

Early changes in contractility indices and fibrosis in two minimally invasive congestive heart failure models[☆]

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Abstract

Background: Heart failure is a common and often fatal disease. Numerous animal models are used to study its aetiology, progression and treatment. This article aims to demonstrate two minimally invasive models of congestive heart failure in a rabbit model and a precise method to assess cardiac performance. **Methods:** Fifty New Zealand White rabbits underwent cervicotomy incision and were then divided into three groups. Aortic regurgitation (AR group) was induced in 17 animals by catheter lesion through the right carotid artery, proximal aortic constriction (AC group) was created in 17 animals by metallic clip placement in the ascending aorta through a neck incision, while 16 animals served as controls (CO group). Eight weeks later, myocardial function and contractility indices were assessed by sonomicrometry crystals. Hearts were then collected for morphometric measurements and left ventricular tissues were subjected to immunohistochemical analysis of fibrosis, necrosis and apoptosis. Statistical analysis was by analysis of variance (ANOVA) with a Dunnett's *post hoc* test or by Kruskal–Wallis test with Dunn's *post hoc* test as appropriate, with significance at $p \leq 0.05$. **Results:** The model of aortic regurgitation indicated early stages of heart failure by volume overload with increased end-diastolic and end-systolic volumes, stroke volume, cardiac output and pressure–volume loop areas. The elastance was higher in the control group compared with that in the AC and AR groups (131.00 ± 51.27 vs 88.77 ± 40.11 vs 75.29 ± 50.70 ; $p = 0.01$). The preload recruitable stroke work was higher in the control group compared with that in the AC and AR groups (47.70 ± 14.19 vs 33.87 ± 7.46 vs 38.58 ± 9.45 ; $p = 0.01$). Aortic constriction produced left ventricular concentric hypertrophy. Fibrosis appeared in both heart failure models and was elevated by aortic constriction when compared with that in controls. Necrosis and apoptosis indices were very low in all the groups. Clinical signs of congestive heart failure were not present. **Conclusions:** The two heart failure models we describe were relatively simple to create and maintain, minimally invasive, accurate, inexpensive and, importantly, had a low mortality rate. These models rapidly induced deterioration of contractility indices and onset of fibrosis, the hallmarks of early myocardial dysfunction associated with heart failure. Sonomicrometry assessments were able to detect early contractility changes prior to clinical signs.

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1. Introduction

Congestive heart failure is the main cause of death in Western countries. Although considerable improvements with drug and surgical treatments have been achieved, mortality from heart failure remains high. Numerous animal

models are used to study the aetiology, progression and treatment of heart disease.

Research on congestive heart failure has focussed on identification, quantification and description of tissue lesions; examination and judgement of different therapeutic modalities; and comprehension of the mechanisms of heart failure [1,2]. The development and standardisation of congestive heart failure models have been very useful to this process. Numerous methods of inducing congestive heart failure in animal models have been described previously, such as myocardial infarction [3] and rapid heart pacing [4], although less invasive methods are needed. The most common way to induce congestive heart failure in animal

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models is with pressure overload through transverse aorta constriction (TAC). By comparison, arteriovenous fistula and induction of mitral valve regurgitation are the most common ways to induce congestive heart failure with volume overload [5]. Other investigators have reported a model combining volume and pressure overload in rabbits by creating aortic lesions and regurgitation with aortic constriction. In this combined model, they created aortic constriction through a lateral thoracotomy [6,7], adding surgical stress and perhaps higher mortality rates. Importantly, none of these models accurately or completely reproduces the natural disease progression of congestive heart failure. Each different model has distinct properties, and therefore the investigator has to choose the model that best reproduces the aspect of heart failure under investigation or design a new model.

These models have been characterised by documenting changes in myocardial contractility and fibrosis [8]. Currently, pressure dimension curves and left ventricular contractility indices derived by sonomicrometry are not often incorporated into congestive heart failure models.

This article aims to demonstrate two minimally invasive models capable of detecting early congestive heart failure in a rabbit model. The volume-overload model was created by induction of aortic regurgitation while the pressure-overload model was created by constricting the ascending aorta. The changes in myocardial contractility assessed by sonomicrometry crystals and post-mortem myocardial fibrosis were used as indices of heart failure. We hypothesise that myocardial indices of contractility and histological alterations would precede the clinical signs of congestive heart failure in these experimental models.

2. Material and methods

2.1. Creation of the congestive heart failure model

Fifty male and female New Zealand White rabbits (body weight: 3.0 ± 0.4 kg) were anaesthetised with ketamine (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}) and were placed in the dorsal decubitus position. Cefazolin (50 mg kg^{-1} , intramuscular) was administered to the skin incision as prophylactic antibiotics. Local anaesthesia (2% xylocaine) was administered prior to cervicotomy incision, and animals were then divided into three study groups. In the aortic constriction group (AC, $n = 17$), the ascending aorta was partially constricted proximal to the brachiocephalic trunk with a metallic clip ($4.5 \text{ mm} \times 1.5 \text{ mm}$; Fig. 1). In the aortic regurgitation group (AR, $n = 17$), a 4-mm diameter catheter was placed into the carotid artery and advanced retrograde into the left ventricle (LV) perforating an aortic valve cusp while continuously monitoring the arterial blood pressure until an increase of 50% on wave pulse from initial measurement (systolic pressure–diastolic pressure) was observed (Fig. 1). A sham control group (CO, $n = 16$) was also exposed to identical surgical procedures except that no aortic constriction or aortic valve perforation was performed.

After the surgical procedure, all animals received indomethacin (2 mg kg^{-1}) as an analgesic, and they were kept under close observation until total recovery from

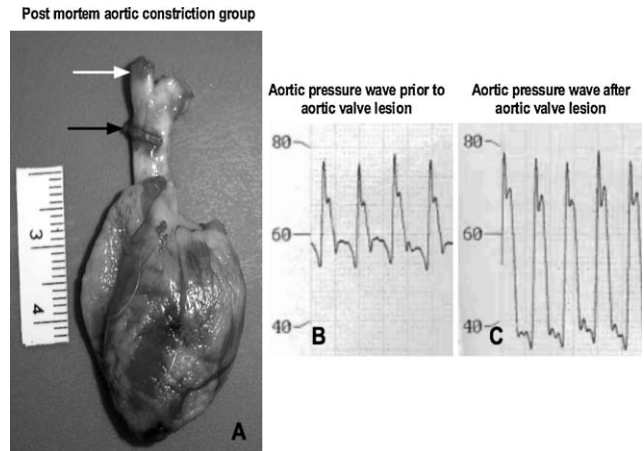


Fig. 1. (A) Photograph of a representative heart in the aortic constriction group showing proper positioning of a metallic clip on the ascending aorta (black arrow), where the clip is positioned proximal to the brachiocephalic trunk artery (white arrow). The metallic clip is 4 mm long and 1.4 mm wide. No animal had metallic clip internalisation. (B) Photograph of a representative aortic pressure waveform tracing before aortic valve lesion. (C) Photograph of a representative aortic pressure waveform showing the 50% increase in wave pulse after successful aortic valve lesion.

anaesthesia, following which the animals were maintained in appropriate cages in a temperature-controlled room and received food and water *ad libitum*. All the animals were examined on a regular basis and the weight, activity and pain were assessed for 8 weeks after the initial procedure. All procedures were performed in accordance with the 'Guiding Principles for Research Involving Animals and Human Beings'. The Committee on Animal Research at our institution approved the protocol.

2.2. Experimental protocol

After 8 weeks, the animals were anaesthetised with ketamine (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}) and were placed in the dorsal decubitus position. Local anaesthesia (2% xylocaine) and fentanyl (0.1 mcg kg^{-1}) were administered prior to skin incision. The animals were ventilated with pressure control and 40% inspired oxygen fraction. A median sternotomy and pericardiotomy were performed to expose the heart. Two sonomicrometry crystals (2 mm, Sonometrics Corp., London, ON, Canada) were placed on the epicardial surface at opposite ends of the short axis of the LV to measure changes in ventricular dimension. One high-fidelity transducer-tipped catheter (2Fr, Millar Instruments, Houston, TX, USA) was inserted into the LV through the apex.

2.3. Data acquisition

The baseline haemodynamic measurements were taken for 5 min. Pressure–volume (PV) loops were generated using the Sonoview software package (Sonometrics Corp., London, ON, Canada) using volume data acquired from sonomicrometry and the pressure catheter. The inferior vena cava was occluded and released to generate descending PV loops. Each occlusion was repeated twice for data acquisition.

2.4. Tissue collection

Immediately after sonomicrometry measurements were completed, the animals were euthanised with lethal doses of pentobarbital. The heart was removed and weighed. The ventricular septum and lateral wall thickness were measured at the level of the papillar muscle. Cross-sectional and longitudinal ventricular specimens were obtained and placed into 10% buffered formalin, embedded in paraffin and cut into 6- μ m sections. All hearts were visually inspected to confirm lesions on the aortic valve in the AR group or for ascending aorta clip position and constriction in the AC group (Fig. 1).

2.5. Necrosis, fibrosis and apoptosis assessment

Multiple sections from each block of the heart were stained with haematoxylin and eosin (H&E) and Masson's trichrome stain. The fibrosis was evaluated by two blinded observers using dedicated image software (Bio ColorScanner 2, Campinas, Brazil). The ratio of fibrosis to total ventricular tissue was assessed and expressed as a percentage. Apoptosis was determined using TdT-mediated dUTP nick-end labelling (TUNEL) technique (In situ Cell Death Detection Kit, La Roche Diagnostics, Mannheim, Germany), counterstaining with bisbenzimidazole (HOE-33342) and phalloidin (both Sigma, Taufkirchen, Germany). A blinded, experienced pathologist (AAS) evaluated total necrosis focus and TUNEL-positive cardiomyocytes nuclei. The necrosis/total tissue ratio was expressed as a percentage. The total number of TUNEL-positive cardiomyocytes nuclei were divided by the total number of cardiomyocytes and expressed as percentage.

2.6. Myocardial function calculations

Systolic, diastolic and overall contractile functions were calculated from PV loops using the Cardiosoft software package (Sonometrics Corp.). End-systolic elastance is an index of systolic contractility calculated from the slope of the regression line that fits the end-systolic points on the PV loops. Diastolic stiffness is the end-diastolic pressure–volume relationship (EDPVR), obtained from the exponential fit of the end-diastolic pressure–volume points on the PV loop. Preload recruitable stroke work (PRSW) is a preload-independent index of contractility. PRSW represents the linear relationship between stroke work and end-diastolic volume. The pressure volume area (PVA) is the sum of stroke work (SW) and elastic potential energy (PE) of the LV. The PVA represents the total mechanical energy generated by ventricular contraction. Mathematically, $PVA = PE + SW$, also $PE = P_{ES} (V_{ES} - V_0)/2 - P_{ED}(V_{ED} - V_0)/4$, where P_{ES} , end-systolic pressure; P_{ED} , end-diastolic pressure; V_{ES} , end-systolic volume; V_{ED} , end-diastolic volume and V_0 , theoretical volume when no pressure is generated. The PVA has a highly linear correlation with myocardium oxygen consumption for each beat. This relationship holds true under a variety of loading and contractile conditions [9].

2.7. Statistical analysis

All data are reported as mean \pm standard deviation and Kolmogorov–Smirnov test for normality was performed.

Analysis of statistical significance was performed using one-way analysis of variance (ANOVA) with Dunnett's *post hoc* test or Kruskal–Wallis test with Dunn's *post hoc* test as appropriate. Significance was accepted at $p < 0.05$. The fibrosis results from both observers were analysed with standard concordance correlation test to compare their assessments (MedCalc for Windows version 10.4.0.0, Belgium). Analyses were performed with statistical package (GraphPad Prism for Mac, version 5, San Diego, CA, USA).

3. Results

3.1. Haemodynamic parameters

After initial pilot studies to establish the model, aortic regurgitation was induced in 17 animals, proximal aortic constriction was induced in 17 animals and 16 animals were in the control group. The surgical mortality was only 5%. The haemodynamic data after 8 weeks of aortic constriction or aortic valve regurgitation are shown in Table 1. There was no difference in segmental shortening fraction, heart rate, stroke work or EDPVR among the three groups. However, 8 weeks after surgery, the AR group had increased end-systolic volume, end-diastolic volume, stroke volume, cardiac output, left ventricular volume at 60 mmHg and 6 mmHg, and PVA when compared with the AC and CO groups during the same time period (Table 1, Fig. 2). No animal had clinical symptoms of congestive heart failure, but after 8 weeks, the elastance and PRSW were lower in the AR and AC groups compared with that in controls (Table 1, Figs. 3 and 4).

3.2. Tissue measurements

In the AC group, the septum and lateral wall thickness was greater and showed uniform concentric hypertrophy 8 weeks after surgery compared to AR and CO groups (Table 1, Fig. 3). The AR group displayed eccentric hypertrophy compared to the AC and CO groups.

3.3. Histological parameters

In general, the incidence of observed necrosis was very low and was comparable in the three groups. However, the percentage of fibrosis was higher in the AC group when compared with that the CO and AR groups (Table 2, Fig. 4). The apoptotic index was very low and was comparable in the three groups (Table 2). The concordance correlation coefficient for fibrosis observations from two blinded observers was 0.96 (95% confidence interval (CI): 0.90–0.98; $p < 0.01$).

4. Discussion

The creation of stable and reliable models of congestive heart failure is necessary for further investigation of drug therapies, development of new surgical techniques and understanding the physiologic alterations under these conditions. Research associated with heart failure has focused on identification, quantification and characterisation of the injured tissue; evaluation of different therapeutic

Table 1
Haemodynamic data 8 weeks after surgery.

	CO group (n = 16)	AC group (n = 17)	AR group (n = 17)	p value
Left ventricle end-systolic pressure (mmHg)	77.06 ± 16.29	81.82 ± 13.97	78.91 ± 11.24	0.67
Left ventricle end-diastolic pressure (mmHg)	7.05 ± 1.79	6.90 ± 2.21	8.73 ± 4.75	0.20
End-diastolic volume (ml)	2.16 ± 0.87	2.22 ± 0.65	3.64 ± 1.59 ^a	0.01
End-systolic volume (ml)	1.50 ± 0.70	1.61 ± 0.60	2.63 ± 1.18 ^a	0.01
Stroke work/beat (erg × 10 ³)	0.23 ± 0.15	0.24 ± 0.10	0.32 ± 0.20	0.20
Heart rate (bpm)	166.53 ± 32.52	167.92 ± 20.35	191.57 ± 35.96	0.11
Cardiac output (ml min ⁻¹)	70.00 ± 40.00	80.00 ± 20.00	150.00 ± 90.00 ^a	0.01
Stroke volume (ml)	0.49 ± 0.16	0.47 ± 0.10	0.78 ± 0.52 ^a	0.01
LV septum thickness (mm)	3.98 ± 1.00	5.21 ± 0.79 ^a	4.66 ± 0.95	0.01
LV lateral wall thickness (mm)	4.83 ± 1.31	5.94 ± 0.82 ^a	5.17 ± 0.71	0.02
PVA (ml mmHg)	63.82 ± 29.76	62.54 ± 14.97	102.87 ± 56.60 ^a	0.01
PRSW (slope of the regression line)	47.70 ± 14.19	38.58 ± 9.45 ^a	33.87 ± 7.46 ^a	0.01
Elastance (slope of the regression line)	131.00 ± 51.27	88.77 ± 40.11 ^a	75.29 ± 50.70 ^a	0.01
V ₀ (volume axis interception)	0.86 ± 0.41	0.83 ± 0.46	1.21 ± 0.56	0.15
EDPVR (slope of the regression line)	0.80 ± 0.55	1.07 ± 1.21	0.79 ± 0.54	0.82
EDPVR (constant)	2.01 ± 1.68	2.65 ± 1.62	2.21 ± 1.60	0.13
Segmental shortening (%)	8.93 ± 2.88	8.34 ± 2.11	8.41 ± 3.06	0.24
TAU (ms)	63.06 ± 17.25	58.05 ± 21.14	58.15 ± 26.27	0.51
dP/dt max (mmHg seg ⁻¹)	1714.66 ± 739.27	1534.68 ± 488.14	1390.12 ± 457.81	0.45
dP/dt min (mmHg seg ⁻¹)	1389.71 ± 714.02	1188.53 ± 458.48	1168.24 ± 285.52	0.85
Left ventricle volume at 6 mmHg (ml)	1.96 ± 0.87	2.09 ± 0.85	3.31 ± 1.44 ^a	0.01
Left ventricle volume at 60 mmHg (ml)	1.92 ± 0.85	2.40 ± 0.93	3.61 ± 1.67 ^a	0.01

Values are mean ± SD.

^a Compared to CO group.

modalities; and understanding the mechanism of heart failure [5]. The purpose of this study was to introduce and describe two new and easily reproducible, minimally invasive techniques for creating a rabbit model of chronic heart failure. In this model, we showed that both the aortic constriction and regurgitation models display early physiologic and histologic hallmarks of chronic heart failure without overt clinical symptoms. Both models of heart failure showed worsening of early myocardium contractility indices, such as

elastance and PRSW. The histological findings also support the contractility indices as demonstrated by an increase of fibrosis with structural fibre derangement. However, the animals did not develop congestive heart failure during the 8-week study period. Animals exposed to constriction of the ascending aorta showed uniform concentric hypertrophy, with increased septal and lateral LV wall thickness compared to controls and those in the AR group. The AR model demonstrated increased cardiac output, stroke volume, PVA, left ventricular volume at 60 mmHg and 6 mmHg, end-systolic and end-diastolic volumes, as compared to the control and aortic constriction groups. These results typify early clinical findings with aortic regurgitation. Lastly, the rabbit models described relatively simple to create and maintain, inexpensive, accurate and, importantly, have a low mortality rate.

Mechanical constriction of the aorta has been used for many years to produce pressure-overload hypertrophy in a number of different species. In general, the extent of hypertrophy and the likelihood of progressing to heart failure increases as the band is placed closer to the heart [10]. There are only a few reports in the literature using ascending aorta constriction, and all of them used large animals such as dogs and lambs [11,12]. Transverse aortic constriction is now done routinely in mice by several groups, but the technical difficulty of that surgical procedure limits availability of this model. In addition to the microsurgical skills required to create aortic constriction in mice, the ability to provide mechanical ventilation when the murine thorax is open, and the requirement for tracheal intubation and low-volume, high-rate mechanical ventilation mandates additional time and expense associated with these procedures.

The surgical instrumentation of rabbit models is considerably easier than rodents because of their larger size, and, conversely, rabbits require considerably less space than larger species such as dogs and lambs. Further, our non-

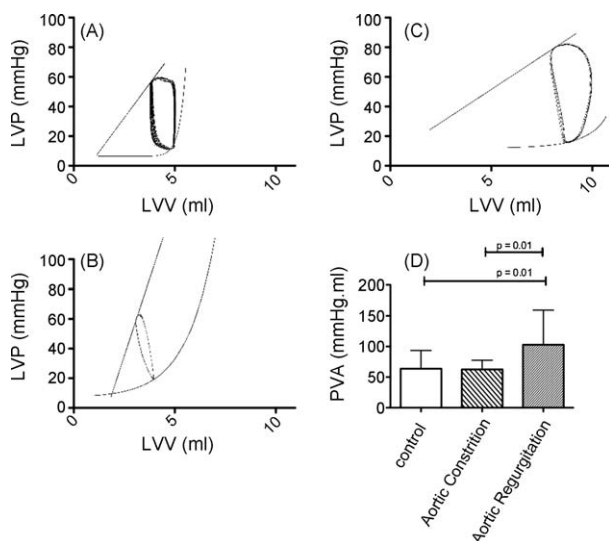


Fig. 2. Representative graphs of left ventricular pressure–volume area (PVA) and pressure–volume (PV) loops from an animal in the control group (A), the aortic constriction group (B), and the aortic regurgitation group (C). PVA is the total mechanical energy generated by ventricular contraction and is the sum of potential energy (PE) and stroke work (SW), which is the area encompassed inside the PV loop. (D) A graph of PVA values (mean ± SD) demonstrating a significant increase in PVA for the aortic regurgitation group.

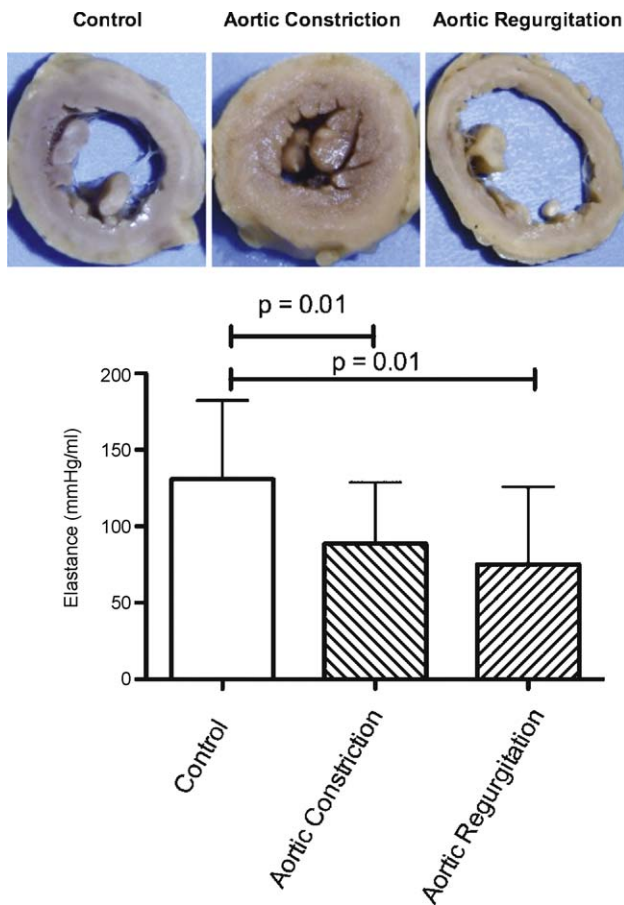


Fig. 3. (A) Representative macroscopic left ventricular slices at papillar muscle level from each group. Note the left ventricular cavity of the control group appears normal, but the aortic constriction group displays concentric hypertrophy, and the aortic regurgitation group with enlargement of ventricle cavity and eccentric hypertrophy. (B) Graph of elastance force for the three groups. Values are mean \pm SD. Note that both heart failure groups had lost significant elastance compared to controls in only 8 weeks.

invasive methods use extrapleural access through the neck in which mechanical ventilation is not necessary to create either ascending aorta constriction leading to pressure-overload hypertrophy or aortic valve lesions leading to aortic regurgitation. Lastly, we experienced a very short learning curve for both methods, requiring only three animals to establish each heart failure model. Thus, the minimally invasive methods we used to create a model of progressive heart failure in rabbits seems useful and practical in many ways and is easily reproducible.

Using echocardiography parameters to evaluate myocardial function, previous studies have shown depression of myocardial function, decreased wall thickness and segmental shortening in animal models of heart failure using either pressure- or volume-overload methods [13]. While echocardiography is less invasive, it requires a crude post-imaging analysis system in order to generate numeric values. Sonomicrometry is an alternate method available for the direct assessment of myocardial function and contractility indices in animal studies, although to our knowledge, there are few published studies employing this technique and none in congestive heart failure models. Sonomicrometer crystal

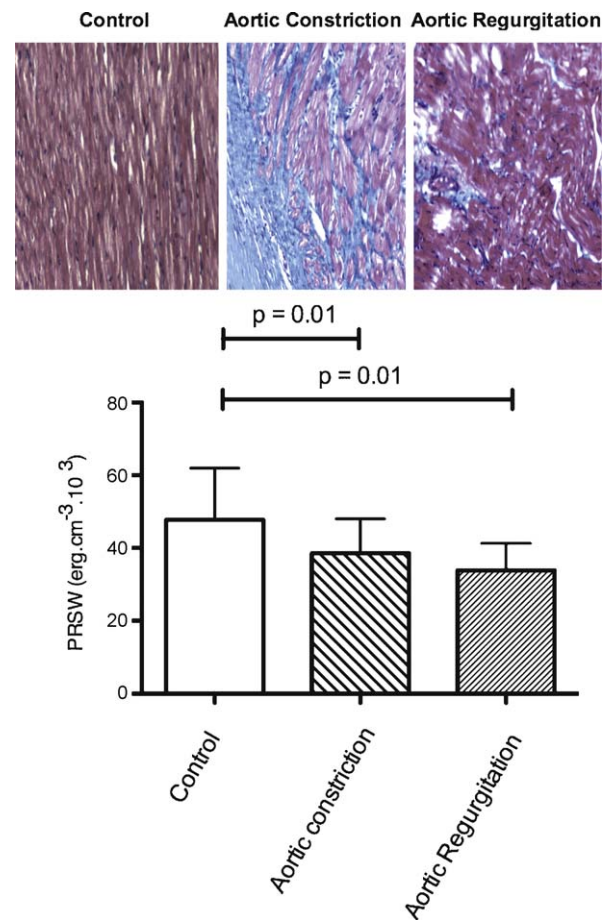


Fig. 4. (A) Representative histological plates from control hearts showing normal distribution of fibers. In contrast the plates from aortic constriction and aortic regurgitation hearts show extensive disarrangement of fibers and the appearance of fibrosis (blue), particularly in the aortic constriction group where the proportion was elevated compared to the aortic regurgitation group or controls. Graph of preload recruitable stroke work (PRSW) for the three groups. Values are mean \pm SD. Note that both heart failure groups had lower PRSW compared to controls in only 8 weeks.

measurements provide an affordable, high-resolution (15 mcm) method of quantifying wall thickness and segmental shortening in real time. Furthermore, no post-imaging analysis is required since measurements are already collected in millimeter distances. More importantly, the same crystals used to make volume measurements provide collateral evaluation of ventricular volume/dimension and pressure–volume area, another important index of cardiac performance and loading. In addition, sonomicrometer crystal measurements can provide contractility indices such as PRSW and elastance. In contrast, these contractility indices are not available with echocardiography which relies instead on dp/dt to assess contractility. Indeed, it has been shown that PRSW and elastance indices have a close relationship to myocyte function [14,15]. A side-by-side assessment of myocardial function and contractility indices using echocardiography and sonomicrometry in the same animals and model would be an interesting comparison and would represent a potential future direction that is possible in our model.

Table 2
Necrosis, fibrosis, and apoptotic index 8 weeks after surgery.

	CO group (n = 16)	AC group (n = 17)	AR group (n = 17)	p value
Necrosis	0.00 ± 0.00	0.15 ± 0.52	0.01 ± 0.02	0.40
Fibrosis	0.39 ± 0.49	5.91 ± 4.54 ^a	2.61 ± 2.57	0.01
Apoptotic index (%)	0.001 ± 0.00	0.001 ± 0.01	0.002 ± 0.01	0.96

Values are mean ± SD. Necrosis and fibrosis are expressed as total tissue ratio percentage. The apoptotic index is calculated by dividing the number of TUNEL-positive cardiomyocyte nuclei by the total number of cardiomyocytes and multiplying that value by 100.

^a Compared to CO group.

The AC group showed a consistent increase in the left ventricular septum and lateral wall thickness compared with that in the AR and control groups. This information concurs with the previous data and is similar to that observed in clinical practice [16]. The AC group maintains LV volume with higher LV septum and lateral wall thickness, indicating concentric hypertrophy. This remodelling does not change cardiac output, stroke work or PVA, and thus, by inference, myocardial oxygen consumption does not change either. This model of aortic constriction also demonstrated a rapid increase in ventricular mass and fibrosis, and these alterations might worsen with an extension of the observation period as was previously described by Hu and colleagues [10]. Diastolic function was unaltered during our study period; however, the systolic function had deteriorated, as indicated by lower elastance and PRSW, indicating that myocardial dysfunction was present. This finding is also consistent with that of Liao and colleagues in a murine model that used transverse aortic constriction and echocardiography evaluation [16].

A previous study by Lygate and colleagues using a rat model of aortic constriction showed that only 60% developed congestive heart failure after surgery. They attributed the 40% failure rate in this model to constriction band internalisation and showed how the original perivascular constricting suture eventually migrated into and resided within the aortic lumen [17]. In our experiments, the aortic constriction was done by placing a metallic clip (4 mm long and 1.4 mm wide) proximal to the brachiocephalic trunk. All animals were inspected after euthanasia to verify for proper clip position and check for aortic clip internalisation. No animal experienced migration or internalisation of the metallic clip. This durability might be due to the metallic clip utilisation itself, since the width of the clip is larger than the stitch routinely employed in constriction aortic models as described by Lygate and colleagues and suggests that using the clips may offer a greater success rate and a more reproducible model. In another rabbit model, Pogwizd and colleagues describe creation of aortic valve lesion followed by aortic constriction 14–28 days later through left thoracotomy after which congestive heart failure developed variably within 3–6 months [6]. Notably they did not describe operative mortality, which might be high with an invasive left thoracotomy. In addition, the period for congestive heart failure development was inconsistent, varying from 3 to 6 months.

Typically, aortic constriction is performed at the transverse aorta, but, in our model, the aorta was constricted at

the ascending aorta with no increase in mortality. Previous reports with aortic proximal constriction were performed in larger animals with similar findings, but we did not find any reports using smaller animals [11,12]. A benefit of our animal model is that it produces a similar pattern of aortic stenosis as is observed in clinical practice, but in smaller animals with lower maintenance costs for the experiment and investigator [18]. Of note, Huang and colleagues used acute ascending aorta occlusion for an ischaemic precondition protocol and reported a mortality rate of about 21% with New Zealand White rabbits. In this study, they applied brief periods of aortic constriction with an open chest surgery and subsequent myocardial ischaemia, but even in the control group (brief period of aortic constriction with no myocardial ischaemia) they reported operative mortality of 23% [19]. This suggests that open chest procedures alone might account for increased mortality compared to that in our minimally invasive model.

Cardiac output was higher in the AR group by 8 weeks after surgery when compared with that in the control and AC groups, although myocardial contractility indices had worsened. In addition, the AR group showed higher volumes at 60 mmHg and 6 mmHg, indicating a higher ventricular capacitance. This suggests that these animals were compensating with increases in end-diastolic and end-systolic ventricular volumes, yet maintained comparable stroke work to the other groups, but with profoundly higher PVA values. Based on this, we can determine that the AR group had a higher potential energy, coupled with higher myocardial oxygen consumption per beat. Essentially, these hearts had become ineffective blood pumps from an energy point of view and demonstrated multiple markers of myocardial dysfunction and progressive heart failure, but had not yet progressed to congestive heart failure [20].

Magid and colleagues, in a similar model of aortic regurgitation with a long follow-up period (2.5 years), concluded that diastolic function decreased after 6 months and then remained stable after that period. Interestingly, the systolic function remained stable during the entire 2.5-year study period; however, the systolic and diastolic functions were assessed with echocardiography [21]. Elastance is a well-established preload-dependent contractility index with good correlation to systolic function, and PRSW is an even better index of myocardial contractility that is both preload and afterload independent [14], but neither of these indices is available with echocardiography. Using sonomicrometry, our data showed a decrease in myocardial contractility, as measured by elastance and PRSW in only 8 weeks, suggesting that this model offers an earlier detection of the development of myocardial dysfunction that occurs with heart failure.

In the AR group, we observed and described an eccentric hypertrophy, as is frequently seen in clinical practice in the early stages of aortic regurgitation patients, where sarcomeres are laid down in series and the myofibres are elongated [22]. Liu and colleagues demonstrated intense myocardial fibrosis, especially on the subendocardium, in a rabbit model of aortic regurgitation after 3 years of follow-up [8]. In our study, we created aortic insufficiency using a method similar to that of Liu and colleagues and we found some degree of fibrosis, but it did not exceed that of controls, most likely due a much shorter period of observation.

In the AR group, we observed similar hypertrophy and fibrosis behaviour seen in patients with chronic aortic regurgitation. Over time, patients with aortic regurgitation have progressive left ventricle dilatation, systolic hypertension, increased wall tension and volume/mass ratio. At this time, the elastance decreased indicating early myocardial dysfunction that is largely masked by increased preload. In decompensated aortic regurgitation, the systolic function is clearly decreased and fibrosis is very evident. Our model translates an early stage of aortic regurgitation, and our fibrosis results are expected and mimic those commonly seen in the clinical scenario. We showed low necrosis incidence in AR group; these results are presumably expected since patients with aortic regurgitation have some degree of necrosis solely at the end stages of valve disease [22].

In the AC group, we also showed an increased fibrosis frequently seen in patients with aortic stenosis. Patients with aortic stenosis develop myocardial hypertrophy that is characterised by an increase in gene expression of collagen I and II and fibronectin, which is associated with the activation of the rennin–angiotensin system [23]. Our study also observed some degree of necrosis in the AC group, but was not significant. Patients with aortic stenosis have reduced coronary flow reserve due prolonged ejection time, ventricular hypertrophy, increased ventricular pressure and decreased diastolic time, and thus myocardial perfusion time. Patients with aortic stenosis can have angina and microscopic focal necrosis without coronary artery disease, particularly in situations of increased oxygen demand [23].

We also observed low apoptotic indices in all the three groups, as would be expected. Several studies had reported low apoptotic indices in human cardiomyocytes in patients with idiopathic, ischaemic and valvular-dilated cardiomyopathy. All these reports used TUNEL technique to detect the apoptotic cells, and the patients were not in decompensated congestive heart failure condition [24]. Aker and colleagues showed an increase in apoptotic rate in a model of congestive heart failure using Chinchilla rabbits [25]. Their model used high-rate LV pacing at 400 beats per minute for 3 weeks, and, at the end of the protocol, the animals were showing clinical signs of decompensated congestive heart failure [25]. The apoptotic signalling pathways in cardiomyocytes is an important factor in the transition from compensated to decompensated heart failure [24]; however, in our study, the animals were in the compensated period.

A limitation of this model as currently described was that myocardial contractility indices beyond 8 weeks were not evaluated to observe more changes that are progressive in induced pressure and volume overload. The model could easily be extended by several months to examine progressive stages of heart failure or be ideally maintained for long periods with chronically instrumented sonomicrometer crystals for serial measurements of cardiac performance. Similarly, extending the model would likely allow for observation of progressing fibrosis, onset of necrosis and onset of apoptosis, indicating a transition from compensated to decompensated heart failure state.

This model is inexpensive, with initial easy instrumentation and, after a short learning curve, has a low mortality rate. The animal size is useful for instrumentation handling,

as well as for maintenance purposes. The sonomicrometry equipment is not widely used and the initial costs for acquisition could be a limitation, but, on the other hand, echocardiography equipment has a higher initial investment than the sonomicrometry system.

The perfect animal model of heart failure does not exist. Acute and chronic heart failure models have been developed to reproduce different aspects of this pathology. The need for new and alternative animal models of heart failure that employ different approaches and methods are necessary because they permit focus upon alternative aspects and periods of progressive heart failure. The researcher has to know and understand the limitations of each model before choosing one that best mimics the desired aspect(s) under investigation.

In summary, our findings have shown these two new rabbit models of chronic heart failure to be reproducible, minimally invasive and an accurate way of assessing contractility indices with very low mortality rate during the protocol. Using sonomicrometry methods to assess myocardial contractility is more direct and possibly more accurate than echocardiography indices. The most important finding is the decrease in myocardial contractility indices after just 8 weeks; however, the animals did not develop or show signs of clinical congestive heart failure. The AC group, with increased fibrosis, developed irreversible lesions faster than the AR group.

These findings support the clinical trend towards earlier valve replacement in aortic regurgitation and or aortic stenosis patients with mild left ventricle dilatation, but absent congestive heart failure. These patients are assessed by echocardiography parameters and the timing for the procedure is based on these parameters. We showed in this model that even with normal cardiac output the contractility parameters can be decreased. Using the current methods, this detriment on contractility parameters is not easily measured in clinical practice.

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