Intraventricular Pressure Gradients in Heart Failure

M. GUERRA[1], C. BRÁS-SILVA[1], M. J. AMORIM[1], C. MOURA[1], P. BASTOS[1], A. F. LEITE-MOREIRA[1]

[1]Department of Physiology and Cardiothoracic Surgery, Cardiovascular R&D Unit, Faculty of Medicine, University of Porto, Porto, Portugal

Summary

The aim of the present study was to characterize intraventricular pressure gradients (IVPGs) in an animal model of chronic heart failure. New Zealand rabbits were treated with doxorubicin (heart failure group, n=5) or saline (control group, n=5) and instrumented with pressure catheters placed in the apex and outflow-tract of left ventricle (LV) and with sonomicrometer crystals placed in the apex and base of the LV free wall. In heart failure animals, ventricular filling was delayed and slower when compared with control animals. Moreover, the physiological nonuniformity observed between apical and basal segments in normal hearts was abolished in failing hearts. Simultaneously, physiological IVPGs observed during normal ventricular filling were entirely lost in heart failure animals. During ventricular emptying physiological nonuniformity between apical and basal segments observed in control animals was also abolished in heart failure animals. In failing hearts minimal length occurred later and almost at same time both in apical and in basal myocardial segments. Simultaneously, the characteristic IVPG pattern observed in healthy hearts during systole, which promotes ventricular emptying, was not observed in failing hearts. The present study showed that diastolic IVPGs, a marker of normal ventricular filling, and systolic IVPGs, a marker of normal ventricular emptying, are abolished in heart failure.

Key words

Intraventricular • Gradients • Heart failure • Diastole • Systole • Myocardial segments

Corresponding author

A. F. Leite-Moreira, Department of Physiology and Cardiothoracic Surgery, Cardiovascular R&D Unit, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal. Fax: 00351225513646. E-mail: amoreira@med.up.pt

Introduction

Courtois *et al.* (1988) described for the first time, in a canine model, intraventricular pressure gradients (IVPGs) during diastole and later showed that these diastolic gradients were diminished by ischemia and related to systolic function (Courtois *et al.* 1990). Recently, we extended those observations also in an animal model. We confirmed not only the existence of diastolic but also systolic IVPGs in the left ventricle (LV) and demonstrated, for the first time, that they are modulated by physiological nonuniformity (Brutsaert *et al.* 1987) between basal and apical myocardial segments (Guerra *et al.* 2013). Moreover, we showed that diastolic and systolic IVPGs, a marker of normal segmental function, can be attenuated, lost entirely or even reversed when ventricular emptying is opposed by afterload elevations or after regional acute ischemia (Guerra *et al.* 2013).

Furthermore, Firstenberg *et al.* (2008) showed that improvements in LV systolic and diastolic function, through surgical myocardial revascularization in patients with ischemic cardiomyopathy, correlate directly with the recovery of diastolic IVPGs. Rovner *et al.* (2005) also demonstrated that diastolic IVPGs augmentation and LV relaxation improvement with exercise are significantly impaired in heart failure patients. However, systolic and diastolic IVPGs were never carefully observed and characterized, namely their magnitude and their behavior in the presence of systolic and/or diastolic dysfunction, in chronic models of heart failure (Stugaard *et al.* 1993, Steine *et al.* 2002).

In fact, the practical value of IVPGs in

understanding ventricular pathophysiology is enhanced by the recognition of its general applicability and quantification not only in acute models, but also in chronic models (De Boeck *et al.* 2005, Yotti *et al.* 2005a,b). Diastolic and systolic IVPGs may reflect intrinsic properties and chronic dysfunction of the LV and not only an acute transvalvular flow disorder (Firstenberg *et al.* 2001, Guerra *et al.* 2011b). In this setting, the present study was conducted to characterize diastolic and systolic IVPGs in an animal model of chronic heart failure, and correlate IVPGs with diastolic and systolic segmental LV dysfunction and nonuniformity.

Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, Revised 1996), and all animals received human care in compliance with the European Convention on Animal Care. Study was also approved by the Ethics Committee of our University.

Chronic heart failure model

A well-documented regimen was used for the induction of heart failure due to doxorubicin toxicity (Arnolda *et al.* 1985, Brás-Silva *et al.* 2008). Adult male New Zealand White rabbits (Oryctolagus cuniculus, 3.0±0.2 kg) received doxorubicin *via* a marginal ear vein by bolus injection (1 mg/kg) twice weekly for 8 weeks (n=5) followed by a washout period of 1 week. Control rabbits (n=5) received the vehicle (0.9 % saline) in equivolumetric doses over the same period. All animals were evaluated by echocardiography at the beginning and every two weeks during the administration of doxorubicin or vehicle. Echocardiographic examination was performed as previously described (Fontes-Sousa *et al.* 2009). Briefly, the rabbits were lightly anesthetized with an intramuscular combination of ketamine hydrochloride (2 mg/kg) and medetomidine hydrochloride (0.15 mg/kg), being allowed to breath spontaneously. The animals were placed prone on a table with an area removed so that the ultrasound probe could be brought from below and placed on a shaved area of the anterior chest wall. The echocardiograms were obtained using a 7.5 MHz transducer (Vivid 3 General Electrics echocardiograph, Portugal) and the examination was performed from the right parasternal short-axis view. Two-dimensional guided M-mode tracings were made just below the mitral valve at the level of the papillary muscles for measurements of the left ventricular internal diameter and the left ventricular wall was free in diastole and systole. Three representative cycles were measured and averaged for each rabbit at each time point. Analyzed parameters were: heart rate, anterior and posterior end-diastolic and end-systolic wall thickness, left ventricular end-systolic and end-diastolic diameters, and fractional shortening.

Experimental preparation
Animal preparation

The instrumentation of the animals for hemodynamic studies was performed one week after the last administration of the vehicle (n=5) or doxorubicin (n=5), respectively, as previously described (Leite-Moreira and Correia-Pinto 2001). In summary, animals were premedicated with ketamine hydrochloride (50 mg kg^{-1}, I.M., Ketalar®, Pfizer Limited) and xylazine hydrochloride (5 mg kg^{-1}, I.M., Rompum®, Bayer). A femoral vein was cannulated, and a intravenous solution containing 20 meq KCl and 40 meq NaHCO$_3$ in 500 ml of 0.9 % NaCl was administrated at a rate of 8 ml kg^{-1} h^{-1} to compensate for perioperative fluid losses. A tracheostomy was performed, and mechanical ventilation was initiated (Harvard Small Animal Ventilator, model 683), delivering oxygen-enriched air. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within physiological limits. General anesthesia was maintained by intravenous infusion of midazolam (0.07 mg kg^{-1} h^{-1}, Midazolam B. Braun), fentanil (0.003 mg kg^{-1} h^{-1}, Fentanil B. Braun) and vecuronium (0.1 mg kg^{-1} h^{-1}, Norcuron®, Organon) to achieve adequate depth of anesthesia for absence of a corneal reflex. A 20 gauge catheter was inserted in the right femoral artery and connected to a pressure transducer to monitor heart rate and arterial pressure and to obtain samples for blood gas analysis. The heart was exposed by a median sternotomy, and the pericardium was opened wide. A limb lead electrocardiogram between the left leg electrode and the right arm electrode (DII) was recorded throughout. Transient aortic constrictions were performed by abruptly occluding the aorta with a silk suture placed around the ascending aorta during the diastole separating two heartbeats. This was achieved by pushing a plastic tube against the aorta with one hand while pulling the silk suture with the other hand. Aortic constriction was quickly released to avoid neurohumoral reflex changes in cardiac function (Correia-Pinto *et al.*

2006). Peak systolic pressure of isovolumetric heartbeats, which can be obtained with aortic occlusions, is a sensitive index of left ventricular contractility.

Pressure measurements

Two 3 French high-fidelity micromanometers (SPR-524; Millar Instruments, Houston, TX, USA) were inserted through an apical puncture wound into the LV cavity. One was pulled carefully back towards the endocardium and secured in place with a purse-string suture to measure apical left ventricular pressure (LVP). The other was introduced until we could see the impact from the aortic valve on the pressure trace. The catheter then was pulled back 3 mm below the aortic valve so that it was located in the LV outflow tract to measure basal LVP. The pressure transducers were calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. The zero was set at the level of the right atrium. Recordings were made with respiration suspended at end expiration. Parameters were converted online to digital data with a sampling frequency of 1 kHz. Left ventricular pressures were measured at end diastole (LVP_{ED}), at pressure nadir (LVP_{min}) and at peak systole (LVP_{max}). Peak rates of LV pressure rise (dP/dt_{max}) and pressure fall (dP/dt_{min}), as well as time to dP/dt_{min}, were also measured. Relaxation rate was estimated with the time constant τ by fitting the isovolumetric pressure fall to a monoexponential function. We recorded the IVPG continuously as apical minus outflow-tract LVP. Peak and average (area divided by duration) IVPGs were calculated during diastolic and systolic phases of the cardiac cycle.

Sonomicrometry

Regional ventricular function was measured with two pairs of ultrasonic segment length gauges implanted in the circumferential direction of apical and basal left ventricular anterior midwall and connected to a sonomicrometer amplifier system (Triton Technology, San Diego, CA, USA). At the end of the experiment, the animal was euthanized by administration of an overdose of anesthetic agents, and the positions of the crystals and micromanometers were verified at postmortem examination. Segment lengths were measured at the end diastole (ED length), at dP/dt_{max} and at mitral valve opening (MVO). Minimal segment length ($Length_{min}$) was measured as the minimal length preceding or

coinciding with peak $-dP/dt_{min}$. Fractional shortening was calculated as the percentage segment length change from end diastole to dP/dt_{min}.

Experimental protocol

After complete instrumentation, we allowed the animal preparation to stabilize for 30 min before the beginning of the experimental protocol, which consisted in measuring apex to outflow-tract pressure gradients and apical and basal myocardial segment lengths in control and heart failure animals during baseline and afterloaded heartbeats. The animals were not paced, but heart rate did not vary significantly during the experimental protocol.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software. Group data are presented as means ± SEM and were compared using two-way ANOVA. Student-Newman-Keuls test was selected to perform pairwise multiple comparisons when significant differences were detected. Statistical significance was assumed at a two tailed value of P<0.05.

Results

Cardiac hemodynamics and echocardiography

The hemodynamic features of the experimental groups are summarized in Table 1. In comparison with the control group, the heart failure group presented a lower systolic pressure, dP/dt_{max}, peak systolic isovolumetric pressure and fractional shortenning. The left ventricular filling pressure, as estimated by left ventricular end-diastolic pressure, was increased in failing hearts, whereas the dP/dt_{min} was decreased and the relaxation time constant τ was increased. Furthermore, the echocardiographic evaluation in the heart failure group demonstrated a progressive increase of the end-diastolic (from 14.2±0.3 to 15.2±0.3 mm) and end-systolic (from 9.9±0.2 to 11.1±0.3 mm) short-axis diameters and a reduction in fractional shortening (from 32±1 to 26±1 %) and ejection fraction (from 64±1 to 56±2 %) of the left ventricle. This was consistent with the presence of dilated cardiomyopathy and heart failure. Moreover, in the present study the animals had clear signs of heart failure such as ruffled hair, labored ventilation, ascites and pleural effusion.

Table 1. Systolic and diastolic parameters in control and heart failure animals.

	Control		Heart failure	
	Apex	**Outflow tract**	**Apex**	**Outflow tract**
LVP_{ED} (mm Hg)	3.3±0.2	3.2±0.3	3.9±0.2$^\alpha$	4.0±0.2$^\alpha$
dP/dt_{max} (mm Hg/s)	3791±667	3669±627	1187±182$^\alpha$	1103±155$^\alpha$
LVP_{max} (mm Hg)	80.7±4.6	80.8±4.7	49.1±5.5$^\alpha$	48.6±5.1$^\alpha$
dP/dt_{min} (mm Hg/s)	-3598±664	-4631±564$^\beta$	-1070±241$^\alpha$	-1098±234$^\alpha$
LVP_{ISO} (mm Hg/s)	148.9±9.2	149.7±9.8	84.6±13.5$^\alpha$	82.6±11.3$^\alpha$
Time to dP/dt_{min} (ms)	140±2	129±1$^\beta$	191±11$^\alpha$	190±9$^\alpha$
LVP_{min} (mm Hg)	2.7±0.6	2.0±1.6	2.9±0.4	3.3±0.5
Time to LVP_{min} (ms)	214±8	231±4$^\beta$	268±9$^\alpha$	272±7$^\alpha$
τ (ms)	15.0±1.3	10.6±0.6$^\beta$	30.4±1.9$^\alpha$	28.4±2.2$^\alpha$
ED length (mm)	6.90±0.33	7.35±0.89	7.33±0.54	7.82±1.22
Length at dP/dt_{max} (mm)	6.97±0.27	7.39±0.67	7.44±0.76	7.98±0.79
$Length_{min}$ (mm)	5.89±0.34	6.28±0.78	6.92±1,38	6.72±1,64
Time to $length_{min}$ (ms)	213±10	170±16$^\beta$	268±86$^\alpha$	291±56$^\alpha$
Length at dP/dt_{min} (mm)	6.20±0.32	6.32±0.42	6.99±1.22	7.12±0.96
Length at MVO (mm)	6.00±0.20	6.22±0.23	6.78±0.98	6.98±1.43
Fractional shortenning (%)	12.7±1.9	14.3±1.1	6.2±2.9$^\alpha$	7.9±1.3$^\alpha$
Heart rate (bpm)	170±11		181±7	

$^\alpha$ $P < 0.05$ versus normal; $^\beta$ $P < 0.05$ versus apex. Abbreviations: LVP_{ED}, end diastolic left ventricular pressure; dP/dt_{max}, peak rate of left ventricular pressure rise; LVP_{max}, peak systolic left ventricular pressure; dP/dt_{min}, peak rate of left ventricular pressure fall; LVP_{ISO}, peak systolic pressure in a isovolumetric beat; LVP_{min}, left ventricular pressure nadir; τ, time constant of isovolumetric relaxation rate; ED length, end diastolic segment length; $Length_{min}$, minimal segment length; MVO, mitral valve opening.

Table 2. Apex-to-outflow tract mean pressure gradients (in mm Hg) throughout the cardiac cycle at baseline and during afterload elevation in control and heart failure animals.

	Diastole			Systole	
	Early diastole	**Ventricular filling**	**Atrial contraction**	**Rapid ejection**	**Slow ejection**
Control					
Baseline	-5.1±0.7	+3.4±0.2	+0.6±0.1	+0.5±0.1	-0.9±0.1
Afterload	-1.5±0.3$^\alpha$	+3.7±0.2	+0.5±0.1	-0.8±0.1$^\alpha$	-0.9±0.1
Heart failure					
Baseline	-0.5±0.1$^\beta$	+0.1±0.1$^\beta$	+0.4±0.1	+1.0±0.2	+0.7±0.2$^\beta$
Afterload	-0.5±0.2$^\beta$	+0.1±0.2$^\beta$	+0.4±0.2	+2.3±0.3$^{\alpha,\beta}$	+1.8±0.3$^{\alpha,\beta}$

$^\alpha$ $P < 0.05$ versus baseline; $^\beta$ $P < 0.05$ versus control.
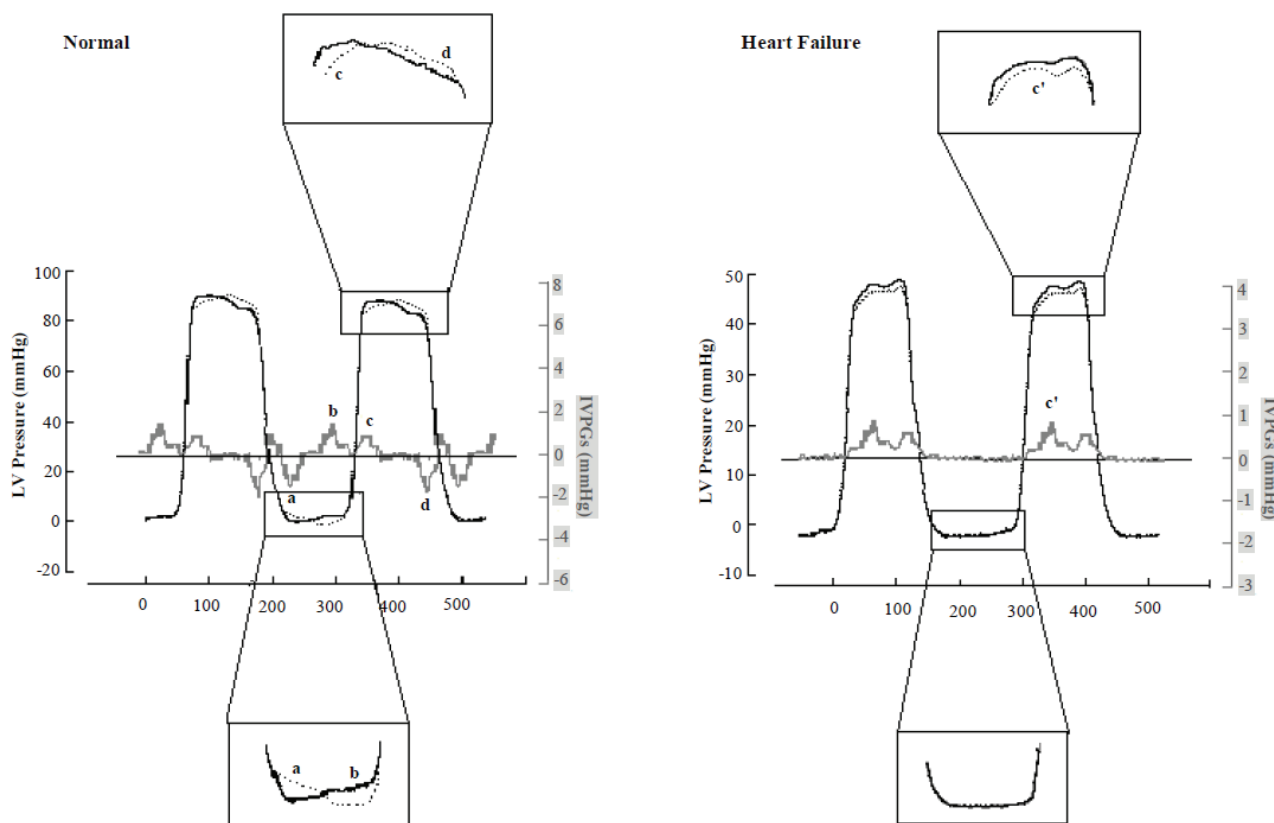
IVPGs changes in heart failure

Figure 1 represents left ventricular apical and outflow tract pressures, as well as, simultaneous recording of the apex-to-outflow tract IVPG along cardiac cycle in two representative examples, one of a normal and one of a heart failure animal. Table 2 summarizes mean IVPGs throughout the cardiac cycle in control and heart failure animals during baseline and afterloaded cardiac beats.

In healthy hearts of control animals, during the rapid phase of ventricular filling, we recorded a −5.1±0.5 mm Hg IVPG from the outflow-tract to the

apex. Later, when apical pressure started to rise, the outflow-tract pressure continued to fall, thus creating a +3.6±0.2 mm Hg apex to outflow-tract pressure gradient. During systole we recorded a biphasic IVPG pattern, manifested as a positive gradient (+0.6±0.1 mm Hg) from apex to outflow-tract during rapid ejection, which was followed by a negative gradient from the outflow-tract to the apex during the slow ejection phase (–0.8±0.1 mm Hg).

Fig. 1. Left ventricular apical (——) and outflow tract (----) pressures and simultaneous recording of apex-to-outflow tract IVPG (gray) along cardiac cycle in a representative normal vs heart failure animal. **Normal:** During diastole we observed a significant outflow tract-to-apex IVPG in the early diastole **(a)** and an apex-to-outflow tract IVPG in the late diastole **(b)**; during early systole we recorded an IVPG from apex-to-outflow **(c)** which inverts during late systole **(d)**. **Heart Failure:** Diastolic IVPGs observed during normal ventricular filling were entirely lost in failing hearts, while during systole we observed only one apex-to-outflow-tract gradient throughout ventricular emptying **(c')**.

In failing ventricles of heart failure animals, diastolic ventricular filling was delayed and slower, as indicated by the increase in times to apical and basal peak dP/dt$_{min}$ and to LVP nadir. Moreover, the nonuniformity (measured as distinct timings at which minimum length was achieved between different ventricular segments during cardiac cycle) observed between apical and basal segments in healthy hearts was abolished in heart failure. In fact, faster outflow-tract relaxation (assessed by dP/dt$_{min}$ and by the time constant τ) observed in healthy hearts was not observed in failing hearts where relaxation occurred at the same moment in the apex and in the outflow-tract (Table 1, Time to LVP$_{min}$). Simultaneously, diastolic IVPGs were entirely lost in heart failure

animals, and no differences were recorded between outflow-tract and apical LV pressures during ventricular filling (Fig. 1, Heart Failure). During ventricular emptying faster contraction of basal in comparison to apical myocardial segments recorded in healthy hearts (assessed by a shorter time to minimal length for a similar myocardial shortening) was also abolished in heart failure animals. In failing hearts minimal length occurred later and almost at same time both in apical and in basal myocardial segments (Table 1, Time to lenght$_{min}$). Simultaneously, the physiological biphasic IVPG pattern observed in healthy hearts during systole was not observed in failing hearts (Fig. 1, Heart Failure). Additionally, the response to afterload elevation was also

distinct in the failing hearts, which may represent a sensitive indicator of LV contractility impairment (Table 2).

Discussion

The present study showed that diastolic IVPGs, a marker of normal ventricular filling, and systolic IVPGs, a marker of normal ventricular emptying, are abolished in failing hearts.

Previously, we demonstrated that diastolic and systolic IVPGs are modulated by physiological nonuniformity between basal and apical myocardial segments and that IVPGs can be attenuated, lost entirely or even reversed when ventricular emptying is opposed by afterload elevations or after regional acute ischemia (Guerra *et al.* 2013). So, we hypothesized that any condition that interferes with the normal segmental function, such as chronic heart failure, might be expected to alter the physiological IVPGs throughout the cardiac cycle. In fact, dilated ventricles are poor suction pumps, aspirating a relatively small volume in early filling and compensating with atrial contraction and a resting tachycardia to maintain cardiac output (Little 2005). Healthy ventricles store greater amounts of elastic energy during systole and are therefore more effective suction pumps during diastole (Gilbert and Glantz 1989). In this context, reduced diastolic IVPGs are the consequence not only of a lower impulse due to depressed elastic recoil, but also of relatively higher deleterious convective forces and impaired ventricular relaxation, which adversely affect LV filling (Nikolic *et al.* 1995).

In our animal model, the progression of cardiac dysfunction was monitored echocardiographically to estimate morphologic and functional alterations during the development of heart failure. Hemodynamic studies performed two weeks after the last administration of doxorubicin and the last echocardiographic evaluation clearly showed the presence of systolic and diastolic dysfunction in heart failure animals. In comparison with the control group, the heart failure group presented significantly lower systolic pressures, dP/dtmax, peak systolic isovolumetric pressures and fractional shortening, consistent with the presence of dilated cardiomyopathy and heart failure. In fact, doxorubicin is an anthracycline widely used in cytostatic treatments which causes a dose-dependent cardiotoxicity and thus has been used to induce heart failure in various animal species (Gomes *et al.* 2012). Doxorubicin-induced cardiomyopathy is characterized by ventricular wall thinning and dilatation, and depressed systolic and diastolic function accompanied by fluid retention and by neurohumoral activation (Chekanov 1999). At the cardiac muscle level, it promotes intrinsic contractile dysfunction and reduced contractile reserve. Furthermore, doxorubicin impairs vascular as well as endocardial endothelial function and induces inflammatory reactions in the heart, leading to thrombosis in the atria and myocarditis (Gaudin *et al.* 1993). Multiple pathways of anthracycline-induced cardiac cellular injury have been proposed such as the release of cardiotoxic substances, which subsequently accumulate in the cardiomyocytes, the generation of free radical, lipid peroxidation, and suppression of DNA, RNA and protein synthesis (Sawyer *et al.* 2010, Adão *et al.* 2013).

In the present study the animals had clear signs of heart failure, which were more compatible with the invasive hemodynamic measurements than with the more modest echocardiographic depression. As echocardiographic recordings were performed every two weeks, the last echocardiographic evaluation was carried out two weeks before the hemodynamic measurements. Given the rapid progression of the disease after six weeks, this might explain the different phenotypes at the two distinct time points. Moreover, echocardiography is not a sensitive method for detecting significant differences of wall thickening in these small hearts.

In heart failure animals, the normal base-to-apex IVPG observed during early diastole was entirely abolished, showing impairment of ventricular filling. Late diastolic apex-to-base IVPG was also lost in failing hearts which may compromise the beginning of next ventricular emptying. In fact, diastolic IVPGs might be causally related to recoil of ventricular walls in conjunction with release of elastic energy stored during the previous systole, which generates suction and contributes to normal filling (Courtois *et al.* 1990). In cardiomyopathic hearts, abnormal diastolic movement of dyskinetic segments during isovolumetric relaxation could impart some motion to blood pooled in the apical and anterior regions of the ventricle and thus contribute to the alteration of the normal diastolic IVPGs (Beppu *et al.* 1988, Delemarre *et al.* 1988). Moreover, some elastic potential energy stored during systole could be prematurely lost during early diastole in failing hearts which may impair ventricular filling and abolish normal early and late diastolic IVPGs (Smiseth *et al.* 1998). Thus, acute or chronic ventricular dysfunction, by

diminishing the amount of functional ventricular myocardium available for contraction and subsequent elastic recoil in one region of the ventricle, should result in loss or diminution of the diastolic IVPG in that region (Guerra *et al.* 2013).

During systole, the normal IVPG pattern observed in healthy hearts, and characterized by an apex to outflow-tract gradient during rapid ejection followed by an outflow-tract to apex gradient during the slow ejection phase, was also lost in failing hearts. In normal hearts, systolic IVPG pattern parallels the physiological aortic-ventricular pressure gradients observed between aorta and the LV with aortic pressure being lower than LV pressure during rapid ejection and higher during the slow ejection phase. In heart failure animals, physiological biphasic IVPGs during systole were not observed, which may impair normal ventricular emptying.

As a potential limitation, we must acknowledge that the present study was performed in anesthetized open-chest and open-pericardium animals with two pressure catheters tied at the level of the LV apex and two myocardial segment length gauges placed in the apex and base of the LV free wall. These manipulations may impair ventricular contractility, as well as, may alter longitudinal function and torsion thereby affecting apex to base gradients. We must however underscore that the catheters and segment length gauges are rather flexible and small, minimizing the negative impact on LV function. Moreover, as both groups (heart failure and control) were submitted to the same surgical manipulation such impact should have been similar, therefore making it acceptable to compare IVPGs in the two groups (Guerra *et al.* 2011a).

In conclusion, regional alterations in IVPGs might be an important factor in the characteristic changes that occur in intraventricular flow in specific cardiac disorders. IVPGs play a physiological role in the healthy heart that is lost in pathological conditions, which may contribute to and represent an early sign of ventricular dysfunction. In this way IVPGs recording and study may give important novel insight into the comprehension of heart failure pathophysiology. Moreover, our findings might be relevant to better understand the pathophysiology of doxorubicin-induced cardiomyopathy so that we can develop efficient protective and/or therapeutic strategies in patients treated with this chemotherapeutic agent.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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