

**Inhaled NO Modifies LV Diastolic Stress  
in the Presence of Vasoactive Agents in Heart Failure**

Shunsuke Natori, Naoyuki Hasebe, Yin-Tie Jin, Tomoyuki Matsusaka,  
Akira Ido, Hironobu Matsuhashi, \*Tadashi Ihara, Kenjiro Kikuchi

First Department of Internal Medicine, Asahikawa Medical College, Asahikawa, Japan

\*Department of Medical Electronics, Suzuka University of Medical Science and  
Technology, Suzuka, Japan

For correspondence and reprint contact:

Naoyuki Hasebe, MD, PhD

First Department of Internal Medicine

Asahikawa Medical College

2-1-1-1 Midorigaoka higashi, Asahikawa

Hokkaido 078-8510 Japan

Phone: +81-166-68-2442

FAX: +81-166-68-2449

E-mail address: [haselove@asahikawa-med.ac.jp](mailto:haselove@asahikawa-med.ac.jp)

Running title: Inhaled NO and Diastolic Stress in Heart Failure

Descriptor Number: 22

Word Count: 3114 words

## Abstract

Nitric oxide (NO) inhalation therapy has been widely used in several diseases with pulmonary hypertension. However, application of NO inhalation therapy remains controversial in heart failure. Cardiovascular effects of inhaled NO were evaluated in dogs before and after induction of heart failure with and without infusion of vasoactive agents. Inhaled NO did not affect the baseline left ventricular (LV) function or the response to isoproterenol in Control or heart failure induced by procainamide. Pulmonary vascular resistance was significantly decreased by inhaled NO in heart failure with infusion of vasoactive agents. Unexpectedly, LV end diastolic pressure was significantly elevated by inhaled NO in heart failure in the presence of infusion of vasoactive agents independent of their types; either the vasodilating agents of acetylcholine and nitroglycerin, or the vasoconstricting agents of norepinephrine and angiotensin-II. The end diastolic LV dimension and wall stress were also significantly increased by inhaled NO, however, those at end systole were not affected. These results suggested that NO inhalation therapy reduced pulmonary vascular resistance, whereas in the presence of additional stress of vasoactive agents, it increased LV preload and end diastolic wall stress in heart failure.

188 words

**Key words;** nitric oxide, heart failure, cardiac output, hemodynamics,  
vasodilation

## Introduction

Nitric oxide (NO) regulates vascular tone and modulates myocardial function (1-6). The effect of inhaled NO (iNO) is believed to be limited to pulmonary vascular beds without significant effects on systemic circulation (7-9). NO inhalation therapy (NO-IT) has been widely applied in various diseases such as persistent pulmonary hypertension of newborn (10.11), primary pulmonary hypertension (12), adult respiratory distress syndrome (13), congenital (14) and valvular heart diseases (15), and transplanted hearts (16). In the treatment of heart failure, however, it is controversial whether NO-IT is effective (17-19) or potentially harmful (20.21). Several papers have demonstrated NO-IT improves hemodynamics (17.18) and exercise capacity (19) in heart failure. In contrast, some papers have warned that NO-IT potentially induces pulmonary edema, or exacerbates cardiac dysfunction in heart failure (20.21).

Previous studies have suggested that NO suppresses myocardial contractility and attenuates positive inotropic responses to  $\beta$ -adrenergic stimulation in myocytes (22.23) and experimental animals (24). The cardiac dysfunction of cardiomyopathy (25) and sepsis (3.26-28) is attributable to excessive production of NO. However, there are few reports demonstrating the direct effects of iNO on cardiac function (29.30).

Inhaled NO potentially dilates pulmonary vascular beds, and in this sense, it must be effective in heart failure with pulmonary hypertension. However, efficacy of iNO may be invalid in heart failure without marked pulmonary hypertension. Accordingly, the current investigation aimed to determine the cardiovascular effects of iNO, particularly on the cardiac function in heart failure lacking pulmonary hypertension. To accomplish this goal, we used a dog model of procainamide-induced cardiac dysfunction (31), which shows serious cardiac dysfunction but does not accompany pulmonary hypertension. We studied

the cardiovascular effects of iNO in the presence or absence of additional stress of vasoactive factors, which characterize clinical syndrome of heart failure. Enhanced sympathetic nervous system was represented by norepinephrine (NE) and isoproterenol (ISO) infusions, and enhanced rennin angiotensin system by angiotensin II (Ang-II) infusion. Using the stepwise infusion of these agents, we modified cardiac function and hemodynamics, and studied the effects of iNO under the various conditions of LV stress. We also investigated whether inhaled NO affected responses to endothelial dependent and independent vasodilators of acetylcholine (Ach) and nitroglycerine (NTG), which have been reported being attenuated in heart failure.

## **Methods**

### Experimental preparation (Fig.1)

Eighteen adult mongrel dogs of either sex, weighing 7.5-16.0 kg, were anesthetized with sodium pentobarbital (20-30 mg/kg, iv). Each dog was intubated and ventilated with oxygen enriched air using a volume ventilator (model 683, Harvard, Southnatick, U.S.A), of which respiratory rate, tidal volume, and end expiratory lung volume were adjusted to maintain arterial blood gas in a physiological range, and those conditions were kept constant throughout the experiment. Thoracotomy was performed in the left fifth intercostal space, and then heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was exposed and a Doppler flow probe was placed on it. Coronary blood flow (CBF) velocity was measured with a 20 MHz pulsed ultrasonic Doppler velocimeter (VF1, Valpey-Fisher, Hopkinton, U.S.A.). A 7 F balloon-tipped catheter was inserted from jugular vein into the main pulmonary artery to measure

pulmonary arterial pressure (PAP). An electromagnetic flow probe was placed on the main pulmonary artery and connected to an electromagnetic flow meter (MFV-1100, Nihon Kohden, Tokyo, Japan). A 7 F catheter was placed in the left carotid artery to measure aortic pressure (AOP) using a strain-gauge manometer (TP-200T transducer, Nihon Kohden, Tokyo, Japan). LV pressure (LVP), LV dP/dt and the logistic time constant of isovolumic relaxation of LV ( $T_L$ ) (32.33) were measured by a catheter-tipped transducer (PC-350, Miller, Houston, U.S.A.) inserted from the right carotid artery into LV. Electrocardiogram was continuously monitored and used to measure heart rate (HR). A 5 MHz transducer for transesophageal echocardiography (SSD-830, UST-5234S-5, Aloka, Tokyo, Japan) was placed just behind LV to monitor changes in LV dimension. The study was reviewed and approved by the Committee of the Ethics on Animal Experiments in Asahikawa Medical College. The dogs were treated in accordance with the guideline of the Committee.

#### Experimental protocol (Fig.2)

We randomized the order of experiments with or without iNO; nine dogs underwent the experiment with iNO then without iNO, whereas the other 9 dogs underwent the reverse sequence. After the stable baseline hemodynamics were recorded, the dose response to each of the following five vasoactive agents was examined in a random order. Each infusion was continued 3 min to obtain a stable hemodynamic change, and it was stepwisely increased up to the maximal dose. The maximal dose of each agent was determined to obtain 20-30% changes in the LV end diastolic pressure (LVEDP) by preliminary experiments. Five vasoactive agents included ISO (Nikken Chemicals Co., LTD., Tokyo, Japan, 0.01, 0.02, 0.05  $\mu\text{g}/\text{kg}/\text{min}$ ), Ach (Daiichi Pharmaceutical Co., LTD., Tokyo, Japan, 2.5, 5, 10  $\mu\text{g}/\text{kg}/\text{min}$ ), NTG (Nippon Kayaku Co., LTD., Tokyo, Japan, 5, 10, 20  $\mu\text{g}/\text{kg}/\text{min}$ ), NE (Nikken Chemicals Co., LTD., Tokyo, Japan, 0.2, 0.5, 1.0  $\mu\text{g}/\text{kg}/\text{min}$ )

and Ang-II (Sigma Chemicals Pty Ltd, Perth, Western Australia, 0.1 0.2, 0.5  $\mu\text{g}/\text{kg}/\text{min}$ ). After the maximal dose was examined, dogs were allowed to recover to the baseline, then the next agent was examined. When the Control experiments were completed, procainamide was infused as described below, and we repeated the same protocol as Control with or without iNO. The application order of vasoactive agents was randomly determined, but kept constant in each dog before and after heart failure with and without iNO.

#### Procainamide-induced heart failure

We used the procainamide-induced heart failure model (31), which was characterized by cardiac dysfunction without pulmonary hypertension. This model allowed us to evaluate the cardiovascular effects of iNO with minimizing the potential effects of pulmonary vasodilation on cardiac function. Procainamide (Daiichi Pharmaceutical Co., LTD., Tokyo, Japan) was continuously infused at a variable dose of 5-10  $\text{mg}/\text{kg}/\text{min}$  to maintain the LV  $\text{dP}/\text{dt}$  by 30~40% of the baseline level.

#### NO administration

NO gas, stored in nitrogen at the concentration of 800 ppm, was mixed into the inspiration limb of a respirator circuit. The inspired concentration of NO was continuously monitored at the point just proximal to the endotracheal tube using a chemiluminescence analyzer (NOA 270B, Severs, Colorado, U.S.A.). NO gas was delivered at the concentration of 70 ppm by titrating the amount of nitrogen. Exhaled gas was scavenged through an absorption column.

## Data analysis and Statistics

Hemodynamic data were monitored on a direct-writing oscillograph and recorded on a multichannel recorder (polygraph system RM 6200, Nihon Kohden, Tokyo, Japan), and continuously digitized and recorded on a personal computer throughout the experiment using a physiological data analyzing system (MacLab, ADInstruments Pty Ltd, NSW, Australia). The logistic model of time constant of isovolumic relaxation ( $T_L$ ) was used as an index of LV diastolic function (32,33). LVED dimension (LVEDD), LV end systolic dimension (LVESD), LV wall thickness at end systole (WTes) and end diastole (WTed) were measured by M-mode transesophageal echocardiography. LV fractional shortening (%FS) was calculated as  $[(LVEDD)-(LVESD)]/(LVEDD) \times 100$ . LV end diastolic and systolic meridional wall stress, WSed and Wses were calculated as follows (34);

$$WS_{ed} = 0.334 \times LVEDP \times LVEDD / W_{Ted} / (1 + W_{Ted} / LVEDD).$$

$$WS_{es} = 0.334 \times LVESP \times LVESD / W_{Tes} / (1 + W_{Tes} / LVESD).$$

The data were stored and analyzed using a personal computer. All values were reported as mean  $\pm$  SEM. Differences between baseline measurements and subsequent values were assessed by repeated-measures ANOVA. If an overall difference was found, comparisons were performed with two-tailed Student's t-test for unpaired data. Data were considered to be significantly different at a p value of 0.05 or less.

## Results

### Baseline hemodynamics and cardiac function

There were no significant differences in the baseline hemodynamics and cardiac function with and without iNO in Control (Table 1). After the continuous infusion of

procainamide, HR, LVP, LV dP/dt, AOP and PAP were significantly decreased, whereas LVEDP and T<sub>L</sub> were significantly increased (Table 1). LVEDD significantly decreased without significant change in LVESD, resulted in approximately -26% reduction in LVEF with procainamide ( $p < 0.01$ ). However, iNO affected neither LV function nor hemodynamics at the baseline even in heart failure.

### Responses to ISO

ISO significantly increased LV dP/dt from  $2975.6 \pm 211.8$  to  $4037.7 \pm 345.0$  mmHg/sec, and HR from  $161.3 \pm 6.6$  to  $203.6 \pm 9.0$  bpm in a dose dependent fashion in Control. In heart failure, the baseline LV dP/dt and HR were significantly decreased, however the dose-response to ISO was well preserved (Fig 3). More importantly, iNO did not significantly affect these positive inotropic and chronotropic responses to ISO even in heart failure.

### Cardiovascular effects of vasoactive agents in Control

Vasodilating agents, both Ach and NTG significantly decreased AOP and LVP. NTG significantly decreased PBF and PAP. In contrast, Ach significantly increased both PBF and PAP. As a result, PVR was significantly increased with NTG, (e.g. from  $22.6 \pm 3.1$  to  $24.5 \pm 3.4$  mmHg/L/min with  $20 \mu\text{g/kg/min}$  NTG  $p < 0.01$ ), in contrast, PVR was slightly decreased with Ach (Fig 4-A). LVEDP was significantly decreased dose dependently with NTG (from  $4.7 \pm 0.6$  to  $3.0 \pm 0.5$  mmHg with  $20 \mu\text{g/kg/min}$  NTG ( $p < 0.01$ )), however, it was not significantly affected with Ach. LVEDD and LVESD were slightly decreased, and %FS was slightly increased with both agents. Despite of these differences in Ach and NTG, iNO did not significantly affect their hemodynamic changes in Control.

Vasoconstricting agents, both NE and Ang-II significantly increased AOP, LVP,

LVEDP, and dP/dt. CBF and PBF were significantly increased, however PAP was not significantly changed with NE. PVR was significantly decreased with NE (e.g. from  $23.8 \pm 2.5$  to  $17.4 \pm 2.2$  mmHg/L/min with  $1 \mu\text{g/kg/min}$  NE ( $p < 0.05$ )). In contrast, PAP and LVEDP were significantly increased, PBF was significantly decreased, thus PVR was not significantly changed with Ang-II. LVEDD was slightly increased, but LVESD was slightly decreased, accordingly %FS was significantly increased with NE. In contrast, LVEDD, LVESD, and %FS were slightly increased with Ang-II. Despite of these hemodynamic differences in NE and Ang-II, iNO did not significantly affect their hemodynamic responses in Control.

#### Cardiovascular effects of vasoactive agents in heart failure

In contrast to Control, PVR (mmHg/L/min) was significantly decreased with iNO in heart failure independent of types of vasoactive agents (Fig 4-A);  $24.2 \pm 3.4$  to  $18.9 \pm 2.6$  by NTG  $20 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $26.7 \pm 4.5$  to  $23.0 \pm 4.1$  by Ach  $10 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $25.0 \pm 3.3$  to  $22.0 \pm 3.5$  by NE  $1 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $28.2 \pm 2.0$  to  $24.2 \pm 2.0$  by Ang-II  $0.5 \mu\text{g/kg/min}$ ,  $p < 0.05$ . LVEDP (mmHg) was significantly increased with iNO in heart failure independent of types of vasoactive agents (Fig. 4-B);  $4.6 \pm 1.1$  to  $5.7 \pm 1.1$  by NTG  $20 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $6.1 \pm 1.4$  to  $7.4 \pm 1.7$  by Ach  $10 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $11.0 \pm 2.4$  to  $12.9 \pm 2.4$  by NE  $1 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $11.8 \pm 2.4$  to  $13.1 \pm 2.7$  by Ang-II  $0.5 \mu\text{g/kg/min}$ ,  $p < 0.05$ .

Table 2 shows the changes in LVEDD and %FS at the maximum dose of each vasoactive agent compared to Control. In contrast to Control, iNO increased LVEDD significantly in heart failure independent of types of vasoactive agents (Table 2).

The WSed ( $10^3 \text{ dyn/cm}^2$ ), but not WSes, was significantly increased with iNO in heart failure (Fig. 5);  $2.73 \pm 0.54$  to  $3.45 \pm 0.69$  by NTG  $20 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $4.67 \pm 0.57$  to  $5.69 \pm 0.38$  by Ach  $10 \mu\text{g/kg/min}$ ,  $p < 0.05$ ;  $6.78 \pm 1.02$  to  $8.25 \pm 1.37$  by NE  $1 \mu\text{g/kg/min}$ ,

$p < 0.05$ ;  $11.24 \pm 1.43$  to  $13.67 \pm 1.57$  by Ang-II  $0.5 \mu\text{g}/\text{kg}/\text{min}$ ,  $p < 0.05$ .

The remaining hemodynamic parameters were not significantly affected by iNO.

## Discussion

The major findings of the current investigation are as follows: (i) Inhaled NO does not influence cardiac function or hemodynamics at baseline in Control or heart failure conditions. (ii) The positive inotropic and chronotropic actions of  $\beta$ -adrenergic stimulation are not affected by iNO in Control or heart failure conditions. (iii) Only in heart failure, iNO significantly reduces PVR in the presence of additional stress of vasoactive agents, but simultaneously increases LV wall stress at end diastole.

### Effects of iNO on ventricular function

Negative inotropic effect of NO has been reported in several in vitro experiments (22-24), normal (4) and impaired (35) human hearts. However, the direct cardiac effect of iNO has not been confirmed in the previous reports in normal (29) and failed hearts (17-21). We confirmed iNO did not affect cardiac function in Control or heart failure conditions, i.e. no changes in peak positive and negative  $dP/dt$ , ejection indexes of transesophageal echocardiography and active relaxation as assessed by  $T_L$ . The direct coronary vasodilating effect of iNO is unlikely, because CBF was not changed. Attenuation of responses to  $\beta$ -adrenergic stimulation by iNO was not observed in the current setting. The half-life of NO in vivo is less than 1 s (7-9). We believe the main action of iNO is limited to the pulmonary vascular beds without direct effects on cardiac function.

## Reduction of PVR by iNO

Inhaled NO did not affect the baseline PVR in Control or heart failure conditions. Even in the presence of additional stress of vasoactive agents, iNO did not affect PVR in Control. These findings indicate that the effect of iNO is not much powerful on the modification of basal pulmonary vascular tone. However, iNO counteracts the additional stress of vasoactive agent, independent of their types in heart failure.

The decreases in PVR with iNO were similarly observed with both endothelium dependent Ach and independent NTG in heart failure. This was unexpected. In our previous study using bolus injection, iNO suppressed the response to Ach but not to NTG (36). At that time, we speculated that iNO might suppress intrinsic NO release and attenuated endothelium dependent vasodilation (36). These discrepancies probably depend on the way of administration of agents, bolus injection and continuous infusion. Vasodilating effect of Ach is mediated by M3 receptors and primarily by intrinsic NO, and secondarily by inhibition of NE release from adrenergic nerve endings. Ach dilates the whole pulmonary vascular bed, however, NTG predominantly dilates systemic veins resulting in systemic venous pooling (37). Therefore, PBF tended to be increased by Ach, but decreased by NTG. In either case, additional pulmonary vasodilating effects of iNO caused further reduction of PVR. Inhaled NO may predominantly dilate smaller vasculature adjacent to alveoli.

NE and Ang-II, the major humoral factors characterizing heart failure, differently act in pulmonary circulation. PBF was increased by NE, but decreased by Ang-II. NE shows  $\alpha$ -adrenergic vasoconstriction and  $\beta$ -adrenergic inotropic action. Ang-II shows vasoconstriction directly by Ang-II type 1 receptor stimulation, and indirectly by enhancement of peripheral NE neurotransmission. Independent of the differences in the

changes in PBF by NE and Ang-II, iNO reduces PVR in heart failure conditions.

The responses of PVR to NTG and NE were opposite to what we expected. PVR was determined by the balance of changes in PBF and PAP, therefore the degree of relative changes in both of the indices could make the discrepancy. The changes in PBF were slightly greater compared to those of PAP with NTG, consequently PVR was calculated to be increased by intravenous infusion of NTG. Similarly, PBF was significantly increased but PAP was not significantly affected with NE, consequently PVR was calculated to be decreased by intravenous infusion of NE in the present study using anesthetized dogs.

#### Elevation of LVEDP and end diastolic wall stress by iNO

We observed unfavorable increase of LVEDP by iNO in heart failure. The increases in left atrial or LV filling pressure by iNO have been reported in several studies on heart failure (17.18.20.21), however, the mechanism remains unknown. One explanation is a negative inotropic action of NO (4.22-24). However, the increases in LVEDP were observed not only with NTG and Ach, which decreases LV dP/dt, but also with NE and Ang-II, which increases LV dP/dt. The  $\beta$ -adrenergic inotropic response was not altered by iNO. These findings suggest that the increase in LVEDP by iNO is independent of further depression of LV function in heart failure.

An alternative and plausible explanation is volume shifts from the selectively dilated pulmonary vasculature to the failing LV. Inhaled NO reduces right ventricular afterload, which increases right ventricular cardiac output, subsequently increases LV preload. It increases LV cardiac output by Frank-Starling mechanism. However, with an impaired LV function, failure to increase forward cardiac output leads to elevation of LVEDP. We confirmed the specific increases in the indices of diastolic LV loading, i.e. LVEDP, LVEDD

and WSeD with iNO in heart failure. We believe that an increase in LV preload secondary to pulmonary vasodilation is the major mechanism of increased LVEDP. Of note, iNO showed beneficial hemodynamic effects in patients implanted with an LV assist device, who need additional support only of right ventricle (38.39).

#### Procainamide-induced heart failure

In order to investigate the effects of iNO on cardiovascular hemodynamics in heart failure, we tried to minimize the prominent pulmonary vasodilating effects of iNO, which would potentially buffer the effects of iNO on cardiac function. Thus, we chose procainamide-induced heart failure as a model without pulmonary hypertension but with serious cardiac dysfunction. A continuous infusion of procainamide causes marked cardiac suppression, moderate vasodilation and heart rate reduction, due to its potent sodium channel blocking and weak autonomic ganglia blocking actions. Consequently, this model dose-dependently shows a marked cardiac dysfunction, moderate hypotension, bradycardia, and most importantly, no pulmonary hypertension. The pulmonary vasodilating effect of iNO could be buffered in this heart failure model lacking pulmonary hypertension. We could study the effects of iNO on LV preload under the condition of stepwisely modified cardiac function and hemodynamics with the additional vasoactive agents in this model.

#### Study limitations

In the assessment of the effects of the various vasoactive agents, we used the time constant of isovolumic relaxation calculated by a logistic regression, which was relatively load independent (32.33). In contrast, indices of contractility we used were relatively load dependent. Therefore, we couldn't simply conclude the increase in LVEDP by iNO was

independent of further depression of LV function in heart failure. However, iNO itself did not significantly affect LVEDP, LV dP/dt, LVEF or LVSV in heart failure. Only in the presence of vasoactive agents, iNO increased LVEDP either with preload reducing agent of NTG or with afterload increasing agent of NE. In the presence of these agents, the relationship between LVEDP and LVSV seemed to shift right upward on the almost identical curve with iNO (data not shown). We believe iNO increases LV preload without significantly affecting LV contractility in heart failure.

We used an experimental heart failure model without pulmonary hypertension to minimize the prominent pulmonary vasodilating effects of iNO, which would potentially buffer the effects of iNO on cardiac function. Pulmonary hypertension is one of the hallmarks of heart failure. Therefore, our results are not applicable to every condition of clinical heart failure.

In summary, the effects of iNO on cardiac function and hemodynamics are minor in normal subjects. The cardiac actions of  $\beta$ -adrenergic stimulation are not affected by iNO. Despite the reduction of PVR in heart failure, iNO could significantly increase LV end diastolic stress. Its major mechanism appears to be volume shifts from the pulmonary vascular beds to the failing LV, which cannot sufficiently increase forward cardiac output, rather than direct effects on the myocardium. Therefore, we may not be able to expect the full-beneficial effects of iNO in heart failure with serious LV dysfunction without significant pulmonary hypertension.

## References

1. Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ Res.* 1978;61:866-79.
2. Ignarro LJ. Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ Res.* 1989; 65: 1-21.
3. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med.* 1993;329: 2002-12.
4. Paulus WJ, Vantrimpont PJ, Shah AM. Paracrine coronary endothelial control of left ventricular function in humans. *Circulation.* 1995;92:2119-26.
5. Michel T, Smith TW. Nitric oxide synthases and cardiovascular signaling. *Am J Cardiol.* 1993;72:33C-38C.
6. Hare JM, Colucci WS. Role of nitric oxide in the regulation of myocardial function. *Prog Cardiovasc Dis.* 1995;38:155-66.
7. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM. Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation.* 1991;83:2038-47.
8. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. *Circ Res.*

1990;66:1561-75.

9. Rimar S, Gillis CN. Selective pulmonary vasodilation by inhaled nitric oxide is due to hemoglobin inactivation. *Circulation*. 1993;88:2884-7.
10. Roberts JD, Polaner DM, Lang P, Zapol WM. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet*. 1992;340:818-9.
11. Kinsella JP, Neish SR, Shaffer E, Abman SH. Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet*. 1992;340:819-20.
12. Pepke Zaba J, Higenbottam TW, Dinh Xuan AT, Stone D, Wallwork J. Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet*. 1991;338:1173-4.
13. Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled nitric oxide for the adult respiratory distress syndrome. *N Engl J Med*. 1993;328:399-405.
14. Roberts JD Jr, Lang P, Bigatello LM, Vlahakes GJ, Zapol WM. Inhaled nitric oxide in congenital heart disease. *Circulation*. 1993;87:447-53.
15. Girard C, Lehot JJ, Pannetier JC, Filley S, Ffrench P, Estanove S. Inhaled nitric oxide after mitral valve replacement in patients with chronic pulmonary artery hypertension. *Anesthesiology*. 1992;77:880-3.

16. Okabayashi K, Triantafillou AN, Yamashita M, Aoe M, DeMeester SR, Cooper JD, Patterson GA. Inhaled nitric oxide improves lung allograft function after prolonged storage. *J Thorac Cardiovasc Surg.* 1996;112:293-9.
17. Loh E, Stamler JS, Hare JM, Loscalzo J, Colucci WS. Cardiovascular effects of inhaled nitric oxide in patients with left ventricular dysfunction. *Circulation.* 1994;90:2780-5.
18. Semigran MJ, Cockrill BA, Kacmarek R, Thompson BT, Zapol WM, Dec GW, Fifer MA. Hemodynamic effects of inhaled nitric oxide in heart failure. *J Am Coll Cardiol.* 1994;24:982-8.
19. Koelling TM, Kirmse M, Di Salvo TG, Dec GW, Zapol WM, Semigran MJ. Inhaled nitric oxide improves exercise capacity in patients with severe heart failure and right ventricular dysfunction. *Am J Cardiol* 1998;81:1494-7
20. Bocchi EA, Bacal F, Auler Junior JO, Carmone MJ, Bellotti G, Pileggi F. Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure. *Am J Cardiol.* 1994;74:70-2.
21. Hayward CS, Rogers P, Keogh AM, Kelly R, Spratt PM, Macdonald PS. Inhaled nitric oxide in cardiac failure: vascular versus ventricular effects. *J Cardiovasc Pharmacol.* 1996;27:80-5.
22. Balligand JL, Ungureanu D, Kelly RA, Kobzik L, Pimental D, Michel T, Smith TW.

Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest.* 1993;91:2314-9.

23. Brady AJ, Warren JB, Poole Wilson PA, Williams TJ, Harding SE. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol.* 1993;265:H176-82.

24. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science.* 1992;257:387-9.

25. de Belder AJ, Radomski MW, Why HJ, Richardson PJ, Bucknall CA, Salas E, Martin JF, Moncada S. Nitric oxide synthase activities in human myocardium. *Lancet.* 1993;341:84-5.

26. Brady AJ, Poole Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol.* 1992;263:H1963-6.

27. Avontuur JA, Bruining HA, Ince C. Sepsis and nitric oxide. *Adv Exp Med Biol.* 1996;388:551-67.

28. Afulukwe IF, Cohen RI, Zeballos GA, Iqbal M, Scharf SM. Selective NOS Inhibition Restores Myocardial Contractility in Endotoxemic Rats; However, Myocardial NO Content Does Not Correlate with Myocardial Dysfunction. *Am. J. Respir. Crit. Care*

*Med.* 2000;162: 21-26.

29. Hayward CS, Kalnins WV, Rogers P, Feneley MP, MacDonald PS, Kelly RP. Effect of inhaled nitric oxide on normal human left ventricular function. *J Am Coll Cardiol.* 1997;30:49-56.
30. Goldstein DJ, Dean DA, Smerling A, Oz MC, Burkhoff D, Dickstein ML. Inhaled nitric oxide is not a negative inotropic agent in a porcine model of pulmonary hypertension. *J Thorac Cardiovasc Surg.* 1997;114:461-6.
31. Alousi AA, Canter JM, Fort DJ. The beneficial effect of amrinone on acute drug-induced heart failure in the anaesthetised dog. *Cardiovasc Res.* 1985;19:483-94.
32. Hiromi M, Miyako T, Shingo Y, Junichi A, Hiroyuki S. Logistic Time Constant of Isovolumic Relaxation Pressure–Time Curve in the Canine Left Ventricle : Better Alternative to Exponential Time Constant. *Circulation.* 1995;92:2318-26
33. Senzaki H, Fetters B, Chen CH, Kass DA. Comparison of ventricular pressure relaxation assessments in human heart failure: quantitative influence on load and drug sensitivity analysis. *J Am Coll Cardiol* 1999; 34:1529-36
34. Yin FC. Ventricular wall stress. *Circ Res.* 1981;49:829-42.
35. Hare JM, Loh E, Creager MA, Colucci WS. Nitric oxide inhibits the positive inotropic response to beta-adrenergic stimulation in humans with left ventricular dysfunction.

*Circulation*. 1995;92:2198-203.

36. Naoyuki H, Shunsuke N, Jin I, Tomoyuki M, Kenjiro K. Inhaled nitric oxide attenuates endothelium dependent pulmonary vascular response and paradoxically enhances left ventricular contraction. *Circulation*. 1998;98:1-141
  
37. Armstrong PW, Walker DC, Burton JR, Parker JO. Vasodilator therapy in acute myocardial infarction. A comparison of sodium nitroprusside and nitroglycerin. *Circulation*. 1975;52:1118-22.
  
38. Hare JM, Shernan SK, Body SC, Graydon E, Colucci WS, Couper GS. Influence of inhaled nitric oxide on systemic flow and ventricular filling pressure in patients receiving mechanical circulatory assistance. *Circulation*. 1997;95:2250-3.
  
39. Jeffery C, Annick L, Michael O', Sangeeta B, Anne P, Jesse B. Hall. The Incidence and Pathogenesis of Cardiopulmonary Deterioration after Abrupt Withdrawal of Inhaled Nitric Oxide. *Am. J. Respir. Crit. Care Med*. 2000;161:1443-1449.

## Figure legends

Fig. 1 Schematic illustration (left) of instrumentation used in assessing hemodynamics and LV function. A 5 MHz transducer for transesophageal echocardiography was placed just behind LV to monitor changes in LV dimension.

Fig. 2 Experimental protocols. Dogs were randomly divided into (A) or (B) groups, and underwent Control protocol following Heart Failure protocol by procainamide infusion. (A) group (n=9) underwent the experiments with iNO then without iNO, whereas (B) group (n=9) underwent the reverse sequence. Vasoactive agents of ISO, NTG, Ach, NE and Ang-II in three doses each were applied in a random order at the first arrow, and repeated in the same order with or without iNO in Control and Heart Failure conditions.

Fig. 3 Dose responses to isoproterenol (ISO) in heart rate (HR) (right panel) and LV dP/dt (left panel) with (○) and without (□) iNO in Control, and with (●) and without (■) iNO in heart failure. ISO increased HR and LV dP/dt dose-dependently. There were no significant differences in the responses of HR and LV dP/dt to ISO with and without iNO. Data are means ± SEM. † p<0.05, ‡ p<0.01 vs NO(-) Baseline, § p<0.05, § § p<0.01 vs NO(+) Baseline, n=18.

Fig. 4. A: Changes in PVR with Ach (upper panel) and Ang-II (lower panel). Increased PVR in heart failure was significantly reduced by iNO. B: Changes in LVEDP with NTG (upper panel) and NE (lower panel). LVEDP was decreased by NTG, and increased by NE, however in either case, it was significantly increased by iNO in

heart failure. Data are means  $\pm$  SEM. †  $p < 0.05$ , ‡  $p < 0.01$  vs NO(-) Baseline, §  $p < 0.05$ , § §  $p < 0.01$  vs NO(+) Baseline, ¶  $p < 0.05$  vs control, \* $p < 0.05$  vs without iNO,  $n = 18$ .

Fig. 5. Changes in end diastolic wall stress (WSed) by vasoactive agents with and without iNO in heart failure. WSed was decreased by NTG and Ach, and increased by NE and Ang-II, in any case, it was significantly increased with iNO.

‡  $p < 0.01$  vs Baseline of NO(-), § §  $p < 0.01$  vs Baseline of NO(+), \* $p < 0.05$  vs without iNO,  $n = 8$ .

Table 1. Effects of inhaled NO on the baseline hemodynamics and cardiac function before and after administration of procainamide

	Control			Procainamide (+)				
	INO (-)	INO (+)	P value	INO (-)		INO (+)	P value	
Heart Rate (bpm)	161 ± 8	158 ± 7	n.s.	103 ± 7	**	99 ± 7	‡	n.s.
mean AOP (mmHg)	84 ± 3	84 ± 3	n.s.	51 ± 3	**	51 ± 4	‡	n.s.
LVP (mmHg)	143 ± 5	144 ± 6	n.s.	92 ± 5	**	92 ± 4	‡	n.s.
dP/dt (mmHg/sec)	2976 ± 257	2849 ± 186	n.s.	1222 ± 88	**	1195 ± 95	‡	n.s.
-dP/dt (mmHg/sec)	-3989 ± 341	-3931 ± 373	n.s.	-1626 ± 151	**	-1538 ± 133	‡	n.s.
mean PAP (mmHg)	13.7 ± 0.7	13.7 ± 0.7	n.s.	13.3 ± 0.8	*	12.9 ± 0.9	†	n.s.
mean PBF (L/min)	0.46 ± 0.1	0.45 ± 0.1	n.s.	0.34 ± 0.1	**	0.33 ± 0.1	‡	n.s.
mean CBF (Hz)	0.80 ± 0.1	0.83 ± 0.1	n.s.	0.49 ± 0.1	**	0.51 ± 0.1	‡	n.s.
LVEDP (mmHg)	5.4 ± 0.8	5.7 ± 0.6	n.s.	7.5 ± 0.6	*	8.0 ± 1.0	†	n.s.
T <sub>L</sub> (msec)	22 ± 2	23 ± 2	n.s.	40 ± 4	**	40 ± 4	‡	n.s.
LVEDD (mm)	30 ± 2	31 ± 2	n.s.	29 ± 1	**	29 ± 1	‡	n.s.
LVESD (mm)	24 ± 2	24 ± 2	n.s.	25 ± 1	n.s.	25 ± 1	n.s.	n.s.
LVEF (%)	49 ± 6	50 ± 6	n.s.	36 ± 4	**	34 ± 4	‡	n.s.

HR, heart rate; AOP, aortic pressure; LVP, left ventricular pressure; PAP, pulmonary arterial pressure; PBF, pulmonary blood flow; CBF, coronary blood flow; LVEDP, left ventricular end diastolic pressure, Tau; time constant of isovolumic relaxation of left ventricle, LVEDD; left ventricular end diastolic dimension, LVESD; left ventricular end systolic dimension, LVEF; left ventricular ejection fraction.

The condition with inhaled NO (INO(+)), without inhaled NO (INO(-)), in Control and after administration of procainamide (procainamide(+))

P value: INO(-) vs INO(+) in Control and procainamide(+)

\*p<0.05, \*\*p<0.01 vs INO(-) in Control, †p<0.05, ‡p<0.01 vs INO(+) in Control, n.s.:not significantly different values are mean ± SE. n=18

Table 2. Effects of inhaled NO on the Changes in LV Dimension with vasoactive agents

		INO (-)	INO (+)
<b>NTG (20 µg/kg/min)</b>			
Control	LVEDD	28.0 ± 2.1	29.3 ± 2.4
	%FS	19.4 ± 1.6	18.6 ± 3.1
Heart Failure	LVEDD	26.3 ± 1.1	28.0 ± 1.2 *
	%FS	9.7 ± 1.9	10.9 ± 1.5
<b>Ach (10 µg/kg/min)</b>			
Control	LVEDD	28.7 ± 1.4	28.7 ± 2.0
	%FS	20.3 ± 2.9	19.4 ± 2.5
Heart Failure	LVEDD	26.0 ± 1.6	27.4 ± 1.9 *
	%FS	16.2 ± 3.9	14.8 ± 3.2
<b>NE (1 µg/kg/min)</b>			
Control	LVEDD	30.4 ± 2.8	30.6 ± 2.6
	%FS	25.7 ± 4.0	24.1 ± 3.9
Heart Failure	LVEDD	29.3 ± 2.1	30.6 ± 2.1 *
	%FS	26.6 ± 2.4	26.8 ± 2.6
<b>Ang-II (0.5 µg/kg/min)</b>			
Control	LVEDD	30.2 ± 1.0	30.6 ± 1.0
	%FS	23.6 ± 3.5	24.5 ± 2.6
Heart Failure	LVEDD	29.4 ± 0.9	31.0 ± 0.6 *
	%FS	17.0 ± 1.9	21.8 ± 1.8

Left ventricular end diastolic dimension (LVEDD) and fractional shortening (%FS) with iNO (INO(+)) and without iNO (INO(-)). \*p<0.05 vs INO(-). values are mean ± SE. n=8

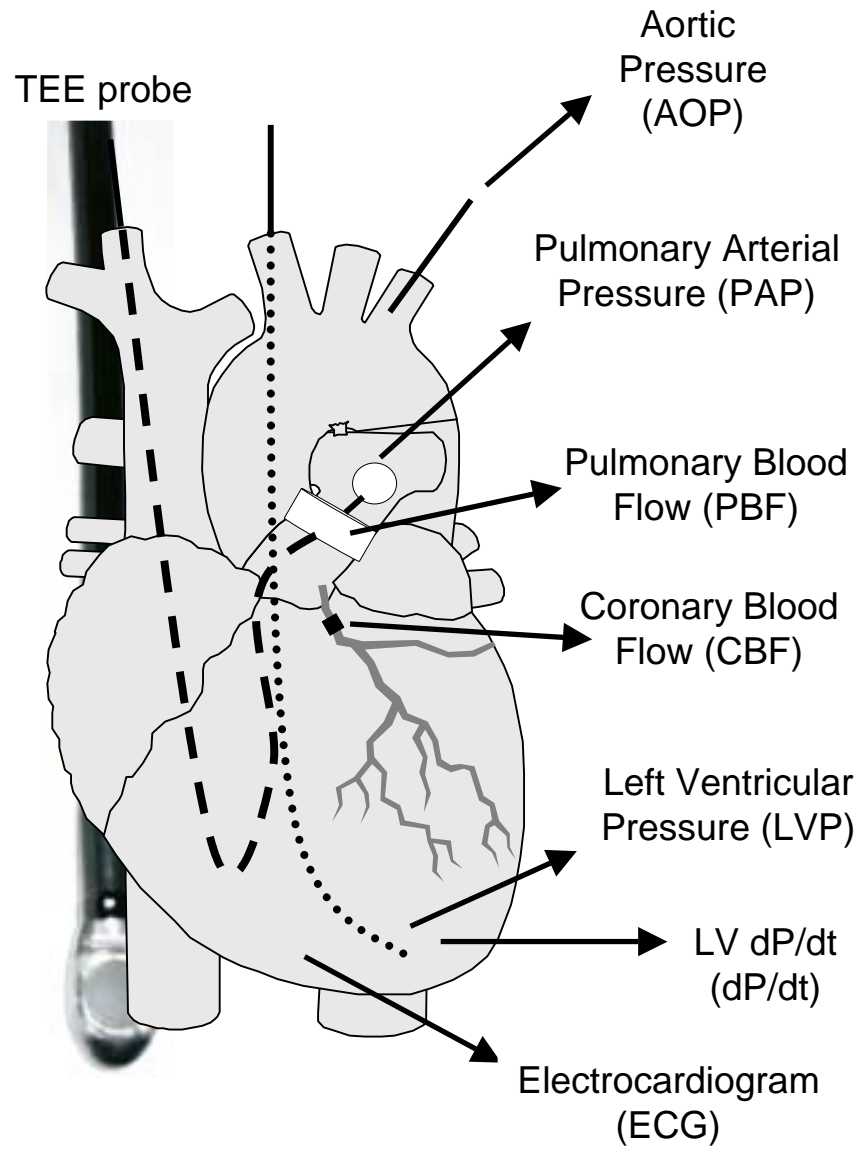


Figure 1

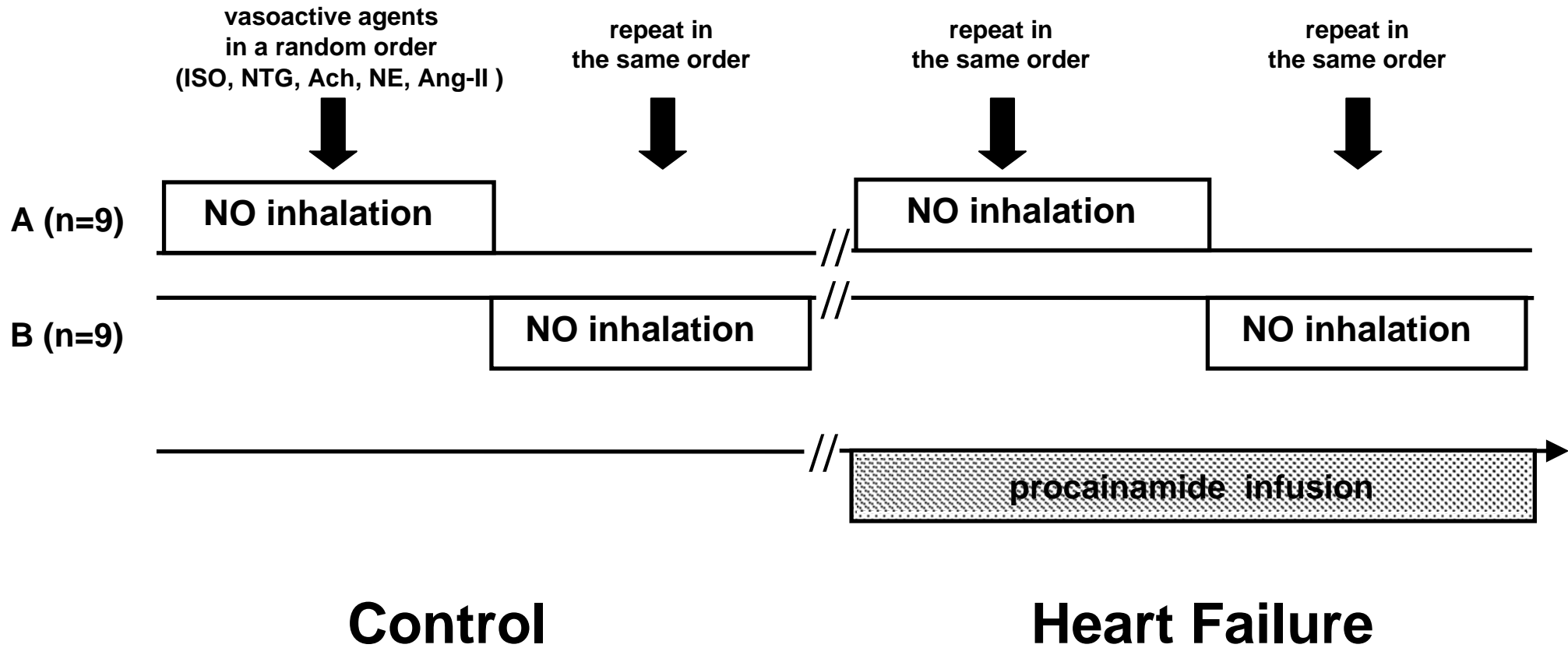


Figure 2

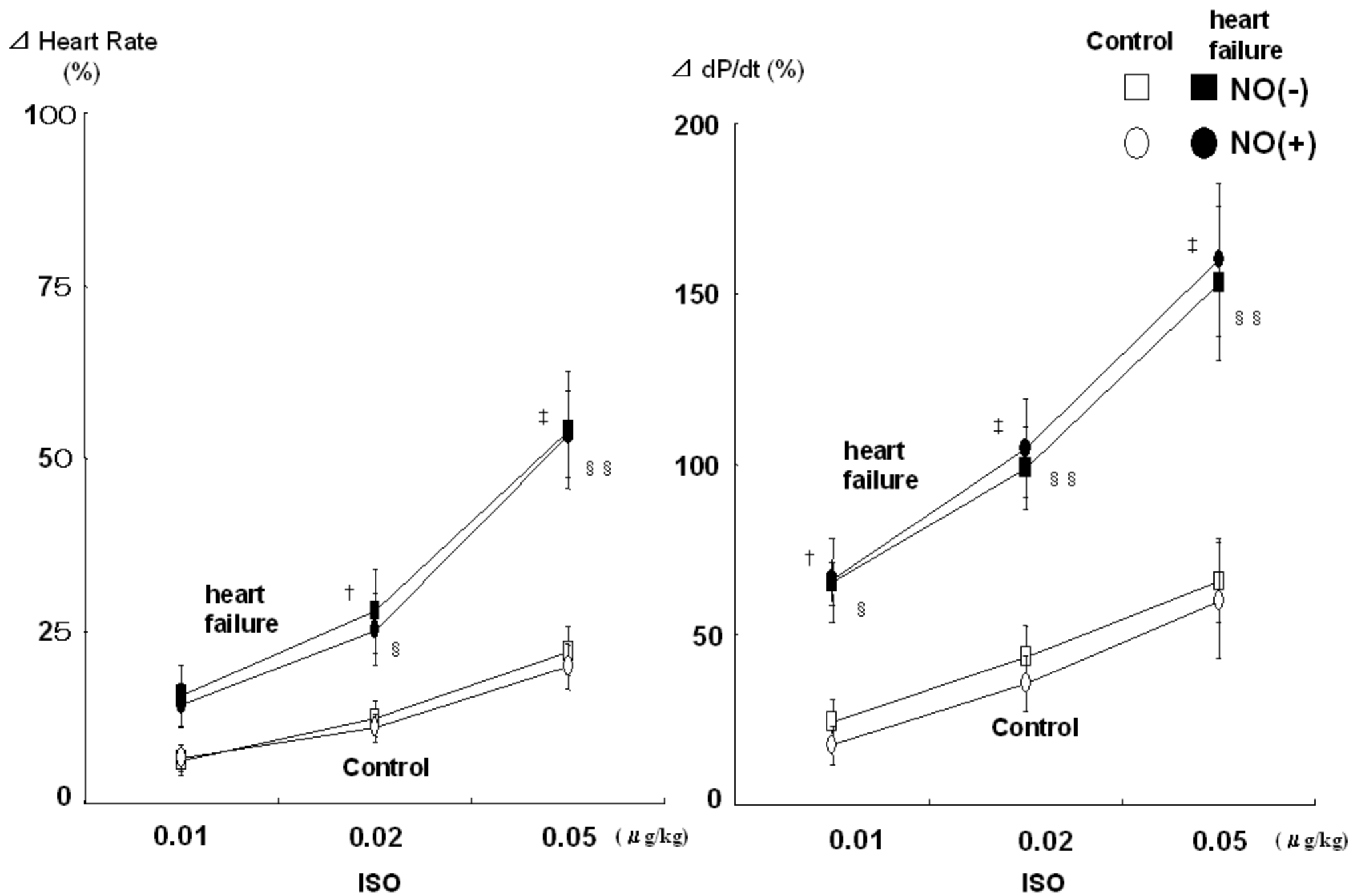


Figure 3

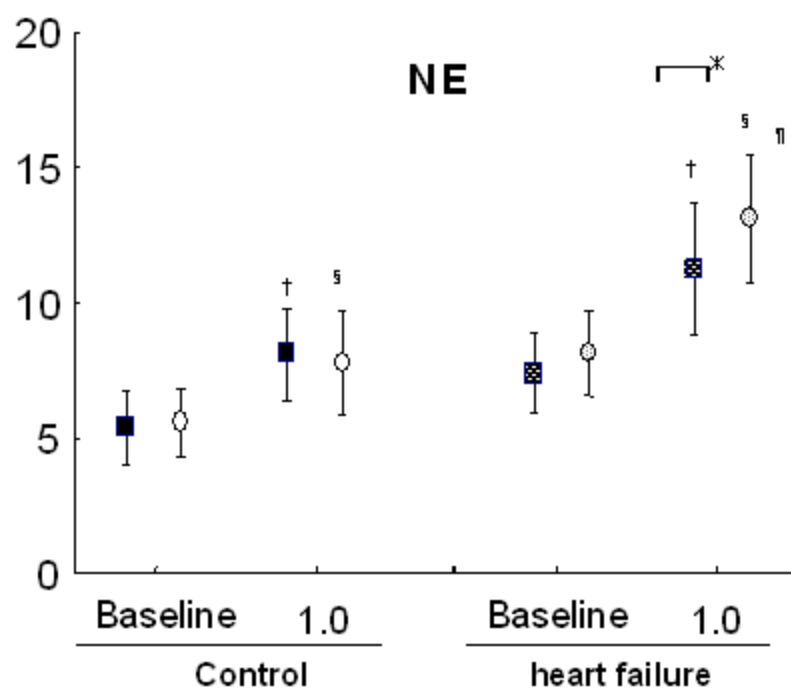
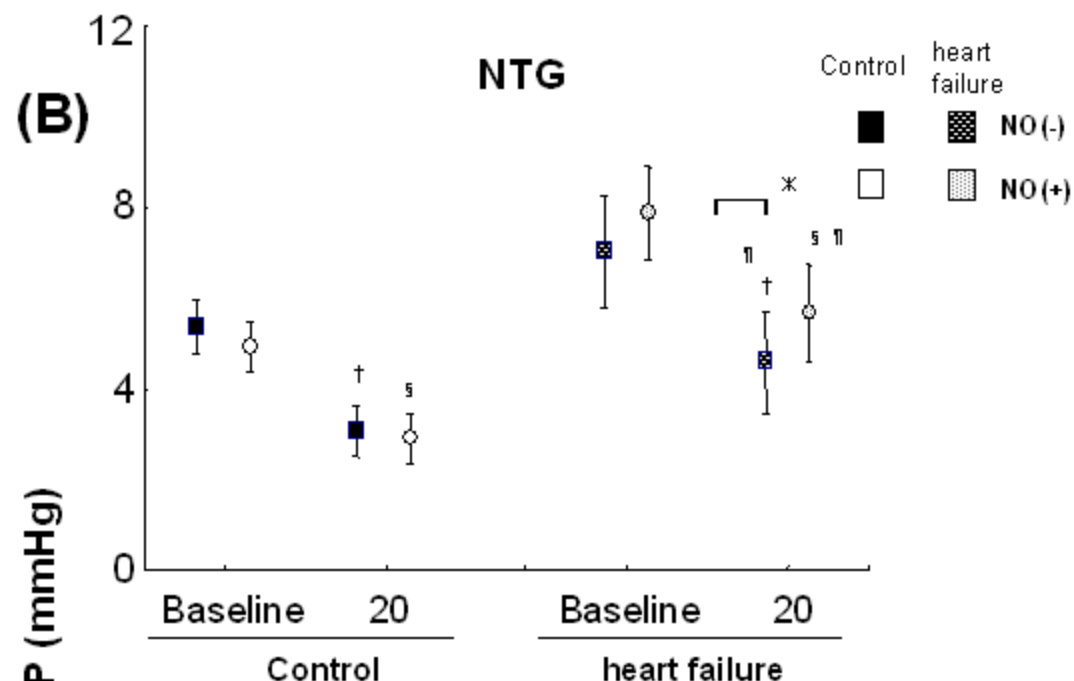
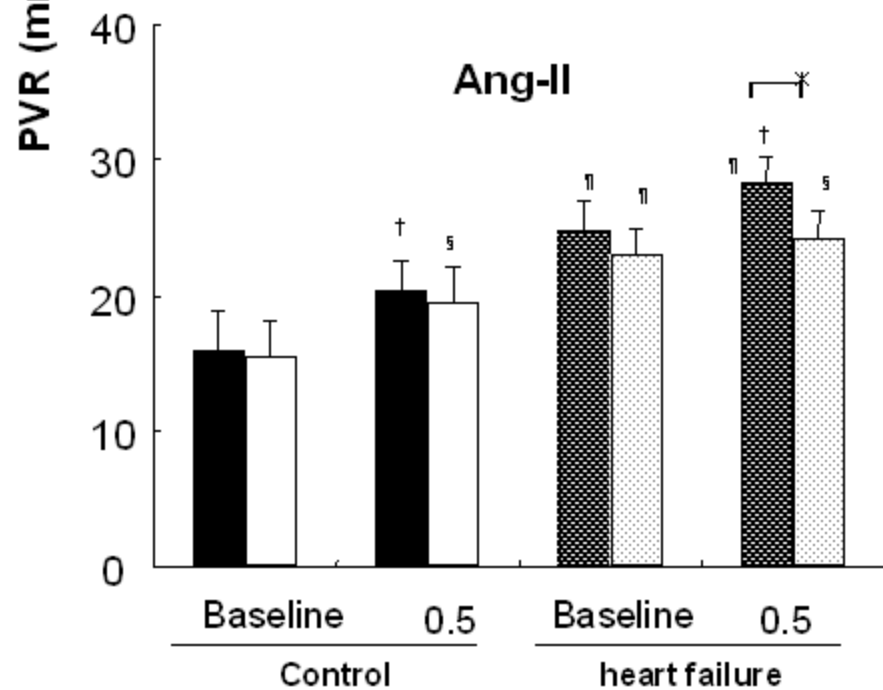
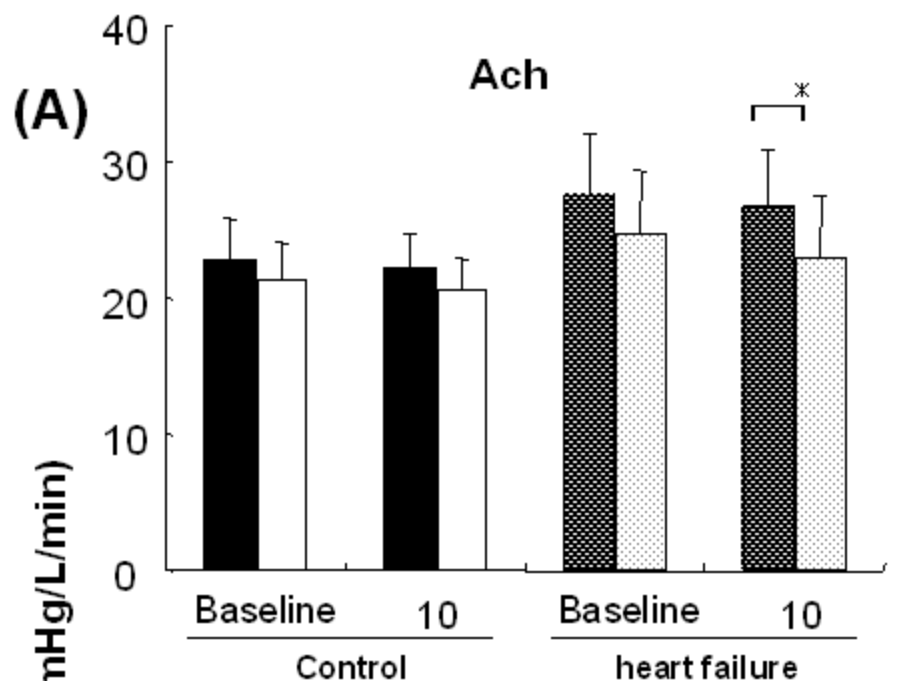


Figure 4

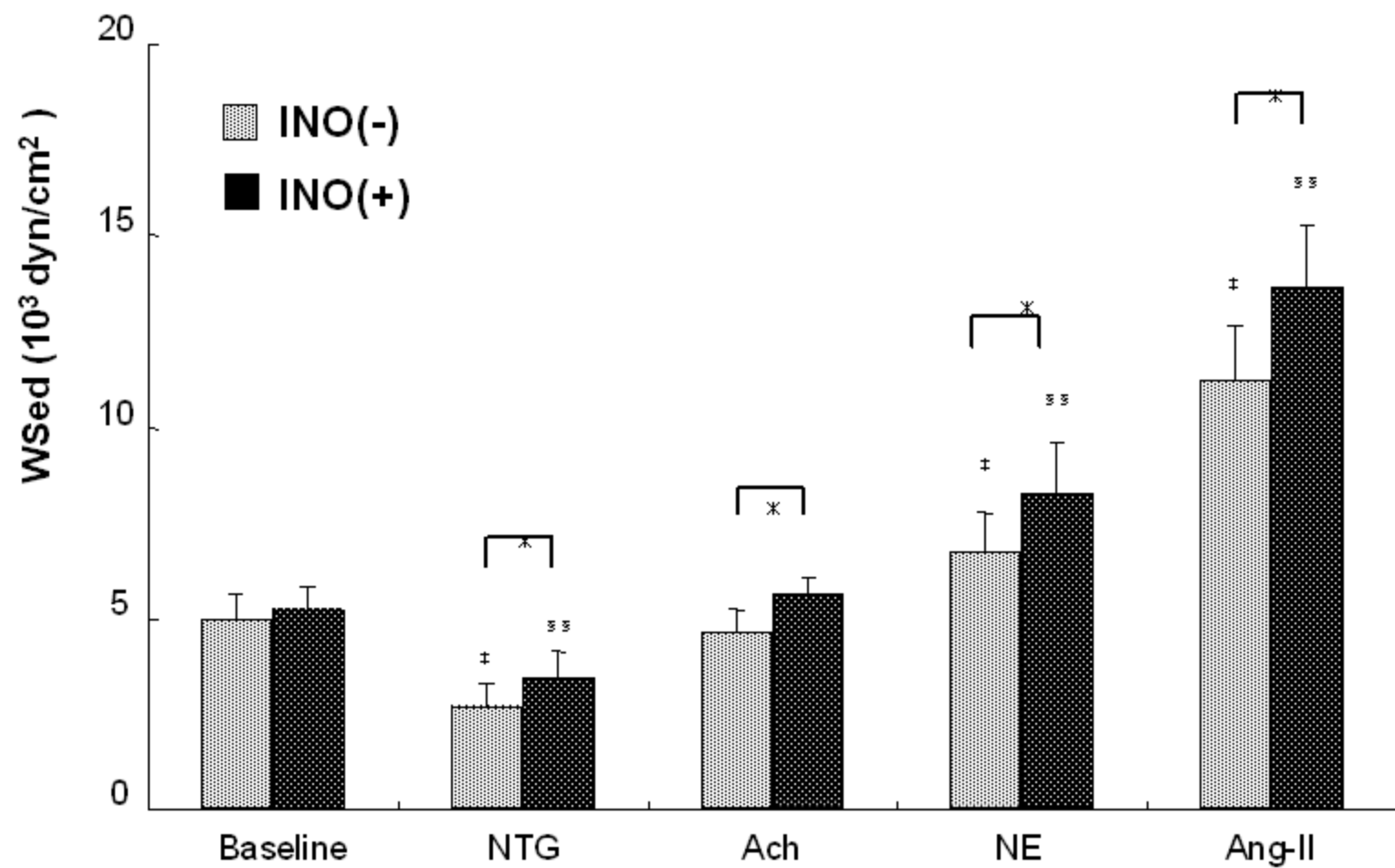


Figure 5