

Research Paper

Pyridoxamine protects against mechanical defects in cardiac ageing in rats: studies on load dependence of myocardial relaxation

Chih-Hsien Wang^{1,2}, En-Ting Wu³, Ming-Shiou Wu⁴, Ming-Shian Tsai⁵, Ya-Hui Ko⁶, Ru-Wen Chang⁶, Chun-Yi Chang⁷ and Kuo-Chu Chang⁶

¹Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan

²Department of Surgery, Hsin-Chu Branch of National Taiwan University Hospital, Hsin-Chu, Taiwan

³Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan

⁴Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

⁵Department of Surgery, E-Da Hospital, Kaohsiung, Taiwan

⁶Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan

⁷Department of Emergency Medicine, National Taiwan University Hospital, Taipei, Taiwan

New Findings

- **What is the central question of this study?**

This study aimed to investigate the hypothesis that pyridoxamine, one of the three natural forms of vitamin B₆, can protect against myocardial relaxation of senescent animals by targeting arterial stiffening and contractile dysfunction of the left ventricle.

- **What is the main finding and its importance?**

We found that treating the senescent rats with pyridoxamine for 5 months might improve myocardial relaxation rate, at least partly through its ability to enhance myocardial contractile performance, increase wave transit time and decrease wave reflection factor.

Our team demonstrated in the past that pyridoxamine attenuated arterial stiffening by targeting the pathogenic formation of glycated collagen cross-links in aged rats. Herein, we examined whether pyridoxamine therapy can protect against mechanical defects in myocardial relaxation by improving arterial wave properties and cardiac contractile performance in senescent animals. Fifteen-month-old male Fisher 344 rats were treated daily with pyridoxamine (1 g l⁻¹ in drinking water) for 5 months and compared with age-matched untreated control animals (20 months old). Arterial wave properties were characterized by wave transit time (τ_w) and wave reflection factor (R_f). We measured the contractile status of the myocardium in an intact heart as the left ventricular (LV) end-systolic elastance (E_{es}). Myocardial relaxation was described according to the time constant of the LV isovolumic pressure decay (τ_e). Pyridoxamine therapy prevented the age-associated prolongation in LV τ_e and the diminished E_{es} in senescent rats. The drug also attenuated the age-related augmentation in afterload imposed on the heart, as evidenced by the increased τ_w and decreased R_f . We found that the LV τ_e was significantly influenced by both the arterial τ_w and R_f ($\tau_e = 16.3902 + 8.3123 \times R_f - 0.4739 \times \tau_w$; $r = 0.7048$, $P < 0.005$). In the meantime, the LV τ_e and the LV E_{es} showed a significant inverse linear correlation ($\tau_e = 13.9807 - 0.0068 \times E_{es}$; $r = 0.6451$, $P < 0.0005$). All these findings suggested that long-term treatment with pyridoxamine might ameliorate myocardial relaxation rate, at

C.-H. Wang and E.-T. Wu contributed equally to this work.

least partly through its ability to enhance myocardial contractile performance, increase wave transit time and decrease wave reflection factor in aged rats.

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Corresponding author K.-C. Chang: Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan. Email: kcchang1008@ntu.edu.tw

Introduction

Ageing is accompanied by increased cardiovascular stiffness, diastolic dysfunction and an increased risk of heart failure (Lakatta & Levy, 2003*a,b*). Myocardial relaxation is a complex process that depends on the early diastolic release of elastic energy that has accumulated during the systole (Ohte *et al.* 1998). Brutsaert & Sys (1989) demonstrated the existence of load dependence of relaxation in the intact heart as a muscular pump. Load dependence manifests as a slower rate of left ventricular (LV) pressure fall in response to haemodynamic loads imposed on the heart, reflecting a progressive delay in cross-bridge inactivation with greater increases in systolic load (Noble, 1968; Karliner *et al.* 1977; Gaasch *et al.* 1980; Hori *et al.* 1985). Meanwhile, the depressed myocardial performance with less deformation of the cardiac musculoelastic structures at end systole will impair isovolumic relaxation of the left ventricle (Brutsaert & Sys, 1989).

Several molecular mechanisms, including oxidative stress, inflammation and post-translational modifications, have been postulated for mechanical defects in cardiovascular ageing (Bernhard & Laufer, 2008). Among these, an increase in protein modification by advanced glycation end-products (AGEs) has been proposed as the primary basis for ageing-associated arterial stiffening and myocardial dysfunction (Lakatta, 1993). Pyridoxamine (PM), one of the three natural forms of vitamin B₆, is a promising agent that can inhibit the AGE formation with few adverse effects (Booth *et al.* 1997). Our team demonstrated in the past that PM attenuated arterial stiffening by targeting the pathogenic formation of AGE-induced aortic collagen cross-links in aged rats (Wu *et al.* 2011).

In the present study, we performed long-term treatment with PM in senescent animals to address the following questions: (i) whether PM will result in prevention of the mechanical defects in myocardial relaxation and cardiac contractile performance in aged rats; and (ii) whether the age-related deterioration in LV isovolumic relaxation can be attenuated by the LV contractile performance during PM treatment. We measured the intrinsic contractility of the myocardium in an intact heart as the LV end-systolic elastance (E_{es} ; Suga *et al.* 1973; Sagawa, 1981). Myocardial relaxation was described according to the time constant of the LV isovolumic pressure decay (τ_e ; Weiss *et al.* 1976;

Yamakado *et al.* 1997). Given that the systemic vasculature plays a pivotal role in myocardial loading (Hori *et al.* 1985; Gillebert & Lew, 1991), we also determined the effects of arterial wave properties on LV diastolic function in senescent animals administered PM. The arterial wave properties were characterized by wave transit time (τ_w) and wave reflection factor (R_f) using the impulse response of the filtered aortic input impedance spectra (Wu *et al.* 2011).

Methods

Animals and catheterization

Specific pathogen-free male Fisher 344 rats, aged 6 ($n = 9$) and 20 months ($n = 15$), were used to evaluate the effects of PM treatment on mechanical defects in cardiac ageing and to investigate the load dependence of myocardial relaxation in senescent animals. The rats were obtained from the colony maintained in the barrier facilities at the Animal Center of the Medical College at National Taiwan University. All rats were allowed free access to Purina rat chow and water, and were housed two or three per cage in an animal room with a 12 h–12 h light–dark cycle. Periodic checks of the cages and body weights were conducted to ensure appropriate administration of the food. At the age of 15 months, the rats were randomly separated into two groups, control ($n = 8$) and experimental ($n = 7$). The 20-month-old untreated control rats were compared with 6-month-old rats to show the effects of ageing on the mechanical events of the heart. Animals in the experimental group were treated daily with PM (1 g l⁻¹ in drinking water) for 5 months and compared with the age-matched untreated control animals to evaluate the effects of PM treatment on the aged heart. During the experiment, each rat was anaesthetized with sodium pentobarbital (50 mg kg⁻¹, i.p.). At the end of the experiment, animals were killed by inhalation of carbon dioxide while still under general anaesthesia. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use committee (IACUC) of the National Taiwan University College of Medicine and College of Public Health.

The general surgical procedures and measurement of the cardiovascular variables in anaesthetized rats were conducted as described previously (Chang *et al.* 2001;

Wu *et al.* 2011). In brief, animals were anaesthetized with sodium pentobarbital (50 mg kg⁻¹, I.P.), placed on a heating pad, intubated, and ventilated with a rodent respirator (model 131; New England Medical Instruments, Medway, MA, USA). The chest was opened through the second intercostal space on the right side. An electromagnetic flow probe (model 100 series, internal circumference 8 mm; Carolina Medical Electronics, King, NC, USA) was positioned around the ascending aorta to measure the pulsatile aortic flow. A high-fidelity pressure catheter (model SPC 320, size 2 French; Millar Instruments, Houston, TX, USA) was used to measure the pulsatile aortic pressure via the isolated carotid artery of the right side, then advanced into the left ventricle to record the LV pressure wave. The lead II ECG was recorded using a Gould ECG/Biotach amplifier (Cleveland, OH, USA). The selective pressure and flow signals of five to 10 beats were averaged in the time domain, using the peak R wave of the ECG as a fiducial point. The aortic pressure and flow signals were subjected to vascular impedance analysis (Wu *et al.* 2011). A single-beat estimation technique was used to calculate the LV E_{es} without altering LV loads (Takeuchi *et al.* 1991; Chang *et al.* 2001). The averaged LV pressure signal was used to calculate the LV τ_e during the isovolumic relaxation period (Weiss *et al.* 1976; Yamakado *et al.* 1997).

At the end of the experiment, each rat was killed to obtain the LV weight. The ratio of the LV weight to body weight was used as an indicator for the degree of cardiac hypertrophy.

Aortic input impedance spectra

The aortic input impedance spectra (Z_i) were obtained from the ratio of ascending aortic pressure harmonics to the corresponding flow harmonics (Fig. 1A and B) using a standard Fourier series expansion technique (Westerhof *et al.* 1972; Milnor, 1989; Nichols & O'Rourke, 2011). The wave transit time (τ_w) was computed by the impulse response of the filtered Z_i (Fig. 1C). This calculation was accomplished by the inverse transformation of Z_i after multiplication of the first 12 harmonics by a Dolph–Chebyshev weighting function with order 24 (Laxminarayan *et al.* 1978). The long vertical arrow in Fig. 1C shows the discrete reflection peak from the body circulation, and the short arrow indicates the initial peak as a reference. Half of the time difference between the long and short vertical arrows approximates the arterial τ_w in the lower body circulation (Sipkema *et al.* 1980; Latson *et al.* 1987). The time domain reflection factor (R_f) was calculated as the amplitude ratio of backward-to-forward peak pressure waves, using the method proposed by Westerhof *et al.* (1972). Therefore, both wave transit time

and the wave reflection factor characterized the wave reflection as it occurred in the rat vasculature.

Left ventricular end-systolic pressure–stroke volume (P_{es} –SV) relationship

In this study, the LV is considered as an elastic chamber with known volume elastance, E_{es} , which represents the LV end-systolic elastance (Sunagawa *et al.* 1984). The LV pressure and ascending aortic flow signals were recorded to construct the ventricular P_{es} –SV relationship with which to calculate the LV E_{es} . In brief, the LV isovolumic pressure curve from an ejecting beat was estimated using the non-linear least-squares approximation technique proposed by Sunagawa *et al.* (1980), as follows:

$$\hat{P}_{iso}(t) = \frac{1}{2}P_{idmax}[1 - \cos(\omega t + c) + P_d] \quad (1)$$

where $\hat{P}_{iso}(t)$ is the estimated isovolumic pressure curve, P_{idmax} is an estimated peak isovolumic developed pressure, ω is an angular frequency, c is a phase shift angle of the sinusoidal curve, and P_d is the LV end-diastolic pressure. The peak R wave of the ECG is used to identify the LV end-diastolic point. The estimated peak isovolumic pressure (P_{isomax}) is the sum of P_{idmax} and P_d . The value of $\hat{P}_{iso}(t)$ is obtained by fitting the measured LV pressure curve segments from the end-diastolic pressure point to the peak positive rate of change of LV pressure (dP_{LV}/dt) and from the pressure point of the peak negative dP_{LV}/dt to the same level as the end-diastolic pressure of the preceding beat (Takeuchi *et al.* 1991). Figure 2B represents schematically the relationship between the ejection contraction (the continuous green line) and the estimated isovolumic contraction (the dashed pink line) in the pressure–time diagram.

The pressure–ejected volume loop (the continuous green line in Fig. 2C) was obtained from the time integration of the aortic flow and the measured LV pressure. Drawing a tangential line from the estimated P_{isomax} to the right corner of the pressure–ejected volume loop yielded a point referred to as the end-systolic equilibrium point (Barnea & Jaron, 1990). The line connecting the estimated P_{isomax} and the end-systolic equilibrium point was the LV P_{es} –SV relationship (the dotted-dashed red line in Fig. 2C). The slope of this red line represented the LV E_{es} , and the volume axis intercept of this line was the effective LV end-diastolic volume (V_{eed}).

Isovolumic pressure relaxation of the LV

The LV end-diastolic point was identified as the peak of the ECG R wave. Myocardial relaxation was examined by following the time course of the LV isovolumic pressure

Table 1. Effects of ageing and pyridoxamine on body weight, left ventricular weight and haemodynamic parameters in male Fisher 344 rats

Parameter	BW (g)	LVW (mg)	LVW/BW	HR (beats min ⁻¹)	P_m (mmHg)	CO (ml s ⁻¹)	R_f	τ_w (ms)
Age (months)								
6 ($n = 9$)	351.1 ± 7.7	604.4 ± 12.3	1.72 ± 0.03	396.4 ± 8.0	110.8 ± 2.8	1.85 ± 0.17	0.39 ± 0.03	21.3 ± 0.5
20 ($n = 8$)	346.9 ± 8.2	708.6 ± 23.6	2.04 ± 0.03	358.1 ± 11.7	109.4 ± 5.4	1.53 ± 0.12	0.61 ± 0.05	16.8 ± 0.7
20 + PM ($n = 7$)	321.4 ± 8.3	607.1 ± 17.6	1.89 ± 0.03	363.2 ± 7.9	101.9 ± 3.9	1.52 ± 0.16	0.49 ± 0.02	19.9 ± 0.5
<i>P</i> value								
6 versus 20	n.s.	<0.01	<0.01	<0.05	n.s.	n.s.	<0.01	<0.01
20 versus 20 + PM	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.	<0.05	<0.05

All values are expressed as means ± SEM. Abbreviations: BW, body weight; CO, cardiac output; HR, basal heart rate; LVW, left ventricular weight; P_m , mean aortic pressure; PM, pyridoxamine; R_f , wave reflection factor; and τ_w , wave transit time.

decline, as described by Weiss *et al.* (1976). The time course of the LV isovolumic pressure decline was defined by the pressure point of the peak $-dP_{LV}/dt$ to 10 mmHg above the end-diastolic pressure (Fig. 3A). The time constant of the LV pressure decay during the isovolumic relaxation period was calculated using the following equation:

$$\ln P_{LV}(t) = \ln P_{LV}(0) - \frac{t}{\tau_e} \quad (2)$$

where $\ln P_{LV}(0)$ is the pressure intercept at the zero time point, and τ_e is the time constant of the LV isovolumic exponential pressure decline that is the inverse negative slope of the $\ln P_{LV}$ versus t relationship (Fig. 3B). The LV isovolumic pressure decline was assumed to be monoexponential; therefore, the linearity of the $\ln P_{LV}$ versus t relationship was examined, and the LV τ_e

was calculated only when the relationship between $\ln P_{LV}$ and t yielded a high linear correlation coefficient. The linearity of the $\ln P_{LV}$ versus t relationship was reflected in the coefficient of determination (r^2) and the relative standard error of the estimate (SEE) calculated from the linear regression between $\ln P_{LV}$ and t .

Statistical analysis

Results are expressed as means ± SEM. One-way ANOVA was used to determine the statistical significance of the results from multiple comparisons of the effects of ageing and PM on cardiac contractility and LV pressure relaxation. Statistical significance was assumed at the level of $P < 0.05$. If the ANOVA results indicated that

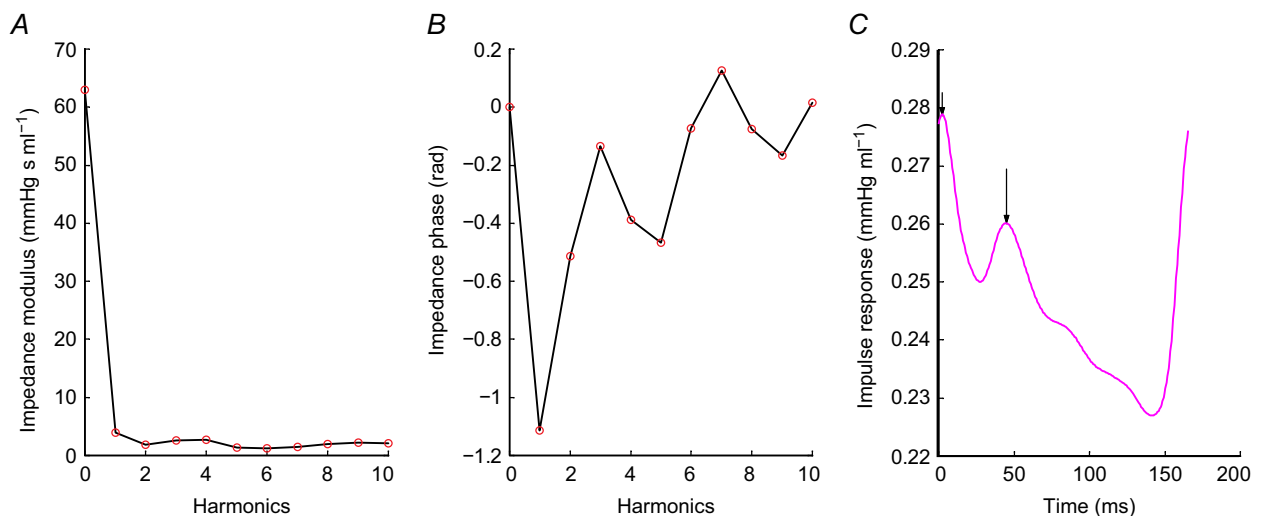


Figure 1. Modulus (A) and phase (B) of the aortic input impedance in a 6-month-old rat and the impulse response function curve (C) derived from the filtered aortic input impedance spectra shown in A and B. In C, the long vertical arrow shows the discrete reflection peak from the body circulation and the short vertical arrow demonstrates the initial peak as a reference. Half of the time difference between the appearance of the reflected peak and the initial peak approximates the arterial wave transit time (τ_w) in the lower body circulation.

a cardiodynamic variable differed significantly in different groups, Tukey's honestly significant difference (HSD) method was used to determine the groups of rats that obtained divergent mean values for that variable.

Results

Table 1 shows the effects of ageing and PM on body weight, LV weight and haemodynamic data in the male Fisher 344 rats. Non-significant changes in body weight were observed in the PM-treated animals. The age-related increase in LV weight was attenuated by PM therapy, which resulted in a decrease in the ratio of LV weight to body weight. Pyridoxamine therapy produced no alteration in the blood glucose level between the 20-month-old control animals ($101.2 \pm 5.8 \text{ mg dl}^{-1}$) and the 20-month-old treated rats ($103.7 \pm 5.3 \text{ mg dl}^{-1}$). The age-induced decline in basal heart rate (HR) was also unchanged in animals administered PM. The mean aortic pressure (P_m) and cardiac output (CO) did not change significantly as animals aged, nor did these haemodynamic data change in response to PM treatment. However, treatment of the old rats with PM produced an increase in arterial τ_w and a decrease in R_f .

Figure 1 exemplifies the aortic input impedance spectra and impulse response function in a single 6-month-old

rat. The aortic impedance modulus fell steeply from a high value at zero frequency to extremely low values at frequencies that fluctuated around the aortic characteristic impedance (Fig. 1A). The aortic impedance phase shown in Fig. 1B indicates the delay between the corresponding pressure and flow components. Figure 1C shows the impulse response function curve derived from the filtered aortic input impedance spectra. Half of the time difference between the long and short arrows approximates the arterial τ_w in the lower body circulation.

The continuous red curve in Fig. 2A and the continuous green curve in Fig. 2B show the measured ascending aortic flow signal and the LV pressure waveform, respectively, of the same 6-month-old rat as in Fig. 1. In Fig. 2B, the dashed pink line represents the isovolumic pressure curve at the end-diastolic volume, which was estimated by fitting a sinusoidal function to the isovolumic portions of the measured LV pressure. The dotted-dashed red line in Fig. 2C indicates the LV P_{es} -SV relationship line, from which we calculated the LV E_{es} .

Figure 3 shows the LV τ_e of the same 6-month-old rat as in Fig. 1. The continuous red line in Fig. 3A represents the measured LV pressure waveform and the dashed green line is its derivative, dP_{LV}/dt , which is shown divided by 50. The LV τ_e is the inverse negative slope of the $\ln P_{LV}$ versus t relationship (Fig. 3B); thus, the LV τ_e represents

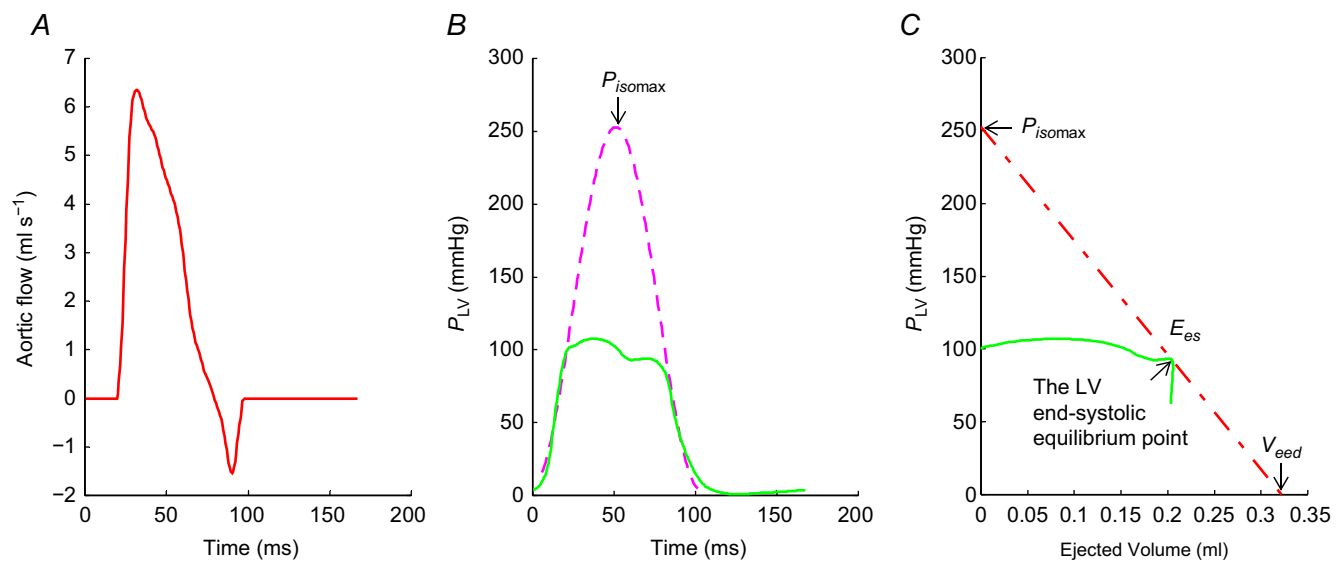


Figure 2. The ascending aortic flow (A) and left ventricular (LV) pressure (B) in the same 6-month-old rat as shown in Fig. 1, and the LV end-systolic pressure–stroke volume (P_{es} -SV) relationships (C)

In B, the dashed pink line represents the isovolumic pressure curve at an end-diastolic volume, which is estimated by fitting a sinusoidal function to the isovolumic portions of the measured LV pressure. In C, drawing a tangential line from the peak LV isovolumic pressure (P_{isomax}) to the right corner of the pressure-ejected volume loop yields a point referred to as the end-systolic equilibrium point. The dotted-dashed red line connecting P_{isomax} to the end-systolic equilibrium point constructs the LV P_{es} -SV relationship, which has a slope of the LV end-systolic elastance (E_{es}) and a volume intercept of effective LV end-diastolic volume (V_{eed}).

the time required for the LV pressure to reduce from a specific pressure to 37% of that pressure. In this case, the LV τ_e was 9.15 ms, with an r^2 of 0.9972 and an SEE of 0.53%.

Figure 4 shows the effects of ageing and PM on the chamber properties of the LV, which were derived from its P_{es} –SV relationship. Ageing had no effects on P_{isomax} (Fig. 4A) but increased V_{eed} (Fig. 4B), causing a significant reduction in LV E_{es} (Fig. 4C). In PM-treated animals, we observed non-significant differences in P_{isomax} between the older animals and their untreated age-matched controls. In contrast, after 5 months of PM treatment, we observed an attenuation of the increase in V_{eed} and a reduction in E_{es} in the hearts of the aged animals.

Figure 5 shows the effects of ageing and PM treatment on the LV isovolumic relaxation, including peak $-dP_{LV}/dt$ and the LV τ_e . Pyridoxamine treatment prevented the significant reduction in peak $-dP_{LV}/dt$ associated with increasing age (Fig. 5A). Treatment of the 20-month-old rats with PM also attenuated the age-associated increase in LV τ_e (Fig. 5B). The linearity of the $\ln P_{LV}$ versus t relationship was reported as an average r^2 of 0.9961 ± 0.0005 and an average SEE of $0.72 \pm 0.07\%$ across all rats studied ($n = 24$).

Figure 6A shows the prediction of LV isovolumic pressure decay from the timing and magnitude of the arterial wave reflection in senescent rats treated with PM. Taking LV τ_e as the dependent variable and arterial R_f and τ_w as the two independent variables,

multiple linear regression was employed to fit the data. The correlation among the three parameters reached significance ($\tau_e = 16.3902 + 8.3123 \times R_f - 0.4739 \times \tau_w$; $r = 0.7048$, $P < 0.005$). Figure 6B shows the inverse relationship between the LV τ_e and the LV E_{es} during the ageing process ($\tau_e = 13.9807 - 0.0068 \times E_{es}$; $r = 0.6451$, $P < 0.0005$). In contrast, the peak $-dP_{LV}/dt$ was not affected by both the arterial wave reflections and the LV contractile performance.

Discussion

To the best of our knowledge, this is the first study to show that PM might improve myocardial relaxation rate from 15 months onward, at least partly through its ability to enhance myocardial contractile performance, increase wave transit time and decrease wave reflection factor in aged rats.

Cardiac intrinsic contractility was evaluated by LV E_{es} because of its independence of the preload, afterload and heart rate in a given constant contractile state of the ventricle (Suga *et al.* 1973; Sagawa, 1981). The LV E_{es} can be determined by the ratio of P_{isomax} to V_{eed} . Because we observed non-significant alterations in P_{isomax} as a function of age, the increased V_{eed} became the predominant factor responsible for the reduced E_{es} in the aged rats (Fig. 4). A decline in P_{isomax} of 20.7% and an increase in V_{eed} of 24.7% indicated that the

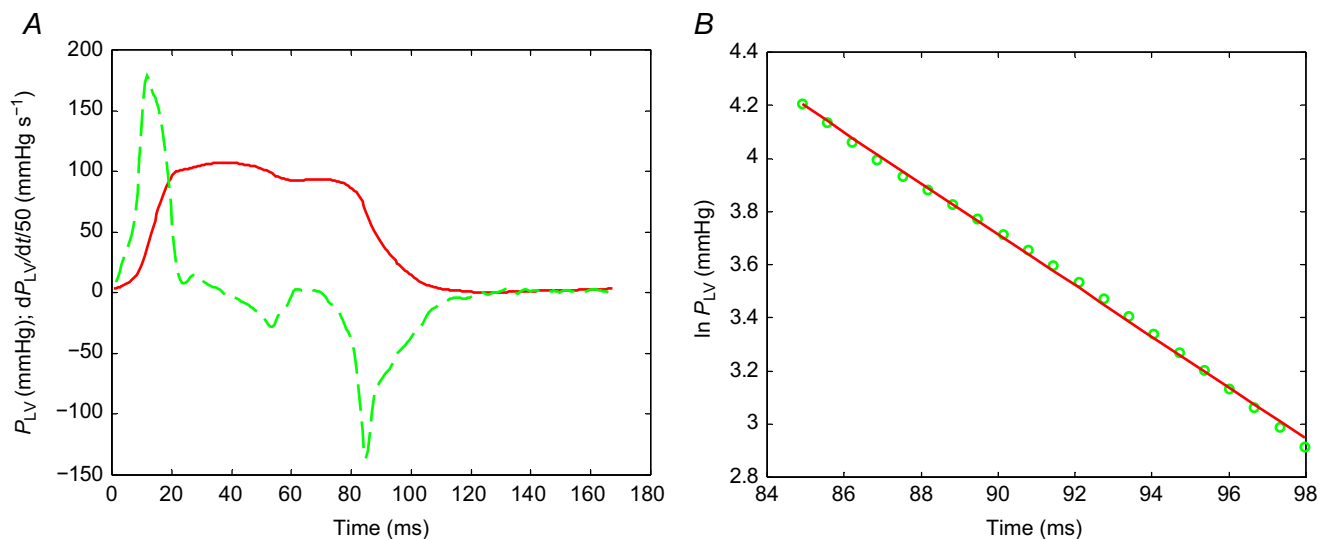


Figure 3. Calculation of the time constant of the LV isovolumic pressure decay.

In A, the continuous red line represents the measured LV pressure waveform in the same 6-month-old rat shown in Fig. 1. The dashed green line is its derivative, dP_{LV}/dt . In B, the time course of the LV isovolumic pressure decline is defined by the pressure point of the peak $-dP_{LV}/dt$ to 10 mmHg above the end-diastolic pressure. The time constant of the LV isovolumic pressure decay (τ_e) was calculated as the negative inverse slope of the $\ln P_{LV}$ versus t relationship. In this case, the LV τ_e was 9.15 ms, with an r^2 of 0.9972 and a relative standard error of the estimate of 0.53%.

aged myocardium is incapable of producing sufficient pressure to support E_{es} . Following exposure to PM for 5 months, the age-associated changes in V_{eed} improved cardiac contractile status, as shown by a 37.7% increase in E_{es} . As for the LV isovolumic relaxation, our study results indicate that ageing is associated with a reduction in peak $-dP_{LV}/dt$ (Fig. 5A) and an increase in LV τ_e (Fig. 5B). A reduction in peak $-dP_{LV}/dt$ indicates slower early pressure relaxation, whereas an increase in LV τ_e indicates slower late pressure relaxation (Brutsaert & Sys, 1989). Treatment of the senescent rats with PM resulted in an improvement in LV isovolumic pressure relaxation, as indicated by an increase in peak $-dP_{LV}/dt$ of 25.1% and a decline in LV τ_e of 14.5%. We show here that PM intervention has the potential to protect the aged hearts from the deterioration

in LV intrinsic contractility and LV isovolumic pressure decay.

Relaxation of the heart is governed by the continuous interplay of the sensitivity of the contractile system to the prevailing load (Brutsaert & Sys, 1989). In the present study, we identified a significant inverse linear correlation between the LV τ_e and the LV E_{es} (Fig. 6B). This result indicated that with advancing age the depression of contractile performance decelerated the LV isovolumic pressure decay. Brutsaert & Sys (1989) suggested that depression of the contractile performance will result in less deformation of musculoelastic structures of the LV wall at end systole. Less systolic deformation of the LV wall will diminish the external restoring force that represents stored potential energy to be released during

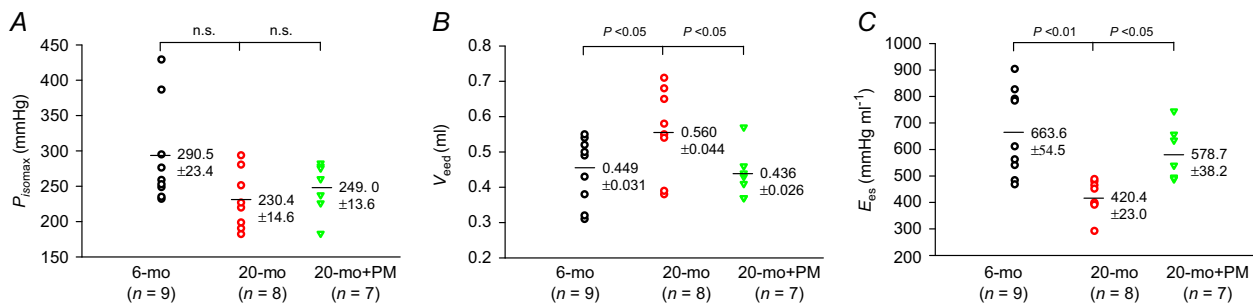


Figure 4. Effects of ageing and pyridoxamine (PM) on peak LV isovolumic pressure (P_{isomax} ; A), effective LV end-diastolic volume (V_{eed} ; B) and LV end-systolic elastance (E_{es} ; C)

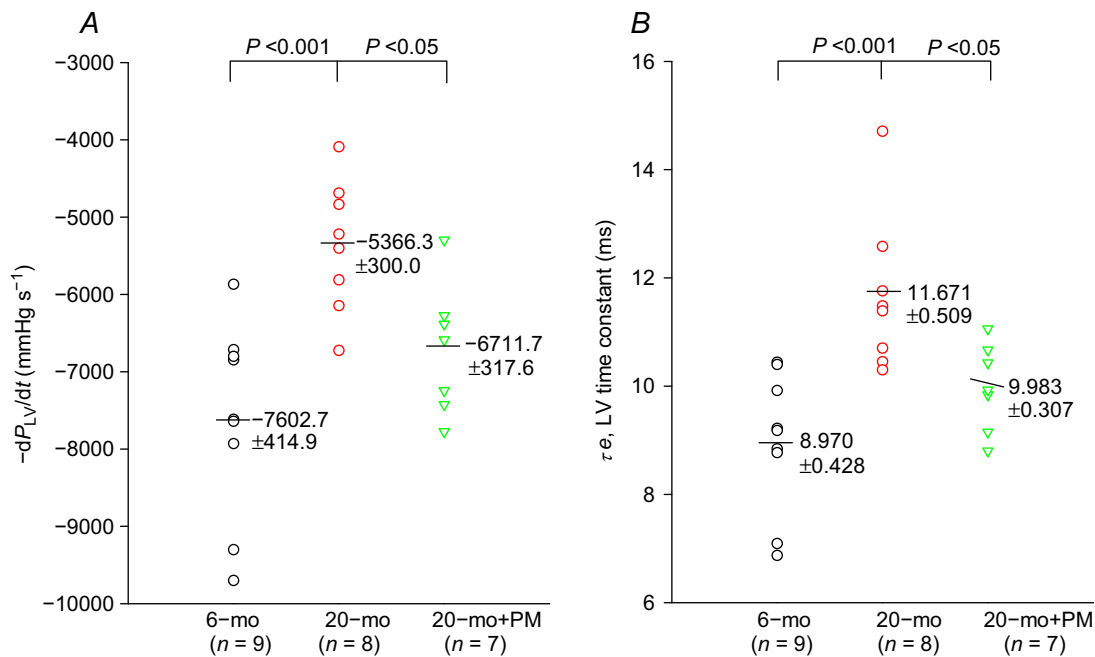


Figure 5. Effects of ageing and PM on peak $-dP_{LV}/dt$ (A) and LV τ_e (B)

relaxation (Rushmer *et al.* 1953). Thus, depression of the contractile performance will lead to a slower rate of LV isovolumic relaxation. After exposure to PM for 5 months, the senescent animals had increased LV E_{es} associated with a reduction in LV τ_e (Fig. 6B). All these findings suggest that PM therapy has the ability to increase myocardial contractile performance of the aged heart, which can enhance the external restoring force and cause recoil of the ventricular wall to acceleration in LV late pressure relaxation.

Another determinant of relaxation in the intact animal is the arterial load imposed on the heart (Brutsaert & Sys, 1989). In the present study, we found that LV τ_e was affected by the timing and magnitude of pulse wave reflection, for arterial τ_w and arterial R_f (Fig. 6A). A reduction in τ_w with age suggested that ageing caused an early return of pulse wave reflection from the peripheral circulation. Ageing also contributed to a significant rise in R_f , augmenting the heavy reflection intensity. As arterial τ_w shortened and arterial R_f was augmented with age, LV τ_e became more prolonged and late pressure relaxation slowed (Fig. 6A). These findings were congruent with the previous finding that ageing substantially influences arterial wave properties to affect LV relaxation detrimentally (O'Rourke, 1990; Murgó &

Westerhof, 1987; Nichols *et al.* 1993; Wu *et al.* 2012). Treatment of the senescent rats with PM for 5 months resulted in an increase in arterial τ_w and a decrease in arterial R_f (Table 1), which was associated with a shorter LV τ_e (Fig. 6A). We show here that PM intervention has the potential to ameliorate arterial wave properties, thereby improving the LV isovolumic pressure relaxation in older animals.

Kangro *et al.* (1997) reported that changes in the elastic properties of the vasculature are associated with corresponding structural changes in the left ventricle. Reduced aortic and arterial distensibility commonly accompany the ageing process (Isnard *et al.* 1989), causing the cardiac muscle cells to adapt to hypertrophy (Safar *et al.* 1987). We found that cardiac hypertrophy occurred in older animals, as manifested by an increased ratio of LV weight to body weight (LVW/BW). A PM-induced decline in LVW/BW indicated that the drug prevented cardiac hypertrophy of the aged rats. Although the cardiac hypertrophy may prolong relaxation with advancing age (Nichols *et al.* 1985), we found no significant linear correlation between the LVW/BW and the LV τ_e in our experimental animals.

Tissue protein modification by AGE plays an important role in the regulation of cardiovascular function in diabetes

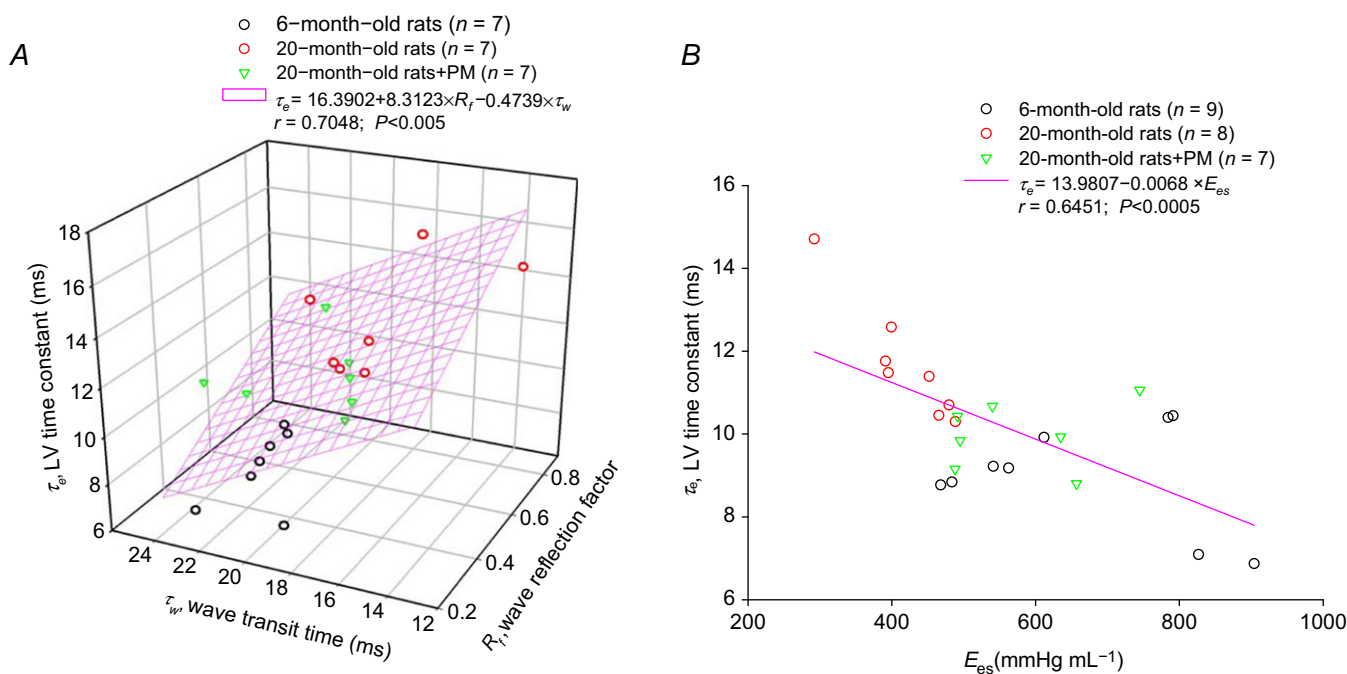


Figure 6. Influences of arterial wave properties and cardiac contractile status on the LV isovolumic pressure relaxation.

In A, a multiple linear regression was employed to fit the data, taking the time constant of the LV isovolumic pressure decay (τ_e) as the dependent variable and the arterial wave transit time (τ_w) and arterial wave reflection factor (R_f) as the two independent variables. The correlation among the three parameters achieved significance. In B, the LV τ_e and the LV end-systolic elastance (E_{es}) showed a significant inverse linear correlation.

and ageing (Avendano *et al.* 1999; Burgess *et al.*, 2001). Bidasee *et al.* (2003, 2004) provided a demonstration that AGE-modified intracellular ryanodine receptors and sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) impair the amplitude of Ca^{2+} transients in the diabetic myocardium. Shao *et al.* (2011) revealed that PM blunts SERCA2a activity loss and attenuates diastolic dysfunction of the diabetic heart. We previously demonstrated a role of PM in improving arterial mechanics by targeting the pathogenic formation of AGE-induced aortic collagen cross-links in old animals (Fig. 3 of Wu *et al.* 2011). Given that ageing is associated with abnormalities in SERCA2a (Cain *et al.* 1998), the effects of PM on SERCA2a expression, phospholamban phosphorylation status and Ca^{2+} regulation in cardiac ageing cells deserve further delineation.

In addition to being an AGE blocker, PM is an inhibitor of a potent blocker of the oxidative degradation of Amadori intermediate (Takatori *et al.* 2004). Pyridoxamine also scavenges a range of toxic carbonyl species derived from glucose and lipid peroxidation (Nagaraj *et al.* 2002; Voziyan *et al.* 2002; Amarnath *et al.* 2004). Thus, PM might act as a protective agent in ageing-induced cardiovascular complications by its ability to decrease oxidative stress. Given that the ability of PM to suppress free radical production in the senescent rats was not investigated, we cannot conclude that the effects of PM observed are due only to inhibition of the AGE formation in the aortas (Wu *et al.* 2011).

Limitations

The estimation of the isovolumic pressure from an ejection beat does have some concerns. The duration of the isovolumic contraction produced by occluding the ascending aorta in diastole is significantly longer than that of the ejecting contraction; therefore, the cardiac cycle length of the estimated isovolumic pressure is shorter than that of the measured isovolumic pressure. However, the predicted peak P_{isomax} shows good correlation with that obtained by aortic occlusion (Sunagawa *et al.* 1980). The advantage of the single-beat estimation technique is that the effects of the specific drug on the LV end-systolic elastance can be measured without altering the ventricular load.

Conclusions

Our study results indicate that the ageing process results in mechanical defects in cardiac contractile performance and myocardial relaxation function in male Fisher 344 rats. Treatment with PM for 5 months ameliorates the contractile dysfunction of the LV in senescent animals, as shown by an increase in E_{es} . Pyridoxamine treatment also

induces an increase in peak $-dP_{\text{LV}}/dt$ and a decrease in LV τ_e , indicating the improvement of early and late pressure relaxation in the aged myocardium. As shown by multiple linear regression analysis, the correlation among the LV τ_e and the arterial τ_w and R_f reached significance. In the meantime, the LV τ_e and the LV E_{es} showed a significant inverse linear correlation. All these findings suggested that PM might protect against the deterioration in arterial wave properties as well as LV contractile performance in older animals, thereby accelerating the isovolumic pressure relaxation of the aged heart.

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Additional information

Competing interests

None declared.

Author contributions

All listed authors (C.-H.W., E.-T.W., M.-S.W., M.-S.T., Y.-H.K., R.-W.C., C.-Y.C. and K.-C.C.) have contributed to the following aspects of the study: conception and design of the experiments; collection, analysis and interpretation of data; and drafting of the article or revising it critically for important intellectual content. All listed authors approved the final version of the manuscript.

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