		(*e*) Describe any sensitivity analyses	Not for this study.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Not for this study.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic,	9

		clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	Not for this study.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	Not for this study.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not for this study.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12,13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14,15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.