Heart Rate Reduction by Ivabradine Reduces Diastolic Dysfunction and Cardiac Fibrosis

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Key Words

Heart rate · Diastolic dysfunction · Collagen · Echocardiography · Hypercholesterolemia

Abstract

Objectives: To determine if heart rate (HR) reduction with ivabradine (IVA), a selective inhibitor of the pacemaker I_f current, prevents cardiac dysfunction associated with dyslipidemia. **Methods:** New Zealand White rabbits received either a standard diet, a 0.5% cholesterol-enriched diet only (CD), or a 0.5% CD with IVA (17 mg/kg/day) for 12 weeks. HR, left ventricular (LV) systolic function, diastolic function and LV regional myocardial performance index (MPI) were studied using echocardiography. Histological analysis included cardiac interstitial fibrosis and collagen type I fibers. Plasma levels of angiotensin II and aldosterone were quantified by immunoassays. **Results:** IVA reduced HR by approximately 11%. IVA improved MPI and attenuated LV diastolic dysfunction (DD) (92% mild and 8% moderate DD with IVA vs. 54% mild and 46% moderate DD in CD group). IVA also reduced atrial fibrosis ($p = 0.027$), ventricular fibrosis ($p = 0.0002$) and ven-

tricular collagen type I ($p = 0.0042$). IVA decreased plasma angiotensin II levels ($p = 0.042$), and both angiotensin II and aldosterone levels were correlated with HR ($p = 0.038$ and 0.008). **Conclusion:** Selective HR reduction with IVA reduces DD and cardiac fibrosis in hypercholesterolemic rabbits. These beneficial effects of IVA support testing pure HR reduction in patients with diastolic heart failure.

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Introduction

Increased resting heart rate (HR) is a risk factor for coronary artery disease, heart failure and mortality [1–3]. Studies have shown the benefits of lower HR on cardiovascular mortality and morbidity in different patient populations [2, 4, 5]. Although it was shown experimentally that stress-related β -adrenergic receptor-mediated increase in HR could promote vascular endothelial dysfunction and atherosclerosis in monkeys [6, 7], the mechanisms linking cardiovascular diseases and HR remain largely unknown. An increase in HR mediated by β -ad-

renergic receptors following activation of the sympathetic nervous system is a common occurrence in heart failure [8], but this may be secondary to the cardiac dysfunction.

Ivabradine (IVA) reduces HR by a direct effect on the sinoatrial node [9]. It is an inhibitor of the F channel [10], which controls the electrical pacemaker activity in the sinoatrial node. IVA is deprived of inotropic and direct vasodilating effects [11–13]. Although the efficacy of IVA in the treatment of myocardial ischemia and angina has been shown [14], no study has directly evaluated its potential benefits on ventricular and atrial dysfunction in the context of dyslipidemia. Hypercholesterolemia has important implications for cardiac function. Elevated aortic afterload accelerated ventricular dysfunction in apolipoprotein E knockout mice [15] and 10 weeks of 0.5% cholesterol-enriched diet impairs systolic and diastolic function in rabbits [16]. Recently, we reported that moderate dyslipidemia led to cardiac dysfunction in addition to endothelial dysfunction in mice [17]. Therefore, in the present study, we have evaluated the effects of chronic HR reduction by IVA on diastolic dysfunction (DD) and cardiac fibrosis in cholesterol-fed rabbits.

Methods

Animals and Experiments

Animal care and procedures complied with the Canadian Council on Animal Care guidelines and were approved by the institutional ethics committee for animal research.

First, to evaluate the dose of IVA needed to induce a reduction in HR of approximately 15%, rabbits were anesthetized (ketamine/midazolam 45/0.75 mg/kg), and electrocardiogram (ECG) radiofrequency transmitters TA10EAF40 (Data Sciences International, Arden Hills, Minn., USA) were placed subcutaneously. In 4 rabbits per group, ECG was monitored by telemetry by alternating data acquisition, 2 rabbits at a time for 24 h. Signals were acquired simultaneously with IOX 2.3.7.5 (Emka Technologies, Paris, France). Recordings were analyzed and HRs calculated with ECG Auto 2.4.0.22 software (Emka Technologies). The dose of IVA was adjusted according to the dose-response curve obtained with the following results: reduction in HR was 1.8% with 1.0 mg/kg/day of IVA, 3.4% with 5.0 mg/kg/day, 9.3% with 10 mg/kg/day, 17.1% with 20 mg/kg/day and 19.4% with 40 mg/kg/day.

Then, 32 male New Zealand White rabbits (2.7–3.0 kg, aged 12–13 weeks) were assigned to receive either a standard diet (control group, $n = 6$), or a 0.5% cholesterol-enriched diet only (CD group, $n = 13$), or a 0.5% CD with IVA (17 mg/kg/day, IVA group, $n = 13$) for 12 weeks. We initially aimed to induce a reduction in HR in the IVA group of approximately 15%.

At 6 and 12 weeks, all rabbits were anesthetized (ketamine 30 mg/kg and xylazine 5 mg/kg) to monitor HR. All rabbits received similar controlled sedation. The dose of anesthetics was first administered according to the weight of each rabbit; moreover, the depth of anesthesia determined by evaluation of reflexes (corneal

and toe pinch, leg withdrawal) and the pattern of respiration were monitored, and the dose of anesthetics was adjusted to reach a similar level of anesthesia in all animals. After 12 weeks (before sacrifice), cardiac function was studied by transthoracic echocardiography. HR and blood pressure were measured using a Millar catheter inserted in the right carotid artery. An arterial blood sample was collected for serum and plasma analyses, and cardiac tissues were harvested after sacrifice.

Ivabradine

IVA hydrochloride (S-16257-2) was provided by the Institut de Recherches Internationales Servier (Courbevoie, France) as a mono-hydrochloride salt. IVA was dissolved in water everyday and distributed in containers to the animals. The concentrations of IVA were regularly adjusted to rabbit body weight and water consumption.

Serum and Plasma Analyses

Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and calcium levels were measured with an automated filter photometer system (Dimension RxL Max, Dade Behring, Deerfield, Ill., USA). Aldosterone levels were measured using a radio immunoassay kit (Coat-A-Count, DPC, Los Angeles, Calif., USA). Angiotensin II was measured using an enzyme immunoassay kit (SPI-Bio, Montigny-le-Bretonneux, France) following delipidation of the samples from the CD and IVA groups.

Echocardiography

A transthoracic echocardiographic study was performed before sacrifice, with rabbits being sedated by intramuscular injection of ketamine and midazolam (45 and 0.75 mg/kg), using a phased-array probe 10S (4.5–11.5 MHz) and a Vivid 7 Dimension system (GE Healthcare Ultrasound, Horten, Norway).

Left ventricular (LV) M-mode spectrum was obtained in parasternal long-axis view to measure LV diameters at both end cardiac diastole (LVDD) and systole (LVDs). LV fractional shortening was calculated as $(LVDD - LVDs)/LVDD \times 100\%$. The Teicholz method was employed to calculate LV volumes and LV ejection fraction.

Pulsed wave Doppler was used to evaluate transmitral flow and pulmonary venous flow in apical 4-chamber view. Transmitral flow was used to measure the peak velocities during early filling (E) and atrial contraction (A) and to calculate the E/A ratio. Pulmonary venous flow was used to measure the systolic flow (S), diastolic flow (D) and reversed atrial flow (Ar). LV basal lateral peak systolic velocities (Sm) and mitral annulus velocities during early filling (Em) and atrial filling (Am) were derived by tissue Doppler imaging. The time intervals from the end of Am to the beginning of Em (b), and from the beginning to the end of Sm (a) were also measured on lateral wall tissue Doppler imaging. LV myocardial performance index (MPI) was calculated by $(b - a)/a \times 100\%$. MPI combines LV systolic and diastolic properties together; it is a pure time interval index, non-geometric and independent of HR, not requiring frequency-based normalization. Higher MPI implies impaired LV myocardial performance [18].

Left ventricular diastolic dysfunction (LVDD) was classified according to published criteria [19] (fig. 1a). To further evaluate LVDD, left atrium (LA) M-mode spectrum was obtained in parasternal long-axis view at the aortic valve level and LA dimensions were measured in both end cardiac diastole and systole. LA frac-

Fig. 1. LV basal lateral MPI. ** $p \leq 0.01$.

tional shortening was calculated as (systolic dimension – diastolic dimension)/systolic dimension $\times$ 100%. The average of 3 consecutive cardiac cycles was used for each measurement.

Oxidative Stress Markers

To evaluate the level of oxidative stress, we used a sensitive and specific gas chromatography-mass spectrometric assay of protein-bound 4-hydroxynonenal bound to thiol proteins (4HNE-P), a product of free radical-induced lipid peroxidation, as previously described [20]. Levels of reduced and oxidized glutathione were determined in 200 μ l of whole blood by capillary electrophoresis according to Serru et al. [21].

Histology

The LA and LV were dissected from the heart and immersion fixed in 10% buffered formalin at 4°C for 24 h and embedded in paraffin. Masson's Trichrome-stained sections were prepared for fibrosis area quantification. Deparaffinized ventricular sections were preincubated with an antigen unmasking solution (Vector Laboratories, Burlingame, Calif., USA), then immunostained with a goat polyclonal antibody against collagen type I (1:10 dilution; Millipore, Billerica, Mass., USA). Species-appropriate biotinylated secondary antibody was applied, followed by streptavidin-horseradish peroxidase complex, visualized with amino-9-ethylcarbazol and counterstained by Mayer's hematoxylin.

The acquisition of 5 histologic images per section was based on complete atrial and ventricular slices, considering every level of tissue wall, without any selection of a specific zone and without any information on the rabbit study group. Images were acquired at 20 $\times$ magnification using a light microscope (BX41, Olympus, Tokyo, Japan) connected to a digital video camera (Qcolor3, Olympus). Automatic computer-based analysis was performed with the same color threshold for all specimens. Data were expressed as percent stained area in the region of analysis.

Statistical Analysis

Data are presented as the mean $\pm$ standard error of the mean for continuous variables and as frequencies and percentages for categorical variables. DD classification was compared across groups using the χ^2 test. Lipid, circulating, echocardiographic and histologic parameters were compared across groups (control

group, CD group and IVA group) using the one-way ANOVA model followed by pairwise comparisons if the global F test was significant at the 0.05 level. For LA fractional shortening, the contrast under the ANOVA model for comparison between the CD and IVA groups was evaluated as an exploratory analysis if the global F test was ≤ 0.10 . Parameters only measured for CD and IVA groups were compared between them using Student's t test. Pearson correlations were performed to investigate the relationships between HR and both aldosterone and angiotensin II. All analyses were done with SAS version 9.1 (SAS Institute Inc., Cary, N.C., USA) and conducted at the 0.05 significance level.

Results

Lipid Levels, HR and Blood Pressure

Lipids

No significant difference between IVA and CD groups was observed at the end of the study for serum levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, glycemia and calcemia (table 1).

Heart Rate

Using telemetric measures in the preliminary study, the dose of IVA was set to 17 mg/kg/day to reach an expected decrease in HR of 15% (see Methods). At 6 weeks of treatment in the main study, echocardiographic measurements under anesthesia showed a mean decrease in HR of 11.4% in the IVA group compared with the CD group (220 ± 7 vs. 245 ± 9 bpm; $p = 0.035$). At 12 weeks, HR was 270 ± 19 , 218 ± 9 and 200 ± 8 bpm in the control, CD and IVA group, respectively ($p = 0.0003$ for IVA vs. controls).

Blood Pressure

Mean blood pressure was not significantly different among the control, CD and IVA groups (table 2).

Cardiac Function

LV Myocardial Performance Index

LV basal lateral MPI was 78.1 ± 4.3 , 100.7 ± 5.4 and $73.4 \pm 3.7\%$ in the control, CD and IVA group, respectively ($p = 0.005$ for IVA vs. CD; $p =$ not significant for IVA vs. controls; fig. 1), indicating that IVA prevented the detrimental effects of the cholesterol diet on LV myocardial performance.

LV Systolic Function

LV fractional shortening and LV ejection fraction were similar in the control, CD and IVA groups (table 2), indicating that the LV global systolic function was well preserved in all cholesterol-enriched diet rabbits.

Table 1. Blood analyses

	Control (n = 6)	CD (n = 13)	IVA (n = 13)
Total cholesterol, mmol/l	0.6 ± 0.1	20.8 ± 1.6**	20.3 ± 2.5**
HDL cholesterol, mmol/l	0.3 ± 0.0	0.8 ± 0.0**	0.8 ± 0.1**
Triglycerides, mmol/l	1.2 ± 0.2	1.6 ± 0.3	1.4 ± 0.2
Glycemia, mmol/l	11.3 ± 0.8	9.9 ± 0.5	9.9 ± 0.6
Calcemia, mmol/l	3.1 ± 0.0	2.9 ± 0.0	3.1 ± 0.1
Angiotensin II, AU/ml	–	16.7 ± 4.8	5.5 ± 1.2***
Aldosterone, pmol/l	386 ± 79	248 ± 44	163 ± 26*
4HNE-P, pmol/mg protein	0.51 ± 0.06	2.72 ± 0.35**	2.60 ± 0.40**
GSH/GSSG	8.7 ± 1.6	15.8 ± 1.4	22.0 ± 3.3*

HDL = High-density lipoprotein; GSH = reduced glutathione; GSSG = oxidized glutathione. * $p \leq 0.01$ versus control; ** $p \leq 0.001$ versus control; *** $p \leq 0.05$ versus CD.

Table 2. Echocardiographic and hemodynamic results after 12 weeks of treatment

	Control (n = 6)	CD (n = 13)	IVA (n = 13)
LV fractional shortening, %	38.1 ± 1.1	40.2 ± 1.4	43.1 ± 1.2
LV ejection fraction, %	72.2 ± 1.4	74.2 ± 1.6	77.5 ± 1.3
Pulmonary venous S wave, cm/s	47.3 ± 4.1	33.9 ± 2.9 ^a	41.1 ± 2.4 ^b
Pulmonary venous D wave, cm/s	33.0 ± 2.0	32.6 ± 1.7	35.1 ± 2.3
Pulmonary venous Ar, cm/s	23.8 ± 2.9	20.5 ± 2.0	19.8 ± 1.1
Mitral E/A ratio	1.34 ± 0.28	1.17 ± 0.12	1.16 ± 0.16
LA fractional shortening, %	26.6 ± 1.6	23.3 ± 1.3	27.6 ± 1.5 ^c
LA systolic dimension, cm	0.93 ± 0.05	1.05 ± 0.04	1.01 ± 0.03
LA diastolic dimension, cm	0.68 ± 0.04	0.81 ± 0.04	0.73 ± 0.04
LV basal lateral MPI	78.1 ± 4.3	100.7 ± 5.4	73.4 ± 3.7 ^c
Systolic blood pressure, mm Hg	70.2 ± 3.3	73.6 ± 2.3	79.5 ± 1.8 ^d
Diastolic blood pressure, mm Hg	50.2 ± 3.2	56.7 ± 2.2	57.3 ± 1.3
Mean blood pressure, mm Hg	56.8 ± 3.1	63.4 ± 2.3	64.7 ± 1.4

S = Systolic flow; D = diastolic flow; Ar = reversed atrial flow; E = peak velocity in early filling; A = peak velocity during atrial contraction. ^a $p \leq 0.01$ versus control; ^b $p = 0.06$ versus CD; ^c $p \leq 0.01$ versus CD; ^d $p \leq 0.05$ versus control; ^e $p = 0.038$ versus CD (for LA fractional shortening, exploratory analysis given $p = 0.10$ for global F test).

LV Diastolic Function

Improved MPI with unchanged LV systolic function suggested that IVA improved diastolic function. Indeed, LV diastolic filling patterns (fig. 2a) were distributed differently among groups ($p = 0.0003$). DD, observed in the CD group, was attenuated by IVA (92.3% of mild DD and 7.7% of moderate DD vs. 53.8% of mild DD and 46.2% of moderate DD for CD rabbits) (fig. 2b). Moderate DD occurred significantly more frequently in the CD group than in the IVA group ($p = 0.027$; fig. 2b). Pulmonary venous S wave was higher in controls than in the CD group

($p = 0.008$), as well as in rabbits treated with IVA versus CD ($p = 0.064$) (table 2). LA fractional shortening was $26.6 \pm 1.6\%$ in the control group (global; $p = 0.10$), $27.6 \pm 1.5\%$ in the IVA group ($p = 0.038$ vs. CD) and $23.3 \pm 1.3\%$ in the CD group (table 2).

Histology – LA and LV Interstitial Fibrosis and LV Collagen Type I

IVA treatment significantly reduced the extent of LA interstitial fibrosis (blue staining; fig. 3a) compared with the CD group [$16.5 \pm 1.1\%$ ($n = 7$) vs. $20.9 \pm 1.4\%$ ($n =$

Fig. 2. LVDD severity. **a** LVDD classification: mild LVDD was defined by $E/A < 1$, or $1 < E/A < 2$, with $S/D > 1$ and $Em < Am$. Moderate LVDD was considered present when $2 > E/A > 1$ with $S/D < 1$. Severe LVDD was diagnosed when $E/A > 2$ with $S/D < 1$. TMF = Transmittal flow; E = peak velocity in early filling; A = peak velocity during atrial contraction; PVF = pulmonary venous

flow; S = systolic flow; D = diastolic flow; MAV = mitral annulus velocity; Em = mitral annulus velocity during early filling; Am = mitral annulus velocity during atrial contraction. **b** Distribution of LVDD severity in control, CD and IVA groups at 12 weeks after treatment. * $p \leq 0.05$, ** $p \leq 0.01$.

9); $p = 0.027$] but did not normalize it (fig. 3b). In contrast, IVA treatment reduced LV interstitial fibrosis (fig. 3a) to the almost normal value of $1.2 \pm 0.2\%$, compared with $5.8 \pm 0.9\%$ in the CD group ($p = 0.0002$; fig. 3b). Ventricular fibrosis was $0.5 \pm 0.1\%$ in the control rabbits ($p = 0.602$ for IVA vs. control rabbits; $p = 0.0008$ for CD vs. control rabbits).

IVA also reduced the extent of LV collagen type I to $2.7 \pm 0.3\%$, compared with $9.6 \pm 1.6\%$ in the CD group ($p = 0.0042$). Collagen type I was $4.8 \pm 0.7\%$ in the normal control rabbits ($p = \text{not significant}$ for IVA vs. control rabbits; $p = 0.0248$ for CD vs. control rabbits).

Circulating Markers

Plasma Levels of Angiotensin II and Aldosterone

Plasma levels of angiotensin II, which were measured in the hypercholesterolemia groups only (table 1), were reduced with IVA compared with the CD group ($p = 0.042$) (fig. 4a). Plasma angiotensin II was significantly correlated with HR at 12 weeks for pooled CD and IVA animals ($r = 0.37$, $p = 0.038$). Aldosterone levels were decreased in the IVA versus the control group ($p = 0.004$;

table 1) and tended to be lower in the IVA versus the CD group ($p = 0.142$). Plasma levels of aldosterone were also significantly correlated with HR for the pooled study animals ($r = 0.46$, $p = 0.008$; fig. 4b).

Oxidative Stress

Values for the lipid peroxidation marker 4HNE-P were higher in both hypercholesterolemic groups compared with controls (vs. CD, $p < 0.0001$; vs. IVA, $p = 0.0002$; table 1). Reduced glutathione/oxidized glutathione ratio in blood, a marker of the thiol redox status, was significantly higher in IVA compared with the control group ($p = 0.004$; table 1), but not with the CD group, nor was the CD group different from controls.

Discussion

This study demonstrates that selective HR reduction with IVA prevented the detrimental effects of hypercholesterolemia on LV myocardial performance. IVA attenuated LV diastolic dysfunction, reduced LA and LV struc-

Fig. 3. Histology of LA and LV interstitial fibrosis. **a** Masson's Trichrome staining of LA and LV sections (images acquired at 20× magnification for each panel). Fibrosis is stained in blue; atrial and ventricular muscle is stained in pink. **b** Quantification by digital image analysis of interstitial fibrosis in the LA and LV sections of control, CD and IVA groups, expressed as the percentage of stained area in the region of analysis. * $p \leq 0.05$, *** $p \leq 0.001$.

tural remodeling (interstitial fibrosis) and reduced LV collagen type I in hypercholesterolemic rabbits. IVA also improved LA fractional shortening. Circulating angiotensin II levels were significantly lowered by IVA and correlated with HR. Aldosterone levels also correlated with HR during treatment and were lower with IVA.

Echocardiography has been successfully used in various rabbit models to study LV remodeling and cardiac function [22, 23]. In the present study, IVA significantly improved LV MPI, which is a simple and non-geometric index that takes into account systolic and diastolic functions independently of HR. In the absence of LV systolic

dysfunction, the significant improvement in LV MPI reflects improved LV diastolic function with IVA. In our current study, chronic reduction in HR by IVA indeed limited LVDD in dyslipidemic rabbits. Our observations are in agreement with previous results reported in dyslipidemic mice [17] and in a rat model of heart failure induced by coronary artery ligation [24]. We found that DD of moderate severity developed in dyslipidemic rabbits, and this was significantly attenuated by the treatment with IVA. These echocardiographic results are concordant with the large reduction in ventricular fibrosis and collagen type I with IVA observed on histology. There-

Fig. 4. a Plasma angiotensin II levels in CD and IVA groups. * $p \leq 0.05$. AU = Arbitrary units. **b** Correlation between HR at 12 weeks and plasma aldosterone levels in animals from pooled control, CD and IVA groups.

fore, there is a significant benefit of selectively lowering HR in the presence of diastolic dysfunction.

We have previously described the prognostic implications of DD detected by echocardiography [25]. We have also previously reported the effects of anesthesia and surgery on diastolic function [26], but all the animals in the present study were submitted to the same degree of anesthesia. The systolic component of the pulmonary venous flow (S wave) has been shown to correlate well with LV filling pressure and with its change over time [27]. Therefore, our assessment of DD was based on the classification recommended by the American Society of Echocardiography, where the pulmonary venous systolic/diastolic flow ratio plays a key role [19].

Elevated concentrations of angiotensin II associated with heart failure have been shown to promote atrial fibrosis [28]. In our dyslipidemia-induced DD model, IVA decreased circulating angiotensin II levels, decreased fibrosis of both the LA and LV walls and decreased collagen type I in LV walls. Reduction in perivascular collagen in ventricular myocardium has been reported with IVA in middle-aged postmyocardial infarction rats [29]. Given the importance of atrial fibrosis in the pathophysiology of atrial fibrillation [30], our observation of reduced atrial and ventricular fibrosis induced by IVA in the setting of dyslipidemia-induced DD potentially opens a new area of investigation for the prevention of this common arrhythmia. Moreover, plasma levels of angiotensin II and aldosterone were positively correlated with HR dur-

ing treatment in our study. These observations are particularly interesting in light of the recent finding that the increased rates of mortality, vascular events and heart failure associated with the use of the lipid-modulating agent torcetrapib were due at least in part to an increase in aldosterone levels [31].

In this hypercholesterolemic rabbit model, the improvement in diastolic function associated with selective HR reduction was associated with IVA-induced reduction in ventricular fibrosis and collagen type I. The improvement in cardiac function and fibrosis [32] could be dependent on the combined reduced levels of angiotensin and aldosterone, which act in synergy during cardiac remodeling [33]. The favorable effects on diastolic function may also in part have been due to IVA-induced increase in diastolic (ventricular filling) time, as described in previous experimental studies [17, 24]; improved myocardial perfusion may also have contributed to the beneficial effects of IVA due to the lengthening of diastole and reduced perivascular collagen [12, 29].

The benefits conferred by IVA were observed independent of effects on hypercholesterolemia and associated oxidative stress. In fact, as expected, circulating levels of cholesterol and of the lipid peroxidation marker 4HNE-P were increased in rabbits fed the hypercholesterolemic diet [34], but were not affected by IVA treatment. However, the possibility that IVA attenuates oxidative stress at the tissue level (i.e. myocardium) cannot be excluded. Indeed, in a previous study conducted in ApoE^{-/-} mice fed

with a cholesterol-rich diet for 6 weeks, IVA-induced HR reduction was associated with decreases in NADPH oxidase activity, superoxide production and lipid peroxidation in the vascular wall [35].

Conclusion

Selective HR reduction with IVA in hypercholesterolemic rabbits attenuates LVDD, reduces LA and LV fibrosis and reduces LV collagen type I. Circulating angiotensin II and aldosterone levels were lowered with IVA and correlated with HR. These beneficial effects of IVA support the evaluation of pure HR reduction in patients with diastolic heart failure.

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Conflicts of Interest

Dr. Tardif holds the Université de Montréal research chair in atherosclerosis. Dr. Tardif has received honoraria from Servier. Dr. Busseuil holds a research fellowship award of the Heart and Stroke Foundation of Canada.

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