

## **Impaired left ventricular function in the presence of preserved ejection in chronic hypertensive conscious pigs.**

Mario Rienzo, Alain Bizé, Dionyssid Pongas, Stéphanie Michineau, Jonathan Melka, Hon Lai Chan, Lucien Sambin, Jin Bo Su, Jean-Luc Dubois-Randé, Luc Hittinger, et al.

► **To cite this version:**

Mario Rienzo, Alain Bizé, Dionyssid Pongas, Stéphanie Michineau, Jonathan Melka, et al.. Impaired left ventricular function in the presence of preserved ejection in chronic hypertensive conscious pigs.. Basic Research in Cardiology, Springer Verlag, 2012, 107 (6), pp.298. <10.1007/s00395-012-0298-9>. <inserm-00770913>

**HAL Id: inserm-00770913**

**<http://www.hal.inserm.fr/inserm-00770913>**

Submitted on 9 Aug 2013

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Impaired left ventricular function in the presence of preserved ejection in chronic hypertensive conscious pigs

Mario Rienzo<sup>1,2</sup>, Alain Bizé<sup>1,2</sup>, Dionyssi Pongas<sup>1,2,3</sup>, Stéphanie Michineau<sup>1,2</sup>, Jonathan Melka<sup>1,2</sup>, Hon Lai Chan<sup>1,2</sup>, Lucien Sambin<sup>1,2</sup>, Jin Bo Su<sup>1,2</sup>, Jean-Luc Dubois-Randé<sup>1,2,3</sup>, Luc Hittinger<sup>1,2,3</sup>, Alain Berdeaux<sup>1,2,3</sup>, Bijan Ghaleh<sup>1,2\*</sup>

<sup>1</sup> Institut Mondor de Recherche Biomédicale INSERM : U955, Université Paris-Est Créteil Val-de-Marne (UPEC), IFR10, 8 Rue du Général Sarrail, 94010 Créteil, FR

<sup>2</sup> Ecole Nationale Vétérinaire d'Alfort Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est Marne-la-Vallée (UPEMLV), Maisons-Alfort, F-94000, FR

<sup>3</sup> Fédération de Cardiologie Assistance Publique - Hôpitaux de Paris (AP-HP), Hôpital Henri Mondor, Hôpital Albert Chenevier, Université Paris-Est Créteil Val-de-Marne (UPEC), 94010 Créteil, FR

\* Correspondence should be addressed to: Bijan Ghaleh <bijan.ghaleh@inserm.fr >

## Abstract

Systolic function is often evaluated by measuring ejection fraction and its preservation is often assimilated with the lack of impairment of systolic left ventricular (LV) function. Considering the left ventricle as a muscular pump, we explored LV function during chronic hypertension independently from increased afterload conditions. Fourteen conscious and chronically instrumented pigs received continuous infusion of either angiotensin II (n=8) or saline (n=6) during 28 days. Hemodynamic recordings were regularly performed in the presence and 1h after stopping angiotensin II infusion to evaluate intrinsic LV function. Throughout the protocol, mean arterial pressure steadily increased by 55±4 mmHg in angiotensin II-treated animals. There were no significant changes in stroke volume, LV fractional shortening or LV wall thickening, indicating the lack of alterations in LV ejection. In contrast, we observed maladaptive changes with 1) the lack of reduction in isovolumic contraction and relaxation durations with heart rate increases, 2) abnormally blunted isovolumic contraction and relaxation responses to dobutamine and 3) a linear correlation between isovolumic contraction and relaxation durations. None of these changes were observed in saline-infused animals. In conclusion, we provide evidence of impaired LV function with concomitant isovolumic contraction and relaxation abnormalities during chronic hypertension while ejection remains preserved and no sign of heart failure is present. The evaluation under unloaded conditions shows intrinsic LV abnormalities.

**MESH Keywords** Angiotensin II ; Animals ; Diastole ; Female ; Hemodynamics ; Hypertension ; physiopathology ; Hypertrophy, Left Ventricular ; chemically induced ; Myocardial Contraction ; Swine ; Ventricular Function, Left

**Author Keywords** isovolumic contraction ; isovolumic relaxation ; hypertension ; left ventricular function

## Introduction

Systolic function is often evaluated by measuring ejection fraction and its preservation is often interpreted as an absence of impairment of systolic left ventricular (LV) function [15]. Usual description of LV function distinguishes systolic from diastolic alterations based on a classic hemodynamic model of the heart [36]. However it has to be emphasized that the classical so-called diastole and systole are tightly coupled [14, 21] and it is well known that concomitant alterations of systolic and diastolic ventricular function occur during ischemic heart disease [22, 26]. Moreover several human studies have reported some alterations in parameters describing systolic function, *e.g.*, mitral annular DTI velocity [2] or long axis systolic DTI velocity [33, 37] while ejection fraction was preserved. Most of these studies analyzed LV function as a hydraulic input-output system as opposed to a muscular pump perspective [7, 23, 38].

In this setting and considering the heart as a muscular pump, we aimed at investigating LV function during chronic hypertension in a large mammal. Previous large animal hypertensive models used renal wrapping [13, 27] or angiotensin II delivered through implanted osmotic pumps [29, 30]. This hypertension was irreversible and the influence of increased afterload *per se* could not be easily distinguished from intrinsic LV alterations. Moreover there is generally missing information about early phases of LV dysfunction development. Alternatively, we focused on the development of LV dysfunction during chronic hypertension in the absence of any sign of heart failure and investigated LV contraction, relaxation and filling independently of increased afterload condition. In the present study, hypertension was induced by continuously infusing angiotensin II during 4 weeks with external peristaltic pumps in chronically instrumented conscious pigs. During the whole protocol, hemodynamic status was regularly investigated and, at each scheduled recording, infusion of angiotensin II was stopped for an hour in order to allow arterial blood pressure to return to pre-treatment values. This novel approach allowed both the characterization of LV function during hypertension and the evaluation of the intrinsic LV properties under unloaded conditions, *i.e.*, in the absence of angiotensin II infusion.

## Methods

The experiments were conducted in accordance with the regulations concerning the use of animals in research.

### **Surgical instrumentation**

Under anesthesia, fourteen female pigs (20–30 kg) underwent left thoracotomy. Fluid-filled Tygon catheters were implanted in the proximal descending thoracic aorta, in the left atrium and in the pulmonary artery for measurement of arterial blood pressure and angiotensin II infusion. A solid-state pressure transducer (P5A, Konigsberg Instruments, Pasadena, CA) was introduced into LV. A flow probe (Transonic Systems Inc, Ithaca, NY) was implanted around the aortic root. Piezoelectric crystals were implanted 1) on opposing LV anterior and posterior endocardial surfaces to measure LV internal diameter, and 2) on opposing LV endocardial and epicardial anterior free wall surfaces to measure wall thickness. All catheters and wires were exteriorized between the scapulae. Animals were sedated with diazepam (0.2–0.4 mg/kg iv) for postoperative care. Enrofloxacin (2 mg/kg/day, im) and long acting amoxicillin (15 mg/kg every two days, im) were administered during the ten days after surgery. Buprenorphine (0.3 mg sc) was administered for five days after surgery. The position of all catheters and crystals was confirmed at autopsy.

### **Hemodynamic measurements**

All hemodynamic data were recorded (1 kHz), digitized and analyzed using HEM v4.2 software (Notocord Systems, Croissy sur Seine, France). Aortic and left atrial pressures were measured with P23XL pressure transducers (Becton-Dickinson, Franklin Lakes, NJ, USA). Cardiac output was measured using a T206 blood flow-meter (Transonic Systems Inc., Ithaca, NY, USA). Left ventricular pressure was cross-calibrated with the left atrial and aortic pressures. The change in LV pressure over time (dP/dt) was computed from the LV pressure signal. Percentage of wall thickening was defined as end-systolic minus end-diastolic thicknesses divided by end-diastolic thickness × 100. The signal of LV wall thickness and LV internal diameter were suitable in 11 out of the 14 instrumented pigs.

LV end-diastole was defined as the initiation of the upstroke of LV pressure tracing after atrial contraction and indicated by the initial increase in LV dP/dt. Aortic valve opening and closure were identified by crossing LV and aortic pressure waveforms. Furthermore these determinations were confirmed by matching the waveforms with the aortic flow signal. The isovolumic contraction time was defined as the time interval between end-diastole and aortic valve opening. The ejection time was defined as the time interval between aortic valve opening and closure. Mitral valve opening was identified by crossing LV and atrial pressure waveforms. The isovolumic relaxation period was defined as the period elapsed from aortic valve closure and mitral valve opening. The filling period was computed from the end of isovolumic relaxation to end-diastole.

### **Mean ejection LV wall stress**

Cylindrical wall stress was calculated as:  $\text{stress} = 1.36 \times (\text{LVP} \times \text{ID}/2h)$ , where LVP is LV pressure, ID is internal diameter (short axis) and  $h$  is wall thickness. The integral of the systolic wall stress over time, so-called mean ejection wall stress, was calculated during the ejection period.

### **Isovolumic relaxation time constant**

The time constant of isovolumic LV pressure (LVP) decay  $\tau$  was calculated using pressure data points during the isovolumic relaxation period and was computed using the logistic method [25, 31]:

$$\text{LVP} = P_A / (1 + e^{t/\tau}) + P_B$$

where  $P_A$  is an amplitude constant and  $P_B$  is the pressure asymptote. The non-linear least squares problem was solved utilizing the Levenberg-Marquardt algorithm.

### **Protocol**

The experiments were conducted 2–3 weeks after surgery when pigs were healthy and afebrile. All animals were studied in the conscious state staying in a sling and care was taken that at least 2 respiratory cycles were included in each recording. A first set of recordings was performed to obtain measurements at Day 0. Then, responses to dobutamine (10  $\mu\text{g}/\text{kg}/\text{min}$ , 5 min) were evaluated. Thereafter, 8 pigs received a continuous angiotensin II infusion (30 ng/kg/min) for 4 weeks using external peristaltic portable pumps. This dose was chosen on the basis of dose-response curves established in preliminary experiments (angiotensin II, 10 to 100 ng/kg/min iv).

Hemodynamic data was regularly recorded at days 3, 7, 14, 21 and 28. Each recording was performed in the presence and 1h after stopping the angiotensin II infusion in order to minimize the impact of changes in loading conditions, *i.e.*, to evaluate the intrinsic LV contractile properties. Dobutamine infusions were repeated at Day 28 in the absence of angiotensin II.

Each animal served as its own control. In addition, six other pigs received saline infusion and served as matched controls.

### **Histology**

Slices from the left ventricle were fixed in 4% formalin and embedded in paraffin for histology. Five  $\mu\text{m}$  thick paraffin-embedded sections were stained with Sirius red for visualization of collagen fibers. Sections were mounted in Eukitt and examined with a bright field microscope. Interstitial fibrosis was quantified and expressed as a percentage of the field area (10 fields analyzed at X10 magnification).

## Statistical Analysis

All results are means  $\pm$  SEM. Statistical analysis was performed using one-way analyses of variance for repeated measures. When overall differences were detected, individual comparisons were performed by Student's *t*-test for paired observations with Bonferroni's correction. A value of  $P < 0.05$  was considered statistically significant.

## Results

### Hemodynamic

Hemodynamic parameters in angiotensin II- and saline-treated pigs are shown in Tables 1 and 2, respectively. The angiotensin II-treated animals did not develop any clinical signs of heart failure such as dyspnea, oedema or ascitis.

In angiotensin II-treated animals, arterial blood pressure significantly increased during the 4 weeks of angiotensin II infusion and this effect remained stable (Figure 1A), confirming the constant effectiveness of angiotensin II infusion. The rises in systolic, diastolic and mean blood pressures averaged  $61 \pm 3$ ,  $50 \pm 3$  and  $54 \pm 3$  mmHg over the 4 weeks, respectively. At Day 28, systolic, diastolic and mean arterial blood pressures reached  $184 \pm 5$ ,  $134 \pm 3$  and  $158 \pm 5$  mmHg, respectively (Figure 1B).

In order to evaluate the intrinsic LV function independently from an increase in arterial blood pressure, angiotensin II infusion was stopped for 1h during each recording. Values of systolic, diastolic and mean arterial pressure returned to Day 0 levels ( $122 \pm 3$  vs  $120 \pm 3$  mmHg,  $85 \pm 4$  vs  $82 \pm 3$  mmHg and  $105 \pm 3$  vs  $101 \pm 3$  mmHg, respectively) (Figure 1C). Calculation of mean ejection wall stress confirmed that the loading conditions were matched between Day 0 and Day 28 when the infusion of angiotensin II was stopped ( $247 \pm 23$  vs  $270 \pm 39$  g/cm<sup>2</sup>, respectively,  $p = \text{NS}$ ).

Heart rate values measured at Day 28 either with or without angiotensin II were significantly increased vs Day 0 ( $93 \pm 4$  and  $107 \pm 8$  vs  $80 \pm 2$  beats/min, respectively).

None of these effects were observed in saline treated pigs.

### Development of LV hypertrophy

Chronic infusion of angiotensin II induced a progressive and significant increase in LV end diastolic wall thickness (Figure 2A). As compared to age and weight-matched control pigs ( $n=6$ ), LV to body weight ratio was significantly increased in angiotensin II-treated animals (Figure 2B), indicating LV hypertrophy. Histological analysis also showed a clear increase in interstitial fibrosis (Figure 2C). Collagen content evaluated with Sirius red was increased from  $6.2 \pm 0.7$  % to  $10.2 \pm 1.2$  % in saline- vs angiotensin II-treated animals ( $p < 0.05$ ).

### Isovolumic relaxation alterations

We observed abnormal evolution of isovolumic relaxation over the 4-weeks of angiotensin II infusion (Table 3). Along with the increase in heart rate at Day 28, total cycle was significantly reduced by 22% in the absence of angiotensin II. However, isovolumic relaxation time remained paradoxically unchanged between Day 0 and Day 28, *i.e.*, the ratio of isovolumic relaxation to total systolic time was significantly increased by 21%. That is, the time devoted to isovolumic relaxation during each minute increased by 36% ( $8.0 \pm 0.3$  vs  $5.9 \pm 0.1$  s per min at Day 28 vs Day 0, respectively). Similar alterations were observed in the presence of angiotensin II at Day 28 as compared to Day 0. Thus, regardless of the presence or absence of angiotensin II, the relative time of isovolumic relaxation was abnormally prolonged at Day 28. These changes were not observed in control pigs (Table 4).

We further analyzed the isovolumic relaxation period by calculating the isovolumic time constant  $\tau$ . Paradoxically,  $\tau$  remained unchanged at Day 28 both in the presence and absence of angiotensin II as compared to Day 0 ( $17 \pm 1$  and  $18 \pm 1$  vs  $17 \pm 1$  ms), *i.e.*, isovolumic relaxation failed to accelerate with the increase in heart rate.

Importantly as increasing heart rate differently influences the various phases of the cardiac cycle, we further analyzed the hemodynamic recordings at different levels of heart rate measured at Day 0 before any angiotensin II infusion. As shown in Figure 3A, the physiological increase in heart rate in these pigs (before starting angiotensin II infusion,  $n=8$ ) induced significant decreases in isovolumic relaxation time, as opposed to the lack of decrease after 4 weeks of angiotensin II infusion.

Concerning dobutamine infusion, at Day 0, isovolumic relaxation time decreased by 18% with the normal increase in triple product of heart rate, LV  $dP/dt_{\text{max}}$  and maximal LV pressure. Interestingly, this response was significantly blunted after 4 weeks of angiotensin II

infusion (-11%) (Figure 3B). Saline-treated animals exhibited similar response between Day 0 and Day 28 (-16% and -17%, respectively).

### **Preserved ejection**

During the 4 weeks of chronic angiotensin II infusion, stroke volume, LV fractional shortening and LV wall thickening were similar both in the presence and absence of angiotensin II as compared to Day 0 (Figure 4). Concomitantly and due to the rise in heart rate, cardiac output increased at Day 28 as compared to Day 0 ( $3.5\pm 0.4$  L/min and  $3.3\pm 0.5$  L/min with or without angiotensin II at Day 28, respectively, vs  $2.7\pm 0.3$  L/min at Day 0,  $p < 0.05$ ).

### **Altered isovolumic contraction**

We next analyzed isovolumic contraction to determine whether it was altered along with isovolumic relaxation. We first focused on time intervals (Table 3). Despite significant decrease in cycle time, absolute values of isovolumic contraction time remained paradoxically unchanged at Day 28 in the absence of angiotensin II as compared to Day 0, *i.e.*, it was significantly increased by 30% when related cycle time, respectively. Thus, the time devoted to isovolumic contraction during each minute was significantly increased by 38% ( $5.9\pm 0.3$  vs  $4.3\pm 0.2$  s per min at Day 28 and Day 0, respectively). These changes were not observed in control pigs (Table 4).

At Day 0 (before starting angiotensin II infusion), these parameters were also analyzed at different levels of heart rate. Isovolumic contraction time decreased when heart rate was rising (Figure 5A). This parameter (expressed as % of cycle) remained unchanged ( $8\pm 1$ ,  $8\pm 1$  and  $8\pm 1$  % of cycle for heart rates at  $80\pm 2$ ,  $3\pm 1$  and  $102\pm 1$  beats/min, respectively). This contrasts with the changes of these parameters observed after 4 weeks of angiotensin II infusion.

Finally, we investigated isovolumic contraction using dobutamine infusion. At Day 0 (Figure 5B), isovolumic contraction time decreased by 45% while the triple product of heart rate, LV  $dP/dt_{max}$  and maximal LV pressure was increased. After 4 weeks of angiotensin II infusion (in the absence of angiotensin II), this response was significantly blunted (-27%). This shows a maladaptive response to inotropic stimulation with a lack of reduction in isovolumic contraction time. Saline-treated animals exhibited similar response between Day 0 and Day 28 (-42% and -44%, respectively).

### **Diastolic filling alterations**

In parallel to the maladaptive response of isovolumic contraction and relaxation, the absolute diastolic filling time was significantly reduced (Table 3). Saline-treated pigs showed similar values between Day 0 and Day 28 (Table 4).

### **Relationship between isovolumic contraction and relaxation**

As illustrated in Figure 6A, there was a linear relationship between the isovolumic relaxation time values (% of cycle time) and corresponding times of isovolumic contraction (% of cycle time) either in the presence or the absence of angiotensin II. Values were measured at Days 0, 3, 7, 14, 21 and 28. This indicates that the development of alterations in isovolumic relaxation mirrored those of isovolumic contraction (Figure 6B). The timing of alterations did not differ between contraction and relaxation. For example, isovolumic contraction time and isovolumic relaxation time (expressed as % of cycle) were both increased up to  $8.9\pm 0.6\%$  and  $12.7\pm 0.4\%$  at Day 14, from  $7.2\pm 0.3\%$  and  $9.8\pm 0.1\%$  at Day 0, respectively.

## **Discussion**

The present study demonstrates impaired left ventricular function in the presence of a preserved ejection in chronic hypertensive pigs as demonstrated by maladaptive changes in isovolumic contraction and relaxation, regardless of changes in loading conditions. These two periods were tightly coupled and simultaneously altered. Indeed, we observed 1) a paradoxical increase in relative durations of isovolumic contraction and relaxation concomitant with the increase in heart rate over the 4 weeks of angiotensin II infusion, 2) both abnormal isovolumic contraction and relaxation responses to dobutamine and 3) a linear correlation between isovolumic contraction and relaxation durations. The link between contraction and relaxation has been previously described in intact animal and human studies investigating the effects of acute load changes, ischemic heart disease, pulmonary hypertension or systemic chronic hypertension [5, 6, 8, 18, 19, 20, 21, 37]. However, they did not distinguish between load influences and intrinsic LV changes nor analyze the consequences of stress conditions such as dobutamine.

In this study, we investigated our animals during chronic hypertension and did not observe any of the usual signs of heart failure. As previously described [9, 11, 17], LV hypertrophy correlates with impaired LV isovolumic relaxation and delayed mitral valve opening. In this pig model, isovolumic relaxation time remained unchanged despite the increase in heart rate observed over the 4 weeks of angiotensin II infusion. This was accompanied by a reduced diastolic filling time. We also evaluated LV pressure decay during isovolumic relaxation by a mathematical model based on a logistic equation, yielding a constant  $\tau$  which shows a stable estimate of relaxation [25, 31]. At Day 28, despite the increase in heart rate,  $\tau$  was abnormally unchanged as compared to Day 0. This contrasts with the normal heart which

accelerates LV relaxation at higher rates as shown by our results obtained at Day 0 at various levels of heart rate and by previous reports [4]. We further investigated isovolumic relaxation during dobutamine infusion and demonstrated again the inability of the heart to adequately adapt to stress.

We next focused on stroke volume, fractional shortening and LV wall systolic thickening. All these parameters were unchanged and cardiac output was not reduced, indicating preserved ejection. However, none of these parameters specifically investigate the isovolumic phase. We therefore turned our attention towards the isovolumic contraction phase searching for alterations independent from ejection. In contrast to the normal heart, isovolumic contraction time remained unchanged. The ratios of isovolumic contraction to total systolic time was paradoxically and significantly increased after 4 weeks of angiotensin II infusion, *i.e.*, the systolic isovolumic contraction failed to accelerate with the increase in heart rate. One could argue that increasing heart rate would differently influence the various phases of the cardiac cycle and therefore explain our results [16]. This is unlikely as the analysis of these time intervals with different levels of heart rate in basal conditions (at Day 0 before starting angiotensin II infusion) clearly shows opposite pattern. Moreover in the presence of dobutamine, the isovolumic contraction time failed to adequately decrease. Again, this contrasts with the normal heart in which there is a linear relationship between this pre-ejection period and R-R interval [34].

In the present study, changes in the two LV isovolumic phases were simultaneous and tightly coupled. There was a linear correlation between isovolumic contraction and relaxation under normal and stress conditions with dobutamine. In agreement with the concept that the heart is a muscular pump rather than a hydraulic pump [3], there was symmetry between isovolumic contraction and relaxation as illustrated in Figure 6B. One could speculate that increased fibrosis and remodeling [1, 24] could participate to these alterations but further investigations are needed.

Several limitations of the present study should be stressed. First, we did not specifically investigate the cellular mechanisms explaining the alteration in isovolumic contraction. Abnormalities in calcium handling and cell shortening may occur [10, 12, 28, 32]. Second, we did not perform echocardiographic studies to measure myocardial deformation and to evaluate left ventricular asynchrony. One could speculate that inappropriate prolonged isovolumic contraction is linked to increased systolic asynchrony with nonuniform activation. This would induce exacerbated ventricular nonuniformity and diastolic asynchrony with altered isovolumic relaxation. Furthermore, isovolumic contraction and cross-bridge detachment during relaxation are energy-consuming processes. In this regard, increased isovolumic time and slow LV relaxation may further jeopardize LV function during the development of the disease and the reduction of myocardial energy reserve. This pig model of hypertension does not induce heart failure *per se* but we believe that it constitutes the basis for future studies and the development of new pharmacological strategies. Finally, it remains unknown whether our results are limited to our experimental setting or rather could be generalized. The evaluation of isovolumic contraction should be re-visited in other animal models and/or humans. The use of pressure volume catheters may have missed subtle alterations in isovolumic contraction as the parameters usually investigated with PV loop do not specifically explore this phase and studies in patients with conductance catheter report the use of low frequency of acquisition (250 Hz) which may reduce the sensitivity of the detection [35].

In conclusion, we provide evidence of impaired LV function with isovolumic contraction and relaxation abnormalities during chronic hypertension while ejection remains preserved and no sign of heart failure is present. The evaluation under unloaded conditions shows intrinsic LV abnormalities.

## Acknowledgements:

This work was supported by grants from INSERM (Mario Rienzo, poste d'accueil 2008), the Société Française d'Hypertension Artérielle (2010), the Fondation de l'Avenir (ET9-529) and the Région Ile de France (CODDIM). We thank the Laboratoire Roche for providing diazepam (Valium®) and Sanofi-Aventis for providing heparin.

## References:

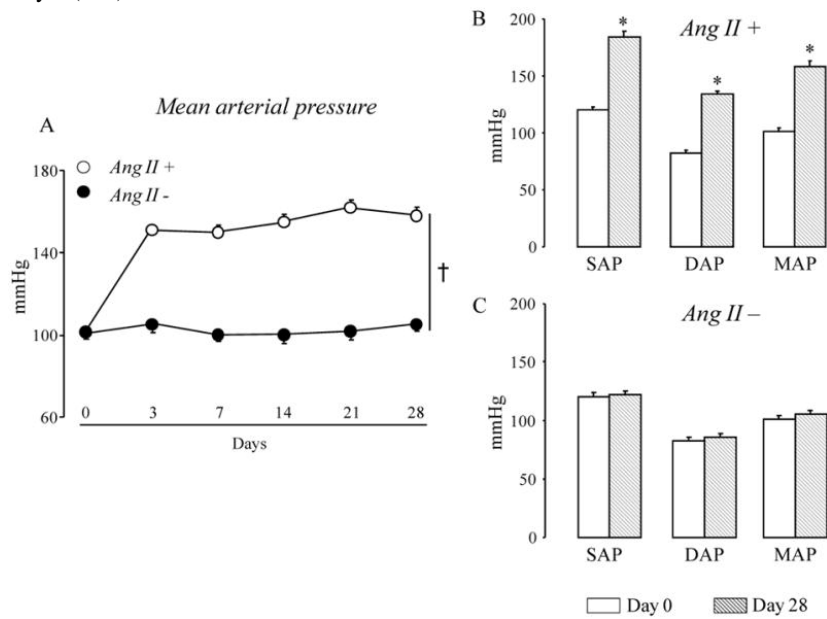
1. Booyens HL, Norton GR, Opie LH, Woodiwiss AJ. 2012; Reverse chamber remodelling following adrenergic-induced advanced cardiac dilatation and pump dysfunction. *Basic Res Cardiol*. 107 : 238 - 10.1007/s00395-011-0238-0
2. Bruch C, Schmermund A, Marin D, Katz M, Bartel T, Schaar J, Erbel R. 2000; Tei-index in patients with mild-to-moderate congestive heart failure. *Eur Heart J*. 21 : 1888 - 95 10.1053/euhj.2000.2246
3. Brutsaert DL. 2006; Cardiac dysfunction in heart failure: the cardiologist's love affair with time. *Prog Cardiovasc Dis*. 49 : 157 - 81 10.1016/j.pcad.2006.08.010
4. Colin P, Ghaleh B, Hittinger L, Monnet X, Slama M, Giudicelli JF, Berdeaux A. 2002; Differential effects of heart rate reduction and  $\beta$ -blockade on left ventricular relaxation during exercise. *Am J Physiol*. 282 : H672 - H679 10.1152/ajpheart.00547.2001
5. Correia-Pinto J, Henriques-Coelho T, Roncon-Albuquerque R, Lourenço AP, Melo-Rocha G, Vasques-Nóvoa F, Gillebert TC, Leite-Moreira AF. 2009; Time course and mechanisms of left ventricular systolic and diastolic dysfunction in monocrotaline-induced pulmonary hypertension. *Basic Res Cardiol*. 104 : 535 - 46 10.1007/s00395-009-0017-3
6. D'Angelo R, Shah N, Rubler S. 1975; Diastolic time intervals in ischemic and hypertensive heart disease: A comparison of isovolumic relaxation time and rapid filling time with systolic time intervals. *Chest*. 68 : 56 - 61
7. De Keulenaer GW, Brutsaert DL. 2007; Diastolic heart failure: a separate disease or selection bias?. *Prog Cardiovasc Dis*. 49 : 275 - 83 10.1016/j.pcad.2006.08.002
8. Dodek A, Burg JR, Kloster FE. 1975; Systolic time intervals in chronic hypertension: Alterations and response to treatment. *Chest*. 68 : 51 - 55 10.1378/chest.68.1.51
9. Douglas PS, Berko B, Lesh M, Reichek N. 1989; Alterations in diastolic function in response to progressive left ventricular hypertrophy. *J Am Coll Cardiol*. 13 : 461 - 7 10.1016/0735-1097(89)90527-5

- 10 . Elliott EB , Hasumi H , Otani N , Matsuda T , Matsuda R , Kaneko N , Smith GL , Loughrey CM . 2011 ; K201 (JTV-519) alters the spatiotemporal properties of diastolic Ca<sup>2+</sup> release and the associated diastolic contraction during  $\beta$ -adrenergic stimulation in rat ventricular cardiomyocytes . *Basic Res Cardiol* . 106 : 1009 - 22 10.1007/s00395-011-0218-4
- 11 . Falcão-Pires I , Palladini G , Gonçalves N , van der Velden J , Moreira-Gonçalves D , Miranda-Silva D , Salinaro F , Paulus WJ , Niessen HW , Perlini S , Leite-Moreira AF . 2011 ; Distinct mechanisms for diastolic dysfunction in diabetes mellitus and chronic pressure-overload . *Basic Res Cardiol* . 106 : 801 - 14 10.1007/s00395-011-0184-x
- 12 . Florea S , Anjak A , Cai WF , Qian J , Vafiadaki E , Figueria S , Haghighi K , Rubinstein J , Lorenz J , Kranias EG . 2012 ; Constitutive phosphorylation of inhibitor-1 at Ser67 and Thr75 depresses calcium cycling in cardiomyocytes and leads to remodeling upon aging . *Basic Res Cardiol* . 107 : 279 - 10.1007/s00395-012-0279-z
- 13 . Gelpi RJ , Pasipoularides A , Lader AS , Patrick TA , Chase N , Hittinger L , Shannon RP , Bishop SP , Vatner SF . 1991 ; Changes in diastolic cardiac function in developing and stable perinephritic hypertension in conscious dogs . *Circ Res* . 68 : 555 - 67 10.1161/01.RES.68.2.555
- 14 . Gillebert TC , Leite-Moreira AF , De Hert SG . 1997 ; Relaxation–Systolic Pressure Relation: A Load-Independent Assessment of Left Ventricular Contractility . *Circulation* . 95 : 745 - 52 10.1161/01.CIR.95.3.745
- 15 . Heusch G . 2009 ; Diastolic heart failure: a misNomer . *Basic Res Cardiol* . 104 : 465 - 467 10.1007/s00395-009-0025-3
- 16 . Heusch G . 2011 ; Heart rate and heart failure – not a simple relationship . *Circ J* . 75 : 229 - 36 10.1253/circj.CJ-10-0925
- 17 . Inouye I , Massie B , Loge D , Topic N , Silverstein D , Simpson P , Tubau J . 1984 ; Abnormal left ventricular filling: an early finding in mild to moderate systemic hypertension . *Am J Cardiol* . 53 : 120 - 6 10.106/0002-9149(84)90695-7
- 18 . Ito T , Hamada M , Shigematsu Y , Kazatani Y , Nishitani K , Matsuzaki K , Daimon F , Ochi T , Kokubu T . 1985 ; The analysis of systolic and diastolic time intervals: a more sensitive non-invasive method in the assessment of left ventricular dysfunction in the patients with essential hypertension . *Clin Exp Hypertens* . 7 : 951 - 63
- 19 . Lavine SJ , Lavine JA . 2006 ; The effect of acute hypertension on left ventricular diastolic pressures in a canine model of left ventricular dysfunction with a preserved ejection fraction and elevated left ventricular filling pressures . *J Am Soc Echocardiogr* . 19 : 1350 - 8 10.1016/j.echo.2006.05.008
- 20 . Leite-Moreira AF , Correia-Pinto J , Gillebert TC . 1999 ; Afterload induced changes in myocardial relaxation: a mechanism for diastolic dysfunction . *Cardiovasc Res* . 43 : 344 - 53 10.1016/S0008-6363(99)00099-1
- 21 . Leite-Moreira AF , Gillebert TC . 1994 ; Nonuniform course of left ventricular pressure fall and its regulation by load and contractile state . *Circulation* . 90 : 2481 - 91 10.1161/01.CIR.90.5.2481
- 22 . Leite-Moreira AF , Lourenço AP , Roncon-Albuquerque R , Henriques-Coelho T , Amorim MJ , Almeida J , Pinho P , Gillebert TC . 2012 ; Diastolic tolerance to systolic pressures closely reflects systolic performance in patients with coronary heart disease . *Basic Res Cardiol* . 107 : 251 - 10.1007/s00395-012-0251-y
- 23 . Little WC . 2005 ; Diastolic dysfunction beyond distensibility: adverse effects of ventricular dilatation . *Circulation* . 112 : 2888 - 90 10.1161/CIRCULATIONAHA.105.578161
- 24 . MacIver DH . 2010 ; Is remodeling the dominant compensatory mechanism in both chronic heart failure with preserved and reduced left ventricular ejection fraction? . *Basic Res Cardiol* . 105 : 227 - 234 10.1007/s00395-009-0063-x
- 25 . Matsubara H , Araki J , Takaki M , Nakagawa ST , Suga H . 1995 ; Logistic characterization of left ventricular isovolumic pressure-time curve . *Jpn J Physiol* . 45 : 535 - 52 10.2170/jjphysiol.45.535
- 26 . Mogelvang R , Goetze JP , Pedersen SA , Olsen NT , Marott JL , Schnohr P , Sogaard P , Jensen JS . 2009 ; Preclinical systolic and diastolic dysfunction assessed by tissue Doppler imaging is associated with elevated plasma pro-B-type natriuretic peptide concentrations . *J Card Fail* . 15 : 489 - 95 10.1016/j.cardfail.2009.09.005
- 27 . Munagala VK , Hart CY , Burnett JC Jr , Meyer DM , Redfield MM . 2005 ; Ventricular structure and function in aged dogs with renal hypertension: a model of experimental diastolic heart failure . *Circulation* . 111 : 1128 - 35 10.1161/01.CIR.0000157183.21404.63
- 28 . Neumann T , Ravens U , Heusch G . 1998 ; Characterization of excitation–contraction coupling in conscious dogs with pacing-induced heart failure . *Cardiovasc Res* . 37 : 456 - 66 10.1016/S0008-6363(97)00246-0
- 29 . Paolucci N , Tavazzi B , Biondi R , Gluzband YA , Amorini AM , Tocchetti CG , Hejazi M , Caturegli PM , Kajstura J , Lazzarino G , Kass DA . 2006 ; Metalloproteinase inhibitor counters high-energy phosphate depletion and AMP deaminase activity enhancing ventricular diastolic compliance in subacute heart failure . *J Pharmacol Exp Ther* . 317 : 506 - 13 10.1124/jpet.105.099168
- 30 . Senzaki H , Gluzban YA , Pak PH , Crow MT , Janicki JS , Kass DA . 1998 ; Synergistic exacerbation of diastolic stiffness from short-term tachycardia-induced cardiodepression and angiotensin II . *Circ Res* . 82 : 503 - 512 10.1161/01.RES.82.4.503
- 31 . Senzaki H , Kass DA . 2010 ; Analysis of isovolumic relaxation in failing hearts by monoexponential time constants overestimates lusitropic change and load dependence: mechanisms and advantages of alternative logistic fit . *Circ Heart Fail* . 3 : 268 - 76 10.1161/CIRCHEARTFAILURE.109.865592
- 32 . Song LS , Pi YQ , Kim SJ , Yatani A , Guatimosim S , Kudej RK , Zhang Q , Cheng H , Hittinger L , Ghaleh B , Vatner DE , Lederer WJ , Vatner SF . 2005 ; Paradoxical cellular Ca<sup>2+</sup> signaling in severe but compensated canine left ventricular hypertrophy . *Circ Res* . 97 : 457 - 464 10.1161/01.RES.0000179722.79295.d4
- 33 . Tan YT , Wenzelburger F , Lee E , Heatlie G , Leyva F , Patel K , Frenneaux M , Sanderson JE . 2009 ; The pathophysiology of heart failure with normal ejection fraction: exercise echocardiography reveals complex abnormalities of both systolic and diastolic ventricular function involving torsion, untwist, and longitudinal motion . *J Am Coll Cardiol* . 54 : 36 - 46 10.1016/j.jacc.2009.03.037
- 34 . Van Der Hoeven GM , Clerens PJ , Donders JJ , Beneken JE , Vonk JT . 1977 ; A study of systolic time intervals during uninterrupted exercise . *Br Heart J* . 39 : 242 - 54 10.1136/hrt.39.3.242
- 35 . Westermann D , Kasner M , Steendijk P , Spillmann F , Riad A , Weitmann K , Hoffmann W , Poller W , Pauschinger M , Schultheiss HP , Tschöpe C . 2008 ; Role of left ventricular stiffness in heart failure with normal ejection fraction . *Circulation* . 117 : 2051 - 2060 Epub 2008 Apr 14 10.1161/CIRCULATIONAHA.107.716886
- 36 . Wiggers CJ . 1942 ; Basic Hemodynamic Principles Essential to Interpretation of Cardiovascular Disorders . *Bull NY Acad Med* . 18 : 3 - 17
- 37 . Yip GW , Zhang Y , Tan PY , Wang M , Ho PY , Brodin LA , Sanderson JE . 2002 ; Left ventricular long-axis changes in early diastole and systole: impact of systolic function on diastole . *Clin Sci (Lond)* . 102 : 515 - 22 10.1042/CS20010196
- 38 . Zile MR , Brutsaert DL . 2002 ; New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function . *Circulation* . 105 : 1387 - 93 10.1161/hc1102.105289

**Figure 1**

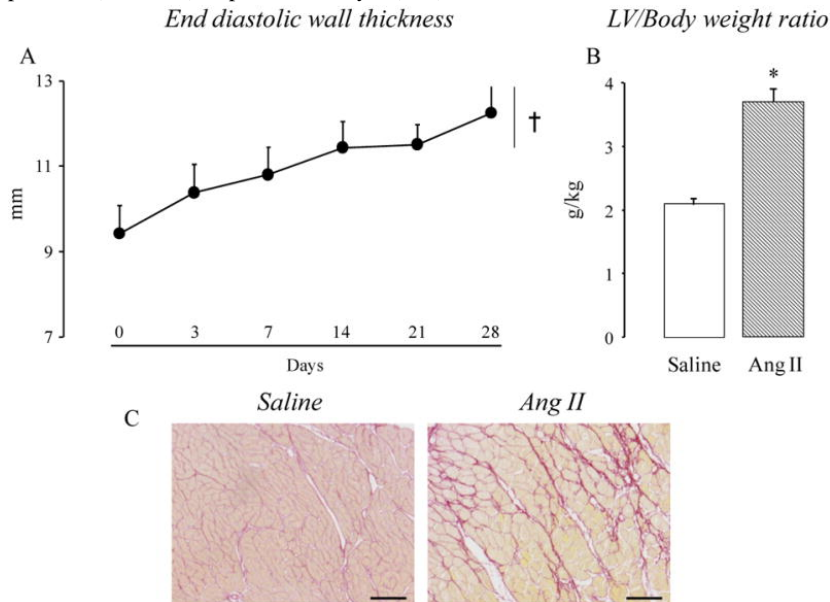
Arterial blood pressure in angiotensin II-treated animals

A) mean arterial pressure measured at Days 0, 3, 7, 14, 21 and 28 of the protocol, in the presence (open circle) and absence (closed circle) of angiotensin II; B) systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressures measured at Day 0 (open bars) and Day 28 (grey bars) in the presence of angiotensin II; C) systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressures measured in the absence of angiotensin II, at Day 0 (open bars) and Day 28 (grey bars). †,  $p < 0.05$ , time effect over the 4 weeks of the protocol (ANOVA). \*,  $p < 0.05$  vs Day 0 (n=8).

**Figure 2**

Myocardial hypertrophy in angiotensin II-treated animals

A) end diastolic wall thickness measured at Days 0, 3, 7, 14, 21 and 28 of the protocol, in the absence of angiotensin II; B) LV to body weight ratio in saline (open bar, n=6) and in angiotensin II-treated (grey bar, n=8) animals. C) Representative staining of collagen (Sirius red staining) fiber network in the left ventricle of saline- and angiotensin II-treated pigs. Scale bars =100  $\mu$ m. †,  $p < 0.05$ , time effect over the 4 weeks of the protocol (ANOVA). \*,  $p < 0.05$  vs Day 0 (n=8).



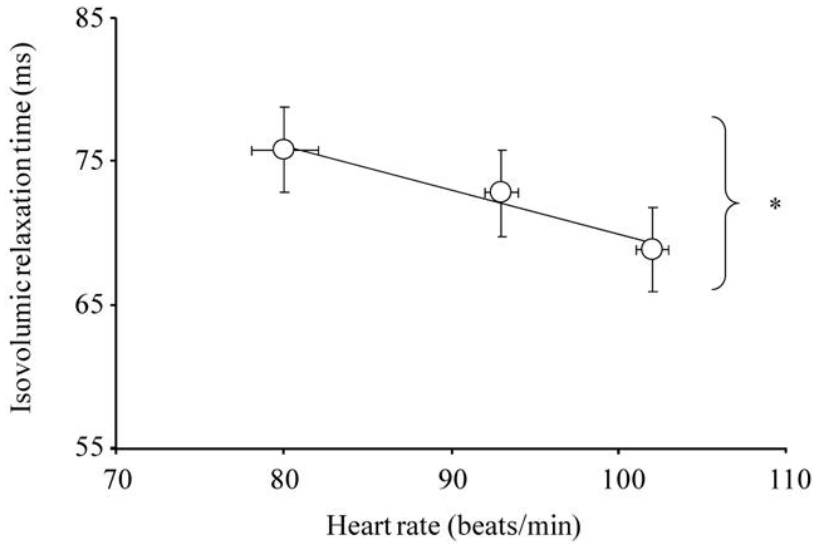


**Figure 3**

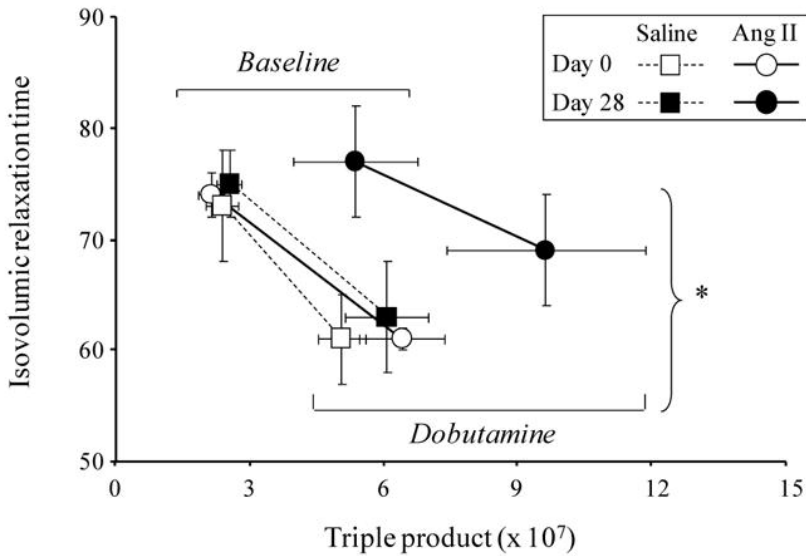
Isovolumic relaxation in saline- and angiotensin-II treated animals

A) Relationship between heart rate and isovolumic relaxation time measured at Day 0 before starting angiotensin II infusion (n=8); \*, p<0.05 by ANOVA; B) relationship between isovolumic relaxation time and the triple product (heart rate x LV dP/dt<sub>max</sub> x LV pressure) in response to dobutamine (10 µg/kg/min, n=6) evaluated at Day 0 (open circles and open squares for angiotensin II- and saline-treated animals, respectively) and Day 28 (closed circles and closed squares for angiotensin II- and saline-treated animals, respectively). Measurements were performed in the absence of infusion. \*: change in isovolumic relaxation time was significantly reduced for angiotensin II-treated animals at Day 28 vs Day 0 (p<0.05) while absolute variations in triple product were similar.

A



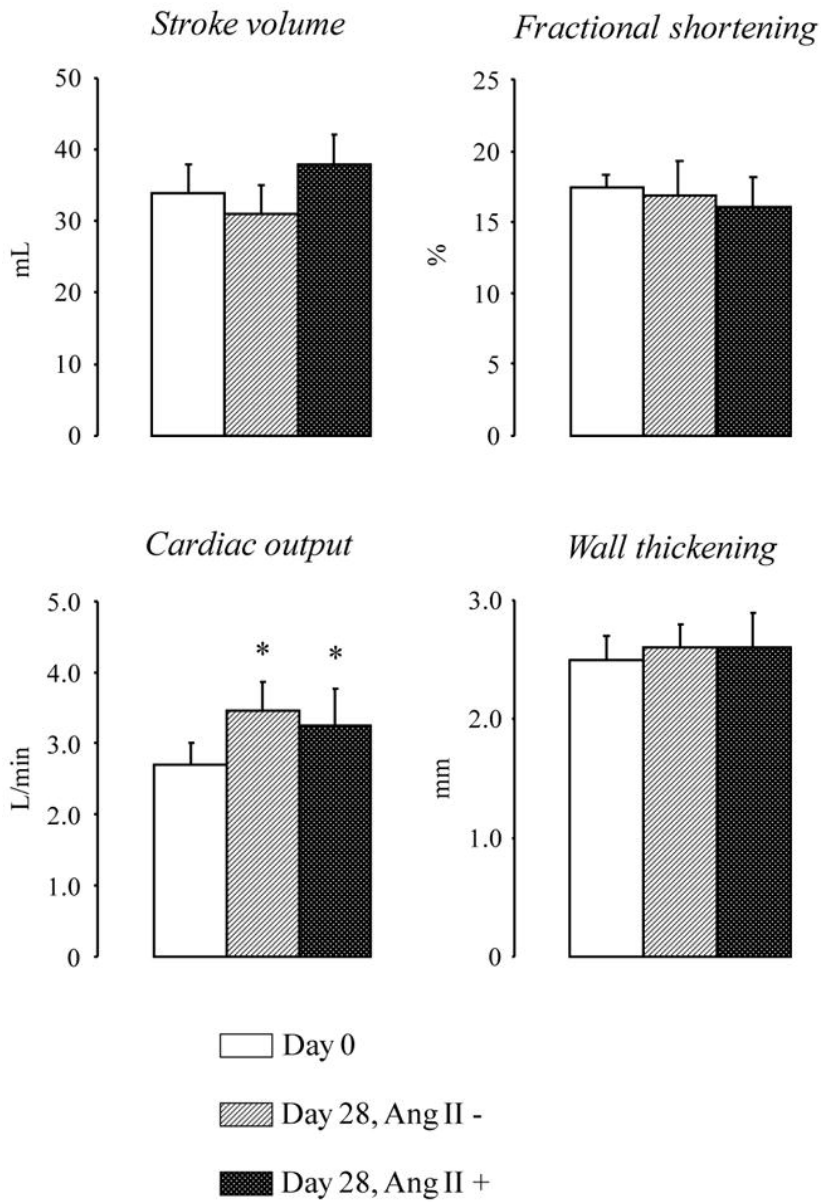
B



**Figure 4**

Ejection in angiotensin II-treated animals

Parameters were evaluated at Day 0 (open bars) and Day 28 (grey bars). A) stroke volume (ml); B) left ventricular fractional shortening (%), C) cardiac output (L/min), D) wall thickening (mm). Values were measured in the presence and the absence of angiotensin II (Ang II). \*,  $p < 0.05$  vs Day 0 (n=8).

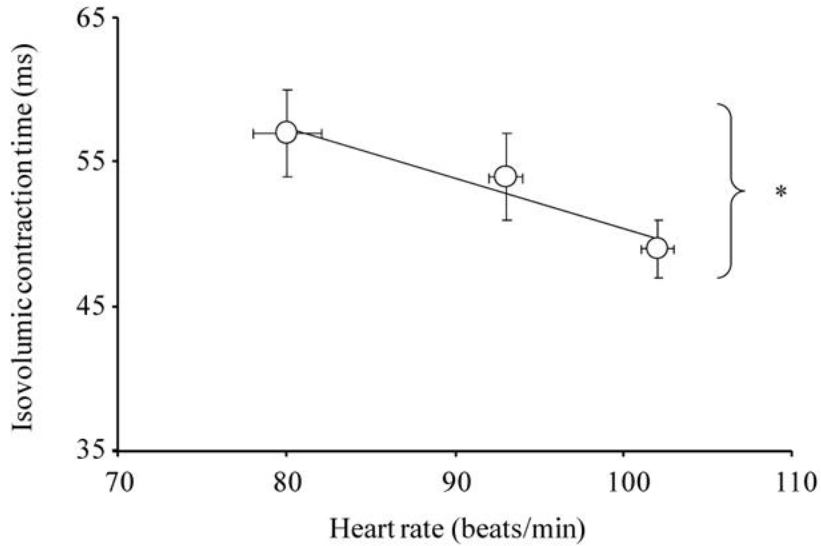


**Figure 5**

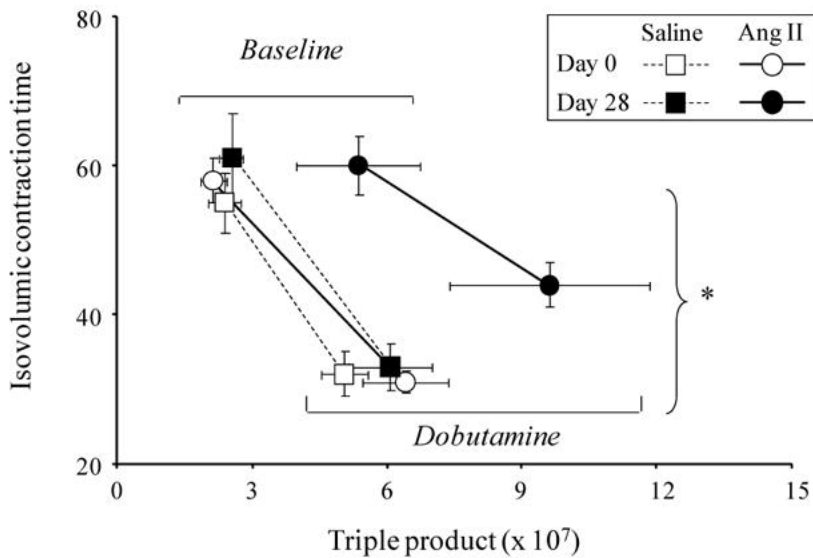
Isovolumic contraction in saline- and angiotensin II-treated animals

A) Relationship between heart rate and isovolumic contraction time measured at Day 0 before starting angiotensin II infusion (n=8); \*, p<0.05 by ANOVA; B) relationship between isovolumic contraction time and the triple product (heart rate x LV dP/dt<sub>max</sub> x LV pressure) in response to dobutamine (10 µg/kg/min, n=6) evaluated at Day 0 (open circles and open squares for angiotensin II- and saline-treated animals, respectively) and Day 28 (closed circles and closed squares for angiotensin II- and saline-treated animals, respectively). Measurements were performed in the absence of infusion. \*: change in isovolumic contraction time was significantly reduced for angiotensin II-treated animals at Day 28 vs Day 0 (p<0.05) while absolute variations in triple product were similar.

A



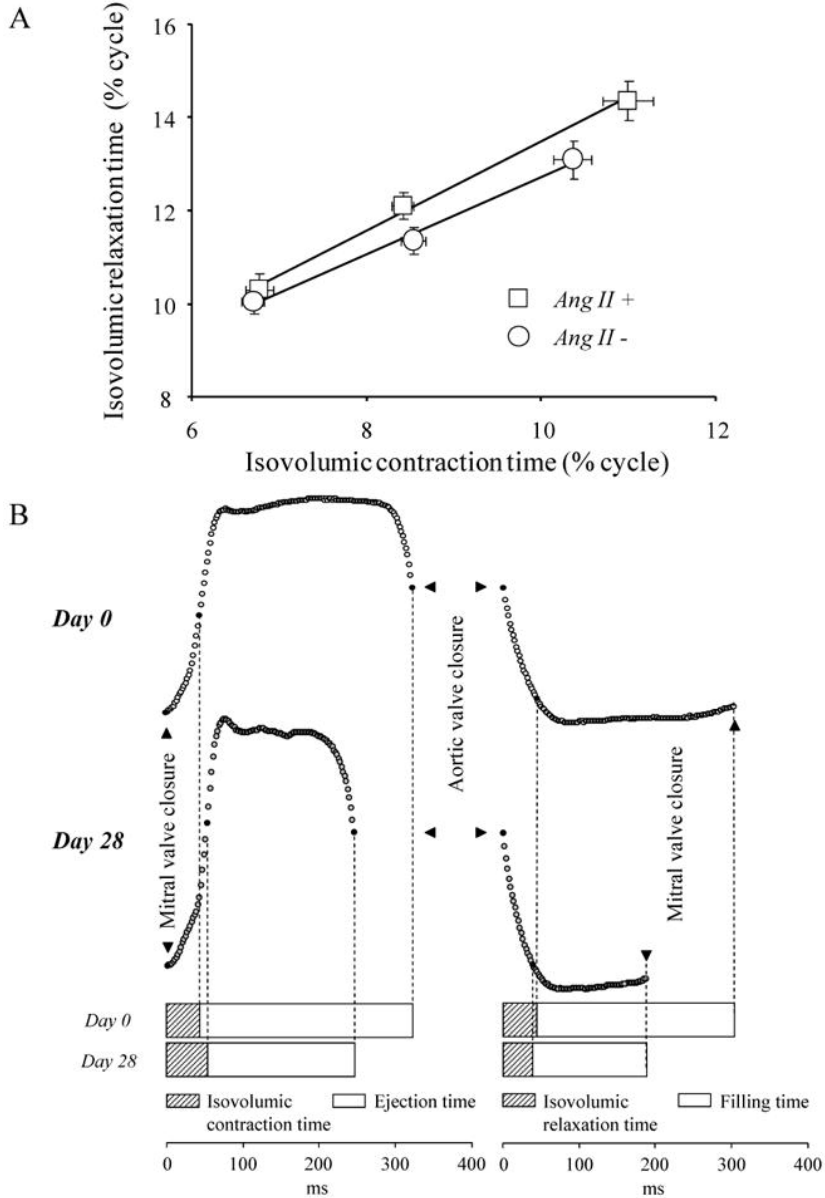
B



**Figure 6**

Relationship between isovolumic contraction and isovolumic relaxation times in angiotensin II-treated animals

A) linear relationship between isovolumic contraction time (% of cycle time) and isovolumic relaxation time (% of cycle time) measured in the presence (open squares,  $y = 0.77x + 4.9$ ) and the absence (open circles,  $y = 0.72x + 5.4$ ) of angiotensin II infusion. Values were measured at Days 0, 3, 7, 14, 21 and 28. B) Representative waveforms of left ventricular pressure measured at Day 0 and after 4 weeks of angiotensin II infusion (Day 28). Dash lines distinguish isovolumic contraction from ejection phase (left panel) and isovolumic relaxation from filling (right panel). Bars on the bottom quantify the relative time-intervals. Similar absolute durations for isovolumic contraction and isovolumic relaxation were observed between Day 0 and Day 28. Despite decreases in cycle time, isovolumic contraction and relaxation durations failed to reduce. Both phases were tightly coupled and changes in isovolumic contraction mirrored those of isovolumic relaxation.



**Table 1**

Hemodynamic parameters in angiotensin II-treated pigs

|   | n | Day 0      | Day 28      |              |
|---|---|------------|-------------|--------------|
|   |   |            | Ang -       | Ang +        |
| Heart rate (beats/min)                  | 8 | 80 ± 2     | 107 ± 8*    | 93 ± 4*      |
| Mean arterial pressure (mmHg)           | 8 | 101 ± 3    | 105 ± 3     | 158 ± 5*     |
| LV pressure (mmHg)                      | 8 | 120 ± 3    | 130 ± 5     | 186 ± 4*     |
| LV end-diastolic pressure (mmHg)        | 8 | 11 ± 3     | 9 ± 2       | 14 ± 2       |
| LV end-diastolic wall thickness (mm)    | 7 | 9,4 ± 0,7  | 12,4 ± 0,5* | 11,8 ± 0,7*  |
| LV end systolic wall thickness (mm)     | 7 | 11,9 ± 0,8 | 15,9 ± 1,1* | 14,4 ± 0,8*  |
| LV wall thickening (mm)                 | 7 | 2,5 ± 0,2  | 2,6 ± 0,3   | 2,6 ± 0,2    |
| LV end-diastolic internal diameter (mm) | 6 | 40 ± 2     | 42 ± 1      | 44 ± 1*      |
| LV end-systolic internal diameter (mm)  | 6 | 33 ± 1     | 35 ± 1      | 37 ± 1*      |
| LV Fractional Shortening (%)            | 6 | 18 ± 1     | 16 ± 1      | 17 ± 2       |
| LV dP/dt max (mmHg/s)                   | 8 | 2305 ± 140 | 3277 ± 316* | 3537 ± 169*  |
| LV dP/dt min (mmHg/s)                   | 8 | -2182 ± 97 | -2277 ± 150 | -3411 ± 105* |

Values are mean ± s.e.m.

\* p&lt;0.05 vs Day 0

**Table 2**

Hemodynamic parameters in saline-treated control pigs

|   | n | Day 0      | Day 28      |            |
|---|---|------------|-------------|------------|
|   |   |            | Saline -    | Saline +   |
| Heart rate (beats/min)                  | 6 | 84 ± 1     | 84 ± 3      | 81 ± 2     |
| Mean arterial pressure (mmHg)           | 6 | 107 ± 2    | 111 ± 2     | 113 ± 3    |
| LV pressure (mmHg)                      | 6 | 132 ± 3    | 135 ± 3     | 132 ± 3    |
| LV end-diastolic pressure (mmHg)        | 6 | 12 ± 2     | 13 ± 3      | 12 ± 4     |
| LV end-diastolic wall thickness (mm)    | 4 | 9,2 ± 0,2  | 10,1 ± 0,4  | 10,2 ± 0,4 |
| LV end systolic wall thickness (mm)     | 4 | 11,7 ± 0,7 | 12,3 ± 0,9  | 12,3 ± 0,8 |
| LV wall thickening (mm)                 | 4 | 2,5 ± 0,7  | 2,1 ± 0,5   | 2,2 ± 0,5  |
| LV end-diastolic internal diameter (mm) | 5 | 42 ± 2     | 45 ± 1*     | 44 ± 1     |
| LV end-systolic internal diameter (mm)  | 5 | 35 ± 2     | 40 ± 2*     | 40 ± 2*    |
| LV Fractional Shortening (%)            | 5 | 18 ± 2     | 18 ± 3      | 18 ± 2     |
| LV dP/dt max (mmHg/s)                   | 6 | 2352 ± 41  | 2318 ± 179  | 2276 ± 42  |
| LV dP/dt min (mmHg/s)                   | 6 | -2560 ± 78 | -2442 ± 101 | -2473 ± 68 |

Values are mean ± s.e.m.

\* p<0.05 vs Day 0

**Table 3**  
Diastolic time intervals in angiotensin II-treated pigs

|  | Day 0    | Day 28    |           |
|--|----------|-----------|-----------|
|  |          | Ang -     | Ang +     |
| Total cycle time (ms)                                  | 754 ± 19 | 587 ± 42* | 654 ± 29* |
| Isovolumic contraction time (ms)                       | 54 ± 2   | 56 ± 3    | 61 ± 3    |
| Ejection time (ms)                                     | 251 ± 6  | 196 ± 7*  | 210 ± 5*  |
| Isovolumic relaxation time (ms)                        | 74 ± 2   | 78 ± 5    | 78 ± 4    |
| Total systolic time (ms)                               | 378 ± 8  | 330 ± 12* | 349 ± 8   |
| Total diastolic filling time (ms)                      | 376 ± 16 | 258 ± 32* | 305 ± 23* |
| Systolic time to total cycle time (%)                  | 50 ± 1   | 57 ± 2*   | 54 ± 2    |
| Diastolic filling time to total cycle time (%)         | 50 ± 1   | 43 ± 2*   | 46 ± 2    |
| Isovolumic contraction time to total systolic time (%) | 14 ± 1   | 17 ± 1*   | 17 ± 1*   |
| Ejection time to total systolic time (%)               | 66 ± 1   | 59 ± 1*   | 60 ± 1*   |
| Isovolumic relaxation time to total systolic time (%)  | 19 ± 1   | 23 ± 1*   | 22 ± 1*   |

Values are mean ± s.e.m., n = 8

\* p<0.05 vs Day 0

**Table 4**  
Time intervals in saline-treated pigs

|  | Day 0    | Day 28   |          |
|--|----------|----------|----------|
|  |          | Saline - | Saline + |
| Total cycle time (ms)                                  | 723 ± 8  | 752 ± 19 | 719 ± 23 |
| Isovolumic contraction time (ms)                       | 51 ± 3   | 54 ± 3   | 53 ± 2   |
| Ejection time (ms)                                     | 250 ± 6  | 251 ± 8  | 244 ± 6  |
| Isovolumic relaxation time (ms)                        | 65 ± 2   | 70 ± 3   | 68 ± 3   |
| Total systolic time (ms)                               | 366 ± 6  | 376 ± 13 | 365 ± 10 |
| Total diastolic filling time (ms)                      | 357 ± 10 | 377 ± 9  | 354 ± 18 |
| Systolic time to total cycle time (%)                  | 51 ± 1   | 50 ± 1   | 51 ± 1   |
| Diastolic filling time to total cycle time (%)         | 49 ± 1   | 49 ± 1   | 50 ± 1   |
| Isovolumic contraction time to total systolic time (%) | 14 ± 1   | 14 ± 1   | 15 ± 1   |
| Ejection time to total systolic time (%)               | 68 ± 1   | 67 ± 1   | 67 ± 1   |
| Isovolumic relaxation time to total systolic time (%)  | 18 ± 1   | 19 ± 1   | 19 ± 1   |

Values are mean ± s.e.m., n = 6

No statistical differences were detected.