demonstrated that presynaptic α_2-adrenergic receptors in the nucleus ambiguus are involved in baroreflex bradycardia.[14] Thus, medetomidine might act on the NTS and/or the nucleus ambiguus, and exert this vagotonic effect on the heart. In contrast, the vagolytic effect on the stomach might depend on other pathways. Robertson and Leslie reported that α_2-adrenergic receptors are also distributed in the dorsal motor nucleus (DMN) of the vagus, which contains preganglionic neurons that control gastric motility and secretion.[13] As dexmedetomidine has been shown to inhibit gastric emptying and gastrointestinal transit in healthy volunteers,[15] medetomidine might act on the DMN and exert vagolytic effects on the stomach. Although further investigations are needed to elucidate the sites of medetomidine action, the organ-specific vagal responses to medetomidine might be associated with different actions of medetomidine on the nuclei.

Effects of Medetomidine on Sympathetic Nerve Activities

In protocol 1, 100 μg/kg of medetomidine significantly suppressed both the cardiac and gastric NE releases. Thus, medetomidine might suppress the whole sympathetic nerve activities. The rostral ventrolateral medulla (RVLM) is known to serve as an important site in mediating the hypotensive and sedative effects of α_2-adrenergic agonist, clonidine.[16] Furthermore, in the supplementary protocol, an α_2-adrenergic antagonist, atipamezole, blocked this medetomidine-induced suppression of sympathetic NE releases. Thus, medetomidine might act on α_2-adrenergic receptors in the RVLM and exert sympatholytic effects to both the heart and stomach.

Restoring the mean arterial pressure to baseline level by infusing phenylephrine scarcely affected medetomidine-induced suppression of NE release both in the heart and stomach. It is possible that 100 μg/kg of medetomidine has already suppressed sympathetic nerve activities to the lowest level, leaving no room for further baroreflex-induced sympathetic suppression. This strong sympatholytic effect might be useful for the treatment of CHF as described in the *Clinical implications* section.

Clinical Implications

Electrical VNS has recently become a new therapeutic option for CHF.[1] However, electrical VNS sometimes causes gastrointestinal adverse effects. Approximately 10% of patients receiving VNS therapy for epileptic seizures complain of nausea.[3] Sanossian and Haut reported chronic diarrhea associated with VNS.[4] As shown in protocol 2, electrical stimulation of the cervical vagus nerve increases both cardiac and gastric ACh releases, and the augmented gastric ACh release might cause nausea and diarrhea in clinical settings. Furthermore, in an animal study, Cho et al. reported that intermittent electrical stimulation of the left cervical vagus nerve induced a 100% incidence of hemorrhagic ulcers in the glandular mucosa of rat stomachs.[17] Thus, hemorrhagic gastric ulcer might be one of the most serious adverse effects caused by electrical VNS therapy in CHF patients, because CHF patients often receive concomitant anti-coagulation therapy, anti-platelet therapy, or both.

Organ-specific vagal activation is one strategy to reduce the above-mentioned adverse effects. Bianchi et al. have reported that endocardial atrioventricular vagal stimulation significantly reduces the ventricular rate acutely during atrial fibrillation in humans.[18] Thus, selective cardiac electrical VNS might be one of the most suitable approaches for vagal activation therapy in CHF patients, if the treatment effects of VNS are exclusively mediated by efferent vagal activation.

Another approach of vagal activation therapy in CHF patients is to use pharmacological agents. An ACh esterase inhibitor is a candidate for pharmacological vagal activation therapy.[19] Because ACh esterase degrades ACh immediately after its release, an ACh esterase inhibitor will block ACh degradation and increase ACh in the synaptic cleft. Kubo et al. have reported that donepezil, an ACh esterase inhibitor against Alzheimer's disease, significantly decreased plasma brain natriuretic peptide levels in patients with subclinical CHF.[20] However, an ACh esterase inhibitor might also cause gastrointestinal adverse effects. Nausea and diarrhea are the most common adverse events related to donepezil therapy,[21] which is similar to that caused by electrical VNS therapy. To prevent these gastrointestinal adverse events, we have to identify a new agent that activates cardiac vagus nerve without stimulating vagal activity in the gastrointestinal tract. However, there is a paucity of information on pharmacological agents that selectively activate the cardiac vagus nerve, partly because of the difficulty in selectively monitoring organ-specific vagus nerve activities. The microdialysis technique enables us to monitor organ-specific vagus nerve activities. The present study demonstrated that medetomidine selectively increased cardiac ACh release without augmenting gastric ACh release, suggesting that medetomidine is able to activate cardiac vagus nerve without stimulating gastric vagal activity. Medetomidine might be a more suitable agent than ACh esterase inhibitors for the treatment of CHF patients, although further investigations are required to examine the effects of medetomidine on other organs.

The sympatholytic effect of medetomidine might also be favorable for cardiac and gastric protection. Because sustained sympathetic overdrive contributes to progressive left ventricular dysfunction and promotes progressive left ventricular remodeling in CHF patients,[22] inhibition of the sympathetic nerve system has been the cornerstone of drug therapy for CHF.[23] Thus, we might be able to use medetomidine as well as β blockers to modify the augmented sympathetic tone in CHF. As medetomidine (or dexmedetomidine) has also been used as an anesthetic agent, sedation with medetomidine (or dexmedetomidine) might be beneficial for the intensive care of CHF patients. Furthermore, sympathetic overdrive also causes mucosal vasoconstriction and reduces the mucosal blood flow in the stomach, potentially leading to gastric ulcers. Because 50–100 μg/kg of dexmedetomidine has an antiulcerative effect equivalent to 25 mg/kg of famotidime,[6] sedation with medetomidine (or dexmedetomidine) might exert a gastroprotective effect in CHF patients while simultaneously conferring cardioprotection. Conversely, the sedative action of medetomidine might render this agent unsuitable for outpatient treatment.

Study Limitations

First, because ACh is degraded by ACh esterase immediately after release, the addition of eserine into the perfusate is required for measuring the in vivo release of ACh. The presence of eserine around the microdialysis fiber could have affected ACh release in the vicinity of the fiber.

Second, in protocol 2, gastric dialysate ACh concentration after hexamethonium injection was slightly but significantly higher than the post-vagotomy baseline level. Thus, the gastric dialysate ACh concentration might partly reflect ACh release from preganglionic nerves.

Third, medetomidine is a chiral imidazole derivative. Although hemodynamic and gastric secretory responses to medetomidine are known to be abolished by an α_2-adrenergic antagonist, atipamezole,[24,25] there is room for a possibility that imidazoline receptors might also be involved in the cardiac vagal activation by medetomidine.

Finally, we did not examine both cardiac and gastric functions

in the present study. According to previous papers, medetomidine can affect both cardiac and gastric functions as follows. Flacke et al. reported that the peak of the first derivative of systolic left ventricular pressure declined after intravenous administration of dexmedetomidine in anesthetized dogs.[26] Savola et al. reported that medetomidine inhibited basal gastric acid and fluid output in conscious rats in a dose-dependent manner while in anesthetized rats, no effect was observed when it was administered intravenously.[25] We need further investigations including chronic experiments to evaluate the effects of medetomidine on both cardiac and gastric functions in CHF conditions.

Conclusions

In the present in vivo study involving rabbits, while medetomidine suppressed both the cardiac and gastric NE releases, it enhanced cardiac ACh release but suppressed gastric ACh release through central actions. Medetomidine might be one of the potential pharmacological agents for vagal activation therapy against heart failure patients without the risk of causing gastric adverse effects.

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Conflict of Interest

None declared.

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Targeting of High Peak Respiratory Exchange Ratio Is Safe and Enhances the Prognostic Power of Peak Oxygen Uptake for Heart Failure Patients

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Background: Peak oxygen uptake ($\dot{V}O_2$) and ventilatory efficiency ($\dot{V}E/\dot{V}CO_2$ slope) measured on cardiopulmonary exercise testing (CPX) are prognostic indicators in heart failure (HF) patients, but peak $\dot{V}O_2$ is influenced by patient effort. In CPX targeting a peak respiratory exchange ratio (pRER; an objective index of effort adequacy) higher than the commonly recommended level, we assessed the safety and prognostic value of CPX parameters compared with non-CPX parameters.

Methods and Results: We studied 283 consecutive HF patients with left ventricular ejection fraction (LVEF) ≤45% (mean, 26.3%) who underwent CPX targeting pRER >1.20. The attained pRER (mean, 1.26) was consistently high irrespective of LVEF, and there was no major exercise-related adverse event. The composite of all-cause death or HF hospitalization occurred in 111 patients (39%) during a median follow-up of 47 months. Among well-known prognostic markers, peak $\dot{V}O_2$ was the most powerful predictor of outcome as both a continuous and an optimal dichotomous variable, followed by $\dot{V}E/\dot{V}CO_2$ slope. On multivariate analysis, peak $\dot{V}O_2$ was a significant independent predictor, whereas $\dot{V}E/\dot{V}CO_2$ slope, B-type natriuretic peptide, and LVEF were not.

Conclusions: In CPX targeting pRER >1.20 for HF patients, peak $\dot{V}O_2$ is the most powerful among well-known predictors, without an increased risk of exercise-related events. These findings advocate a high target pRER in CPX even in advanced HF.

Key Words: Exercise; Heart failure; Oxygen uptake; Prognosis; Respiratory exchange ratio

Despite recent advances in medical therapy, the prognosis in patients with heart failure (HF) remains poor,[1,2] and risk stratification is an important issue. Cardiopulmonary exercise testing (CPX) provides important information on integrative exercise responses involving the pulmonary, cardiovascular and skeletal muscle systems.[3–6] Both exercise intolerance and ventilatory inefficiency, reflected by low peak oxygen uptake ($\dot{V}O_2$) and high minute ventilation–carbon dioxide production relationship ($\dot{V}E/\dot{V}CO_2$ slope), respectively, are associated with poor prognosis, independently of other clinical and hemodynamic parameters.[7–9]

Measurement of peak $\dot{V}O_2$ requires maximum effort and is, therefore, influenced by subjective factors such as patient motivation and exercise termination by the investigator. Peak respiratory exchange ratio (pRER: peak ratio of $\dot{V}CO_2$ to $\dot{V}O_2$) has been used as an objective means of quantifying effort: a value above 1.10 generally indicates good effort, while that below 1.00 indicates poor effort.[10–12] Indeed, peak $\dot{V}O_2$ has been shown to have poor prognostic reliability in cases of low pRER,[13] which can be explained by the underestimation of peak $\dot{V}O_2$.

$\dot{V}E/\dot{V}CO_2$ slope can be derived from submaximal exercise and is independent of subjective factors. Over the last decade, peak $\dot{V}O_2$ has been shown to provide inferior prognostic value compared with $\dot{V}E/\dot{V}CO_2$ slope in HF patients, but the mean pRER was relatively low (1.05–1.10) in those studies.[14–18] Targeting higher pRER than the commonly recommended level (>1.10) would enhance the prognostic power of peak $\dot{V}O_2$, but may increase the exercise-related risk, particularly in advanced HF patients.

The purpose of the present study was to assess the predictive value of peak $\dot{V}O_2$ and the safety in CPX targeting pRER >1.20 for HF patients receiving current optimal medical therapy.

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Table 1. Baseline Subject Characteristics

Characteristics	All patients (n=283)
Age (years)	61.8±13.5
Male sex	81%
Hypertension	59%
Diabetes	43%
BMI (kg/m²)	21.9±3.7
Ischemic	45%
AF rhythm	17%
Serum creatinine (mg/dl)	1.10±0.39
Hemoglobin (g/dl)	13.2±1.7
Serum sodium (mEq/L)	139.1±3.2
Plasma BNP (pg/ml)	291.7±318.1
LVDd (mm)	63.6±9.2
LVDs (mm)	53.7±10.3
LVEF (%)	26.3±8.0
LAD (mm)	45.2±8.1
Medications	
β-blocker	92%
ACEI or ARB	83%
Diuretic	82%
CPX parameters	
pRER	1.26±0.13
Peak work rate (W)	90.7±31.7
Peak $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	17.0±4.4
$\dot{V}E/\dot{V}CO_2$ slope	34.3±8.1

Data given as mean±SD or %.
ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation (and including atrial flutter); ARB, angiotensin-receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CPX, cardiopulmonary exercise testing; LAD, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; pRER, peak respiratory exchange ratio; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}E$, minute ventilation; $\dot{V}O_2$, oxygen uptake.

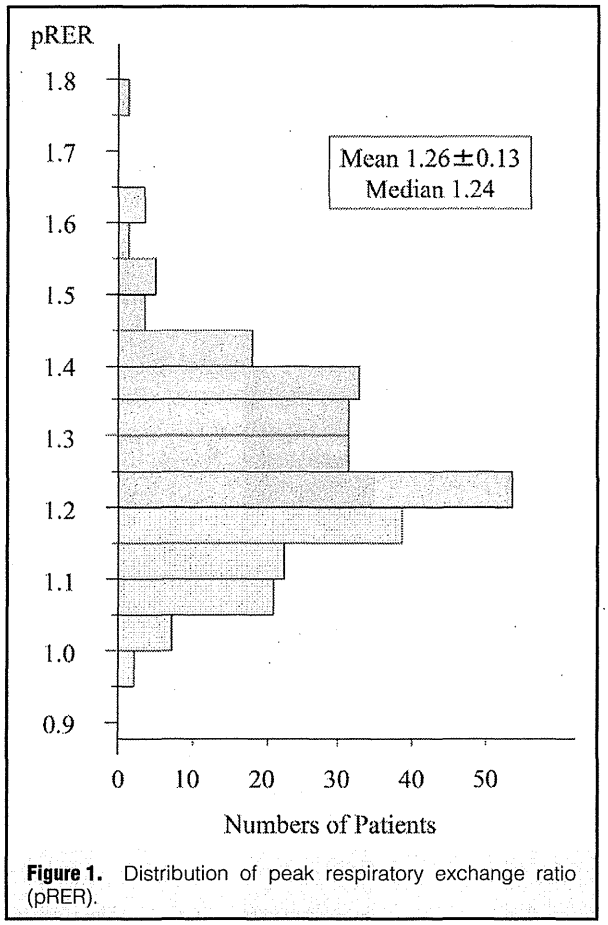

Figure 1. Distribution of peak respiratory exchange ratio (pRER).

Methods

Subjects

Consecutive HF patients who were admitted with HF and who underwent CPX between March 2002 and September 2012 at National Cerebral and Cardiovascular Center, Japan were screened retrospectively. Patients were excluded from the study if they had left ventricular ejection fraction (LVEF) >45%, serum creatinine >2.5 mg/dl, history of myocardial infarction within the preceding 3 months, or significant pulmonary disease. The presence of ischemic heart disease was confirmed on coronary angiography or documentation of myocardial infarction. Treatment for HF was tailored to all patients on the basis of current guidelines and was kept constant throughout the study. The study complies with the Declaration of Helsinki and was approved by the institutional ethics committee, and all patients gave written, informed consent.

CPX

Patients underwent symptom-limited CPX using a cycle ergometer with respiratory gas exchange analysis at a clinically stable stage after treatment with appropriate medications. The testing consisted of an initial 2 min of rest, 1 min of warm-up (0-W load), and full exercise by an individualized ramp protocol with increments of 10–15 W/min. Expired gas analysis was performed throughout testing on a breath-by-breath basis and VE, $\dot{V}O_2$, and $\dot{V}CO_2$ data were stored in a computer hard disk every 6 s for off-line analysis (AE-300S, Minato Medical Science, Osaka, Japan).

In 2001, we examined CPX data of 300 consecutive patients with cardiovascular disease at National Cerebral and Cardiovascular Center, and found that 266 of them achieved the respiratory compensation (RC) point, while 34 did not. All of the patients who did not achieve the RC point had pRER <1.20 (median, 1.06; range, 0.87–1.16). Among patients who achieved the RC point, the median pRER was 1.27 (range, 1.05–1.75), and the RER of the RC point was <1.15 in 77% and <1.20 in 94% (median, 1.10; range, 1.00–1.27). Based on these findings, we have identified the target pRER level as >1.20, and since the beginning of 2002, all subjects in CPX have been strongly encouraged to exercise toward the target pRER at National Cerebral and Cardiovascular Center.

Peak $\dot{V}O_2$ was determined as the higher value normalized to body weight ($ml \cdot kg^{-1} \cdot min^{-1}$) of either the greatest $\dot{V}O_2$ during exercise (smoothed after a 5-point moving average) or the average $\dot{V}O_2$ of the last 3 datapoints (18 s) before termination of exercise. $\dot{V}E$ was plotted against $\dot{V}CO_2$ to represent the $\dot{V}E/\dot{V}CO_2$ slope, excluding the part after the RC point where its slope started to increase.

Non-CPX Parameters

All patients underwent an echocardiographic evaluation at a clinically stable stage after treatment, and LV end-diastolic di-

Table 2. Cox Regression Analysis for Combined Event

Variables	Univariate analysis			Multivariate analysis	
	X^2	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (per 10-year increase)	0.56	1.06 (0.92–1.23)	0.45		
Male sex	0.34	1.16 (0.71–2.02)	0.56		
Hypertension	0.0016	1.01 (0.69–1.48)	0.97		
Diabetes	5.6	1.57 (1.08–2.29)	0.018	1.18 (0.79–1.77)	0.43
BMI (per 1.0-kg/m^2 increase)	0.40	0.98 (0.93–1.04)	0.53		
Ischemic	1.9	1.30 (0.89–1.89)	0.17		
AF rhythm	0.14	0.91 (0.53–1.47)	0.71		
Creatinine (per 1.0-mg/dl increase)	26.8	3.27 (2.13–4.93)	<0.0001	1.74 (1.02–2.93)	0.042
Hemoglobin (per 1.0-g/dl increase)	20.2	0.78 (0.69–0.87)	<0.0001	0.90 (0.78–1.04)	0.14
Sodium (per 1.0-mEq/L increase)	15.2	0.89 (0.85–0.94)	<0.0001	0.96 (0.90–1.02)	0.15
BNP (per 10-pg/ml increase)	21.2	1.012 (1.007–1.016)	<0.0001	1.003 (0.997–1.008)	0.29
LVDd (per 1.0-mm increase)	17.2	1.04 (1.02–1.06)	<0.0001	0.97 (0.89–1.04)	0.41
LVDs (per 1.0-mm increase)	19.5	1.04 (1.02–1.06)	<0.0001	1.07 (0.99–1.17)	0.079
LVEF (per 1.0% increase)	11.7	0.96 (0.93–0.98)	0.0006	1.01 (0.97–1.06)	0.61
LAD (per 1.0-mm increase)	20.8	1.06 (1.03–1.08)	<0.0001	1.03 (1.007–1.06)	0.012
Peak $\dot{V}O_2$ (per 1.0-ml·kg^{-1}·min^{-1} increase)	46.8	0.85 (0.80–0.89)	<0.0001	0.92 (0.86–0.99)	0.026
$\dot{V}E/\dot{V}CO_2$ slope (per 1.0 increase)	27.5	1.06 (1.04–1.08)	<0.0001	1.02 (0.99–1.05)	0.12

CI, confidence interval; HR, hazard ratio. Other abbreviations as in Table 1.

ameter (LVDd), LV end-systolic diameter (LVDs), and left atrial diameter (LAD) were determined on 2-D echocardiography. LVEF was measured on echocardiography, radionuclide ventriculography, or LV angiography. Blood samples were drawn for standard measurements (serum creatinine, sodium, and hemoglobin) and B-type natriuretic peptide (BNP) within 3 days before or after CPX. Plasma BNP concentration was measured by radioimmunoassay (Shionoria BNP kit; Shionogi & Co Ltd, Osaka, Japan).

Endpoints

All patients survived to discharge. Follow-up data were determined from outpatient records, and at least 1 year of follow-up was available in all patients. Endpoints were composite outcome, defined as all-cause death or HF hospitalization and all-cause death, analyzed by time from the date of CPX to first event. HF hospitalization required that a patient had typical symptoms and signs, treatment with diuretics, and at least an overnight hospital stay. Event rate was assessed when patients were dichotomized at an optimal threshold value and were divided according to quartiles of each predictor.

Statistical Analysis

Continuous variables, presented as mean±SD, were compared using unpaired Student t-test, and categorical variables with the chi-squared test. Cox proportional hazard analysis was used to assess the association between variables and outcome. Variables with P<0.1 on univariate analysis were included in multivariate analysis. The optimal dichotomous values were identified on receiver operating characteristic (ROC) curve analysis. The association of specific variables with time to outcome was expressed as hazard ratio and 95% confidence interval (CI). Cumulative events were assessed using the Kaplan-Meier method, and differences in events were compared with log-rank test. P<0.05 was considered to be statistically significant.

Results

Baseline Characteristics

From March 2002 through September 2012, 283 consecutive patients met the inclusion criteria; baseline characteristics for the overall group are listed in **Table 1**. Overall, the mean age was 61.8 years, 81% were men, and 45% had ischemic heart disease. Most of the patients were treated with β-blockers (92%), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (83%), and diuretics (82%).

The median time from HF hospitalization to CPX was 29 days. In CPX, the mean pRER was 1.26±0.13 (median, 1.24; range, 0.95–1.78), and pRER >1.10 and >1.20 was obtained in 89% and in 66%, respectively (**Figure 1**). When patients were divided into subgroups according to age or LVEF (**Table S1**), the mean pRER was >1.20 in all subgroups, except in the subgroup of patients aged ≥75 years (1.19±0.11). During CPX, asymptomatic ST-segment depression occurred in 32 patients (11%), asymptomatic non-sustained (3 or 4 beats) ventricular tachycardia in 7 patients (2.5%), and symptomatic post-exercise hypotension in 4 patients (1.4%), but all events resolved without any treatment during the recovery period. There was no exercise-related death or adverse event requiring hospitalization, including worsening HF, myocardial infarction, or sustained ventricular tachycardia.

Predictors of Composite Outcome

The median follow-up period was 47 months. The composite of all-cause death or HF hospitalization occurred in 111 patients (39.2%), and all-cause death in 48 patients (17.0%). The rate of the combined event was 15.9% at 1 year, 22.9% at 2 years, and 32.0% at 3 years. The all-cause mortality rate was 2.8% at 1 year, 6.5% at 2 years, and 10.3% at 3 years.

On univariate Cox regression analysis, serum creatinine, sodium, hemoglobin, BNP, LVDd, LVDs, LVEF, LAD, peak $\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$ slope, and the presence of diabetes were all significantly related to composite outcome, while age, gender, body mass index, and presence of hypertension, ischemic heart disease, and atrial fibrillation were not (**Table 2**). Among them,

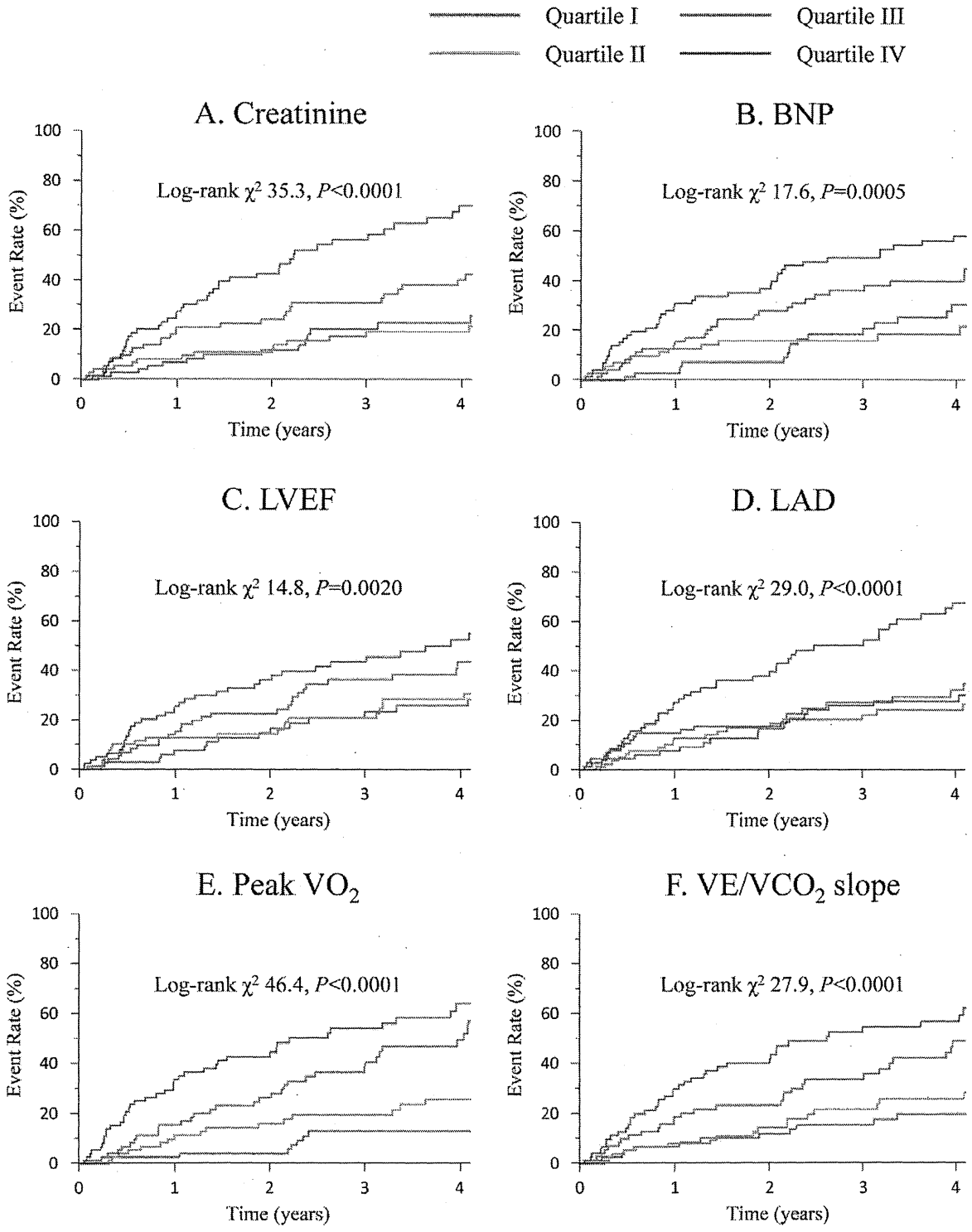

Figure 2. Kaplan-Meier cumulative curves for the combined event according to quartiles of major predictors. Patients were divided according to quartiles of each predictor as follows: (**A**) creatinine: quartile I, <0.83 (n=71); II, ≥0.83 to <1.03 (n=72); III, ≥1.03 to <1.26 (n=71); IV, ≥1.26 mg/dl (n=69); (**B**) BNP: quartile I, <96 (n=69); II, ≥96 to <200 (n=70); III, ≥200 to <370 (n=70); IV, ≥370 pg/ml (n=71); (**C**) LVEF: quartile I, >31 (n=72); II, >25 to ≤31 (n=67); III, >20 to ≤25 (n=77); IV, ≤20% (n=67); (**D**) LAD: quartile I, <40 (n=64); II, ≥40 to <45 (n=77); III, ≥45 to <51 (n=73); IV, ≥51 mm (n=68); (**E**) peak $\dot{V}O_2$: quartile I, >20.0 (n=72); II, >16.5 to ≤20.0 (n=70); III, >13.6 to ≤16.5 (n=70); IV, ≤13.6 ml·kg^{-1}·min^{-1} (n=71); and (**F**) $\dot{V}E/\dot{V}CO_2$ slope: quartile I, <29.0 (n=70); II, ≥29.0 to <32.3 (n=74); III, ≥32.3 to <38.0 (n=69); IV, ≥38.0 (n=70).

Table 3. Predictive Power of CPX Parameters According to pRER

Variables	Univariate analysis			Bivariate analysis	
	X^2	HR (95% CI)	P-value	HR (95% CI)	P-value
Overall (n=283)					
Peak $\dot{V}O_2$ (per 1.0-ml·kg^{-1}·min^{-1} increase)	46.8	0.85 (0.80–0.89)	<0.0001	0.87 (0.82–0.92)	<0.0001
$\dot{V}E/\dot{V}CO_2$ slope (per 1.0 increase)	27.5	1.06 (1.04–1.08)	<0.0001	1.03 (1.004–1.05)	0.021
pRER ≥1.20 (n=188)					
Peak $\dot{V}O_2$ (per 1.0-ml·kg^{-1}·min^{-1} increase)	29.1	0.85 (0.80–0.90)	<0.0001	0.87 (0.81–0.94)	0.0001
$\dot{V}E/\dot{V}CO_2$ slope (per 1.0 increase)	15.9	1.05 (1.03–1.07)	<0.0001	1.02 (0.99–1.05)	0.22
pRER <1.20 (n=95)					
Peak $\dot{V}O_2$ (per 1.0-ml·kg^{-1}·min^{-1} increase)	17.6	0.83 (0.76–0.91)	<0.0001	0.84 (0.76–0.93)	0.0008
$\dot{V}E/\dot{V}CO_2$ slope (per 1.0 increase)	14.7	1.10 (1.05–1.15)	0.0001	1.08 (1.03–1.13)	0.0035

Abbreviations as in Tables 1,2.

Table 4. Cox Regression Analysis for Combined Event as Optimal Dichotomous Value

Variables	Area under ROC curve	Optimal cut-off	Univariate analysis			Multivariate analysis	
			X^2	HR (95% CI)	P-value	HR (95% CI)	P-value
Creatinine	0.660	1.03 mg/dl	22.1	2.50 (1.70–3.75)	<0.0001	1.57 (1.03–2.42)	0.038
Hemoglobin	0.626	13.2 g/dl	13.4	2.03 (1.39–3.00)	0.0002	1.74 (1.14–2.69)	0.011
Sodium	0.624	139 mEq/L	6.3	1.62 (1.11–2.38)	0.012	1.09 (0.73–1.65)	0.66
BNP	0.662	208.0 pg/ml	18.7	2.30 (1.57–3.42)	<0.0001	1.36 (0.90–2.09)	0.15
LVDd	0.635	63 mm	10.1	1.87 (1.28–2.82)	0.0015	1.58 (0.95–2.67)	0.077
LVDs	0.640	52 mm	7.4	1.72 (1.16–2.60)	0.0064	0.94 (0.53–1.68)	0.82
LVEF	0.606	30%	8.8	1.97 (1.25–3.25)	0.0029	1.72 (0.99–3.04)	0.0503
LAD	0.642	47 mm	13.8	2.03 (1.40–2.98)	0.0002	1.89 (1.25–2.89)	0.0024
Peak $\dot{V}O_2$	0.708	15.5 ml·kg^{-1}·min^{-1}	40.4	3.42 (2.34–5.06)	<0.0001	1.82 (1.16–2.90)	0.0086
$\dot{V}E/\dot{V}CO_2$ slope	0.668	34.7	22.2	2.46 (1.70–3.60)	<0.0001	1.52 (1.01–2.31)	0.046

ROC, receiver operating characteristic. Other abbreviations as in Tables 1,2.

Table 5. Cox Regression Analysis for All-Cause Mortality

Variables	Univariate analysis			Multivariate analysis	
	X^2	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (per 10-year increase)	2.3	0.85 (0.69–1.05)	0.13		
Male sex	1.7	1.78 (0.77–5.15)	0.19		
Hypertension	1.5	0.70 (0.40–1.25)	0.23		
Diabetes	0.47	1.22 (0.69–2.16)	0.50		
BMI (per 1.0-kg/m^2 increase)	0.10	0.99 (0.91–1.07)	0.75		
Ischemic	0.46	1.22 (0.69–2.15)	0.50		
AF rhythm	0.36	1.24 (0.59–2.40)	0.55		
Creatinine (per 1.0-mg/dl increase)	8.2	2.84 (1.41–5.44)	0.0042	1.37 (0.59–3.05)	0.45
Hemoglobin (per 1.0-g/dl increase)	4.4	0.84 (0.71–0.99)	0.036	1.02 (0.84–1.25)	0.80
Sodium (per 1.0-mEq/L increase)	11.9	0.86 (0.80–0.94)	0.0006	0.94 (0.86–1.02)	0.16
BNP (per 10-pg/ml increase)	8.5	1.011 (1.004–1.017)	0.0036	1.003 (0.993–1.01)	0.51
LVDd (per 1.0-mm increase)	8.8	1.05 (1.02–1.07)	0.0030	1.01 (0.90–1.13)	0.83
LVDs (per 1.0-mm increase)	9.1	1.04 (1.02–1.07)	0.0026	1.02 (0.92–1.15)	0.71
LVEF (per 1.0% increase)	5.2	0.96 (0.92–0.99)	0.023	1.003 (0.95–1.07)	0.92
LAD (per 1.0-mm increase)	13.8	1.07 (1.03–1.11)	0.0002	1.04 (1.002–1.08)	0.038
Peak $\dot{V}O_2$ (per 1.0-ml·kg^{-1}·min^{-1} increase)	27.7	0.81 (0.75–0.88)	<0.0001	0.85 (0.76–0.95)	0.0041
$\dot{V}E/\dot{V}CO_2$ slope (per 1.0 increase)	11.3	1.05 (1.02–1.08)	0.0008	1.003 (0.96–1.04)	0.89

Abbreviations as in Tables 1,2.

peak $\dot{V}O_2$ was the most powerful predictor of combined event (X^2=46.8; P<0.0001), followed by $\dot{V}E/\dot{V}CO_2$ slope (X^2=27.5; P<0.0001). On multivariate analysis, peak $\dot{V}O_2$, creatinine, and LAD were significant independent predictors of the combined endpoint, while $\dot{V}E/\dot{V}CO_2$ slope, BNP, and LVEF were not.

Figure 2 shows Kaplan-Meier cumulative curves for the combined event according to quartiles of 6 major predictors. As compared with the highest quartile, the lowest quartile had

Table 6. Cox Regression Analysis for All-Cause Mortality as Optimal Dichotomous Value

Variables	Area under ROC curve	Optimal cut-off	Univariate analysis			Multivariate analysis	
			X^2	HR (95% CI)	P-value	HR (95% CI)	P-value
Creatinine	0.610	1.34 mg/dl	17.9	3.85 (2.11–6.94)	<0.0001	1.54 (0.75–3.10)	0.23
Hemoglobin	0.578	13.6 g/dl	7.0	2.31 (1.23–4.64)	0.0082	1.83 (0.87–4.10)	0.12
Sodium	0.638	138 mEq/L	8.9	2.38 (1.35–4.29)	0.0028	1.46 (0.76–2.85)	0.26
BNP	0.662	256.0 pg/ml	10.9	2.63 (1.48–4.80)	0.0009	1.71 (0.91–3.27)	0.095
LVDd	0.628	66 mm	7.9	2.25 (1.28–4.03)	0.0051	1.87 (0.84–4.53)	0.13
LVDs	0.629	53 mm	9.2	2.53 (1.37–4.97)	0.0025	0.99 (0.36–2.61)	0.99
LVEF	0.570	29%	6.8	2.43 (1.23–5.36)	0.0092	1.47 (0.62–3.69)	0.39
LAD	0.676	49 mm	22.1	4.02 (2.24–7.46)	<0.0001	3.50 (1.81–7.01)	0.0002
Peak $\dot{V}O_2$	0.707	16.0 ml·kg^{-1}·min^{-1}	24.7	4.53 (2.45–8.92)	<0.0001	2.19 (1.05–4.73)	0.036
$\dot{V}E/\dot{V}CO_2$ slope	0.657	36.6	14.2	3.05 (1.72–5.42)	0.0002	1.46 (0.76–2.82)	0.26

Abbreviations as in Tables 1,2,4.

a hazard ratio of 6.08 (95% CI, 3.26–12.2) in peak $\dot{V}O_2$ and 2.28 (95% CI, 1.33–4.02) in LVEF. As compared with the lowest quartile, the highest quartile had a hazard ratio of 3.64 (95% CI, 2.11–6.58) in creatinine, 3.17 (95% CI, 1.86–5.59) in $\dot{V}E/\dot{V}CO_2$ slope, 2.63 (95% CI, 1.57–4.56) in LAD, and 2.50 (95% CI, 1.49–4.35) in BNP. The analysis confirmed the superior prognostic value of peak $\dot{V}O_2$ compared with the other predictors.

Predictive Power of CPX Parameters According to pRER

When patients were divided into subgroups according to pRER, peak $\dot{V}O_2$ was greater in predicting the combined endpoint than $\dot{V}E/\dot{V}CO_2$ slope among patients with pRER ≥1.20, whereas peak $\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ slope had a similar predictive value among those with pRER <1.20. On bivariate analysis, peak $\dot{V}O_2$, but not $\dot{V}E/\dot{V}CO_2$ slope, was an independent predictor of the combined endpoint in the high pRER subgroup, while both peak $\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ slope were independent predictors in the low pRER subgroup (**Table 3**).

Predictive Power as a Dichotomous Value

The area under the ROC curve was greater for peak $\dot{V}O_2$ (0.708) than for any predictors including $\dot{V}E/\dot{V}CO_2$ slope (**Table 4**). When patients were dichotomized at each optimal threshold value identified on ROC curve analysis, peak $\dot{V}O_2$ <15.5 ml·kg^{-1}·min^{-1} was the most powerful predictor of outcome, followed by $\dot{V}E/\dot{V}CO_2$ slope >34.7, creatinine >1.03 mg/dl, and BNP >208.0 pg/ml.

On multivariate analysis, peak $\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$ slope, creatinine, hemoglobin, and LAD were significant independent predictors of the combined endpoint, while BNP and LVEF were not (**Table 4**).

Predictors of All-Cause Mortality

On univariate Cox regression analysis, peak $\dot{V}O_2$ was the most powerful predictor of all-cause mortality among well-known predictors as both a continuous and an optimal dichotomous variable (<16.0 ml·kg^{-1}·min^{-1}), and only peak $\dot{V}O_2$ and LAD were significant independent predictors on multivariate analysis (**Tables 5,6**).

Discussion

Peak $\dot{V}O_2$ is an established prognostic predictor in HF patients,[7] but is influenced by subjective factors such as patient effort. Although recent studies consistently reported that $\dot{V}E/\dot{V}CO_2$ slope was greater than peak $\dot{V}O_2$ in predicting outcome, the attained mean pRER, an objective index of effort adequacy, was relatively low (1.05–1.10) in those studies.[14–18] The main finding of the present study is that in HF patients with LV systolic dysfunction receiving current optimal medical therapy, peak $\dot{V}O_2$ has the most powerful predictive value for morbidity and mortality among well-known prognostic markers including $\dot{V}E/\dot{V}CO_2$ slope, when the patients are strongly encouraged to exercise toward a target pRER >1.20. Importantly, we also observed no major exercise-related adverse event despite maximum effort even in patients with advanced LV dysfunction.

pRER >1.10 has been generally considered to be a good effort[10–12] and is used as a target level in clinical practice and trials, including the Heart Failure: A Controlled Trial Investigating Outcomes of exercise traiNing (HF-ACTION) study, the largest trial to test the effect of exercise training in HF patients with LV systolic dysfunction (median LVEF, 25%).[19] Peak $\dot{V}O_2$, however, was shown to be less prognostically reliable in HF patients with pRER <1.15 than in those with pRER ≥1.15.[13] Additionally, Arena et al evaluated the prognostic utility of CPX during 2 distinct time periods and showed that the predictive value of peak $\dot{V}O_2$ was greater during the recent period (mean pRER, 1.14) than during the previous period (mean pRER, 1.09).[20] In a study with a mean pRER of 1.19, Corrà et al reported that peak $\dot{V}O_2$, but not $\dot{V}E/\dot{V}CO_2$ slope, was an independent predictor of major cardiac events, but only a small proportion of their patients (31%) had received β-blocker therapy.[21] To the best of our knowledge, this is the first study evaluating the prognostic value of these parameters in CPX targeting pRER >1.20 (mean, 1.26) for HF patients receiving current optimal medical therapy (β-blockers, 92%). As both a continuous and an optimal dichotomous variable, peak $\dot{V}O_2$ was greater in predicting outcome than any prognostic markers. Furthermore, we found that peak $\dot{V}O_2$ was more powerful in predicting morbidity than $\dot{V}E/\dot{V}CO_2$ slope, the effort-independent predictor, among patients with pRER ≥1.20, while the 2 parameters had a similar predictive value among those with pRER <1.20. These findings suggest that targeting pRER >1.20 enhances the prognostic power of peak $\dot{V}O_2$.

Mezzani et al showed that among the subgroup of patients with reduced peak $\dot{V}O_2$, the composite event rate was significantly lower in patients with low pRER (<1.15) than in those with high pRER (≥1.15).[13] This can be explained by the underestimation of peak $\dot{V}O_2$ because of poor effort in their patients

with low pRER. In contrast to their results, we did not find that patients with pRER <1.20 had better outcome than those with pRER ≥1.20 among the subgroup of patients with reduced peak $\dot{V}O_2$ (<15.5 ml·kg^{-1}·min^{-1}). The most likely explanation is that the present patients with low pRER may have stopped their exercise tests for reasons other than poor effort, mainly exercise-limiting comorbidities associated with advanced disease severity.

In accordance with previous studies,[14–18] we observed the powerful prognostic value of $\dot{V}E/\dot{V}CO_2$ slope as both a continuous and an optimal dichotomous variable, and, particularly among the low pRER subgroup, $\dot{V}E/\dot{V}CO_2$ slope was similar to peak $\dot{V}O_2$ in predicting the combined endpoint. Because of the effort-independent characteristic, $\dot{V}E/\dot{V}CO_2$ slope is considered to be very useful for risk stratification in patients unable to exercise at maximum effort due to comorbidities such as joint disorder and aortic aneurysm.

The present study assessed the predictive value of well-known non-CPX parameters. Plasma BNP is a reliable and established prognostic biomarker in HF patients,[22,23] and previous studies showed that peak $\dot{V}O_2$ and BNP had an similar impact in predicting outcome among HF patients, although they did not report the attained pRER.[24–26] In the present study BNP was an inferior predictor compared with peak $\dot{V}O_2$ in CPX targeting pRER >1.20, and the predictive value was not significant in multivariate analysis including CPX parameters.

It has been demonstrated that LA size is a barometer of LV filling pressure and reflects the burden of diastolic dysfunction,[27] and that LA enlargement is associated with an increased risk of cardiovascular events and death.[28] But there are only limited data comparing CPX parameters with LA size as a prognostic marker, while many studies have compared CPX parameters with LV size or function. We found LAD to be a significant independent predictor of both morbidity and mortality on multivariate analysis. These findings suggest that LA size represents different aspects of HF physiology not reflected by exercise capacity, more greatly than LV parameters or BNP.

The safety of CPX has been evaluated in some studies. Among large series of subjects with and without known disease, serious complications have been reported to be rare.[29] In the HF-ACTION study, there were no deaths, and non-fatal major cardiovascular events occurred in <0.5 per 1000 tests among 2,037 HF subjects (median LVEF, 25%) who completed 4,411 CPX (median pRER, 1.09).[30] The incidence of adverse events, however, is likely to vary depending on the subjects or exercise effort. In this context, the fact that no major exercise-related adverse event occurred in the present patients with advanced LV dysfunction (median LVEF, 24%) and excellent effort (median pRER, 1.24) is an important point. Although further larger studies are needed to confirm the safety, it seems unlikely that CPX targeting a high pRER is associated with an increased exercise-related risk.

Study Limitations

This study has some limitations. First, we examined data obtained from routine CPX with a target pRER >1.20 at our institution, retrospectively. Prospective studies are required to determine the optimal target pRER for the most reliable risk stratification with peak $\dot{V}O_2$. Second, because we studied patients who underwent CPX among those admitted with HF, they were relatively young and the prognosis was less poor compared with general HF patients.[1,2] Similarly, given that the present cohort consisted of HF patients with impaired LV function and without severe renal dysfunction, it remains uncertain whether the present findings apply to HF patients with preserved EF or those with chronic renal failure. Finally, there are some major predictors for HF patients that were not available in this study, including echocardiographic indices of LV diastolic function, particularly tissue Doppler imaging, which has been shown to be a strong prognostic marker, independently of CPX parameters[31] or BNP.[32]

Conclusions

In CPX targeting pRER >1.20 for HF patients receiving current optimal medical therapy, peak $\dot{V}O_2$ had the most powerful predictive value of morbidity and mortality among well-known prognostic markers including $\dot{V}E/\dot{V}CO_2$ slope, BNP, and LVEF, without an increased risk of exercise-related events. The present results advocate a higher target pRER than the commonly used level (>1.10) in CPX even for advanced HF patients.

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Supplementary Files

Supplementary File 1

Table S1. Mean peak respiratory exchange ratio

Please find supplementary file(s);
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Chronic vagal nerve stimulation improves baroreflex neural arc function in heart failure rats

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Kawada T, Li M, Zheng C, Shimizu S, Uemura K, Turner MJ, Yamamoto H, Sugimachi M. Chronic vagal nerve stimulation improves baroreflex neural arc function in heart failure rats. *J Appl Physiol* 116: 1308–1314, 2014. First published March 27, 2014; doi:10.1152/japplphysiol.00140.2014.—We tested whether 6-wk vagal stimulation (VS) treatment improved open-loop baroreflex function in rats after myocardial infarction (MI). The following three groups of Sprague-Dawley rats were examined: normal control (NC, $n = 9$), MI with no treatment (MI-NT, $n = 8$), and MI treated with VS (MI-VS, $n = 7$). Under anesthesia, a stepwise input ranging from 60 to 180 mmHg was imposed on isolated carotid sinus baroreceptor regions, while the responses in splanchnic sympathetic nerve activity (SNA) and arterial pressure (AP) were measured. The response range of percent SNA was greater in the MI-VS than in the MI-NT group (63.8 ± 4.9% vs. 33.1 ± 3.8%, $P < 0.01$). The slope of the AP response to percent SNA was not different between the MI-VS and MI-NT groups (0.611 ± 0.076 vs. 0.781 ± 0.057 mmHg/%). The difference in the response range of AP between the MI-VS and MI-NT groups did not reach statistical significance (40.7 ± 6.2 vs. 26.4 ± 3.5 mmHg). In conclusion, the 6-wk VS treatment significantly improved the baroreflex control of SNA, but the effect was limited for the baroreflex total-loop function due to the lack of significant improvement in the AP response to percent SNA.

carotid sinus baroreflex; open-loop analysis; sympathetic nerve activity; myocardial infarction

PROGRESSION of heart failure is associated with autonomic imbalance characterized by sympathetic overactivity and vagal withdrawal (5, 11). Although mechanisms involved in the autonomic imbalance are not completely elucidated, impaired arterial and cardiac baroreflexes are important contributing factors (1, 3, 26). To correct the autonomic imbalance, vagal stimulation (VS) has been proposed as a therapeutic approach for heart failure (12, 14, 20, 23–25, 29). In a rat model of chronic heart failure following myocardial infarction (MI), 6-wk VS has been shown to improve the survival rate (14). However, whether the VS treatment improves the impaired arterial baroreflex function in MI-induced heart failure remains undetermined. A close association between the dysfunction of the autonomic nervous system and the derangement of the immune system is postulated in the pathophysiology of heart failure (5). Systemic inflammatory responses are observed in heart failure, and pharmacological vagal activation (13) or electrical VS (29) can reduce the inflammatory responses. The VS treatment may ameliorate inflammation in the heart and reduce pathological afferent signals from the damaged heart to the central nervous system. Because cardiac sympathetic afferent activation inhibits the baroreflex control of sympathetic nerve activity (SNA) in heart failure (3), it is postulated that the VS treatment may improve baroreflex function. To gain an insight into the treatment mechanism of VS, the present study examined the effects of 6-wk VS on the arterial baroreflex function in MI rats.

The arterial baroreflex system may be analyzed by dividing it into two principal subsystems: a neural arc subsystem from pressure input to efferent SNA, and a peripheral arc subsystem from SNA to arterial pressure (AP) (16, 18). Under normal physiological conditions, an increase in AP suppresses SNA via the arterial baroreflex, which resultantly decreases AP, and vice versa. This closed-loop feedback operation makes it difficult to separately quantify the characteristics of the neural and peripheral arc subsystems. To circumvent the problem, the present study employed an isolated carotid sinus preparation by which the open-loop baroreflex function can be assessed over the entire operating range of the carotid sinus baroreflex (7–9, 27).

MATERIALS AND METHODS

Animal care was provided in strict accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences*, approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee of National Cerebral and Cardiovascular Center. The following groups of Sprague-Dawley rats were examined: normal control (NC), MI with no treatment (MI-NT), and MI with VS treatment (MI-VS).

Creation of myocardial infarction and device implantation. For the MI-NT and MI-VS groups, the left coronary artery was ligated under halothane anesthesia in 8-wk male rats (14). One week later, the rats in the MI-VS group underwent a second surgery for implanting a radiocontrolled pulse generator (ISE1010C, Unimec) to stimulate the right vagus and an electrocardiogram transmitter (TA10CA-F10, Data Science International) to monitor heart rate (HR). The total weight of the implanted devices was 17 g. This value was subtracted in the measurement of body weight in the MI-VS group. The VS treatment was started from 1 wk after the device implantation (i.e., 2 wk after the MI induction). The right vagus was stimulated using a rectangular pulse of 0.2-ms duration at 20 Hz for 10 s every minute according to our previous study (14). The intensity of VS was adjusted in each rat to reduce HR by 20–30 beats/min. The VS treatment was continued for 6 wk until the day of the acute experiment described below. The VS was ceased when the rat was removed out of the cage for the acute baroreflex study. Because the intraperitoneal anesthesia took ~30 min to develop its sufficient effect and the preparation for venous and arterial lines took 10–20 min, an acute effect of VS might have been lost before the measurement of baseline AP and HR.

Acute baroreflex study. Each rat was anesthetized with an intraperitoneal injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml), and mechanically ventilated with oxygen-supplied room air. Anesthesia was maintained by a continuous intravenous infusion of a diluted solution of the above anesthetic mixture from the right femoral vein. An arterial catheter was inserted

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Table 1. *Number of animals used in the acute baroreflex study*

	NC	MI-NT	MI-VS
Total number	9	15	10
Death during operation	0	2	0
Small biventricular weight[a]	N/A	2	1
Diminished baroreflex function[b]	0	3	0
Electrode failure[c]	N/A	N/A	2
Included in final data analysis	9	8	7

NC, normal control; MI-NT, myocardial infarction with no treatment; MI-VS, myocardial infarction treated with vagal stimulation; N/A, not applicable. [a]Small biventricular weight was defined as normalized biventricular weight of less than 2.2 g/kg, which suggested insufficient myocardial infarction to induce cardiac remodeling. [b]Diminished baroreflex function was defined as a magnitude of arterial pressure response of less than 10 mmHg during the input pressure change from 60 to 180 mmHg. Because some litters of rats do not tolerate the bilateral carotid occlusion, we cannot determine whether the diminished baroreflex was truly attributable to the effect of myocardial infarction. Hence the data from these 3 rats were excluded from the final data analysis. This exclusion does not affect our main conclusion because including the 3 rats would only exaggerate the impaired baroreflex function in the MI-NT group. [c]Electrode failure was defined as the lack of periodic bradycardia during 10 s on and 50 s off vagal stimulation, which suggested broken lead wire. Although nerve injury may also result in the lack of bradycardia, discoloration of the nerve in the cuff electrode was not observed by macroscopic inspection.

into the right femoral artery to measure AP and HR. Another catheter was inserted into the left femoral vein to measure central venous pressure. Body temperature of the animal was maintained at ~38°C using a heating pad and a lamp.

A postganglionic branch of the splanchnic sympathetic nerve was exposed retroperitoneally, and a pair of stainless steel wire electrodes (Bioflex wire, AS633, Cooner Wire) was attached to the nerve. This nerve was selected as representing the sympathetic outflow because the splanchnic vascular bed contributes to approximately one-third of the change in the total vascular conductance in the AP regulation (17). This nerve is thicker than the often-used renal sympathetic nerve and was easy to locate even when the surrounding tissue was edematous in the MI rats. The nerve and electrodes were covered with silicone glue (Kwik-Sil, World Precision Instruments). A preamplified nerve signal was band-pass filtered at 150–1,000 Hz, and then full-wave rectified and low-pass filtered at a cut-off frequency of 30 Hz using analog circuits. A ganglionic blocker hexamethonium bromide (60 mg/kg) was given intravenously at the end of the experiment to confirm the disappearance of SNA and to measure the noise level.

The aortic depressor nerves and the vagal nerves were sectioned, and carotid sinus baroreceptor regions were isolated from systemic circulation bilaterally (19, 21). The isolated carotid sinuses were filled with warmed Ringer's solution through catheters inserted into the common carotid arteries. Carotid sinus pressure (CSP) was controlled using a servocontrolled piston pump. Heparin sodium (100 U/kg) was given intravenously to prevent blood coagulation.

Protocols. After completing the above surgery, the baroreflex-mediated AP response was monitored for more than 30 min. The rat was excluded from the final data analysis in the event that the reflex response to CSP was progressively diminished within this stabilization period. Possible causes for the deterioration include surgical damage to the carotid sinus nerves and brain ischemia due to the bilateral carotid occlusion. Although the vertebral arteries were kept patent, some litters of rats do not tolerate the bilateral carotid occlusion. We occasionally experience diminished baroreflex function even in normal rats. In the present study, three rats assigned to the MI-NT group were excluded from the final data analysis because of diminished baroreflex function (Table 1). This exclusion does not affect our main conclusion, because including the three rats would only exaggerate the impaired baroreflex function in the MI-NT group.

After the stabilization, CSP was decreased to 60 mmHg for 4 min, and then increased stepwise from 60 to 180 mmHg in increments of 20 mmHg every minute (8). The data obtained from two consecutive stepwise CSP inputs were averaged for analysis.

Data analysis. The rats assigned to the MI-NT and MI-VS groups were excluded from the final data analysis when the normalized biventricular weight was less than 2.2 g/kg (based on the mean plus 1 SD of the NC group), because it indicated insufficient development of heart failure due to small MI (Table 1). On the other hand, two rats in the MI-NT group did not survive the acute baroreflex study due to severe heart failure (the normalized biventricular weights were 2.97 and 3.00 g/kg, respectively). Resultantly, 9 rats in the NC group, 8 rats in the MI-NT group and 7 rats in the MI-VS group were used for the final data analysis (Table 1).

Data were acquired at 1,000 Hz using a 16-bit analog-to-digital converter. Mean SNA, AP, and HR values were calculated at each CSP level by averaging the data during the last 10 s of each step. Because the absolute amplitude of SNA varied among animals depending on recording conditions, SNA was presented in percent values in each animal. The SNA value corresponding to the CSP level of 60 mmHg was assigned to 100%. The noise level after the hexamethonium administration was assigned to 0%.

The CSP-SNA, CSP-AP, and CSP-HR relationships approximated an inverse sigmoid curve and were quantified using a four-parameter logistic function as (10):

$$y = \frac{P_1}{1 + \exp[P_2(\mathrm{CSP} - P_3)]} + P_4$$

where y denotes the output (SNA, AP, or HR); P_1 is the response range of y (i.e., the difference between the maximum and minimum values of y); P_2 is the slope coefficient; P_3 is the midpoint of the sigmoid curve on the CSP axis; and P_4 is the minimum value of y (i.e., the lower plateau of the sigmoid curve). The maximum gain or the maximum slope of the sigmoid curve was calculated as $P_1P_2/4$.

The SNA-AP relationship approximated a straight line and was quantified using a linear regression as:

$$\mathrm{AP} = \mathrm{P_a SNA} + \mathrm{P_b}$$

where P_a and P_b are the slope and intercept, respectively.

Statistical analysis. All data are presented as means and SE values. Each parameter was compared among NC, MI-NT, and MI-VS groups using one-way analysis of variance (ANOVA) followed by Tukey test for simultaneous multiple comparisons. Differences were considered significant when $P < 0.05$ (4).

RESULTS

As shown in Table 2, body weight was not different among the three groups. Biventricular weight was significantly heavier

Table 2. *Body weight, biventricular weight, and baseline hemodynamics*

	NC (n = 9)	MI-NT (n = 8)	MI-VS (n = 7)
Body wt, kg	0.486 ± 0.012	0.468 ± 0.013	0.462 ± 0.012
Biventricular wt, g	1.001 ± 0.023	1.292 ± 0.042†	1.289 ± 0.054†
Normalized biventricular wt, g/kg	2.06 ± 0.04	2.76 ± 0.08†	2.80 ± 0.11†
Central venous pressure, mmHg	1.52 ± 0.17	3.14 ± 0.30†	2.41 ± 0.16*
Mean arterial pressure, mmHg	126.6 ± 5.9	110.9 ± 5.8	115.0 ± 4.8
Heart rate, beats/min	408.6 ± 13.1	379.5 ± 12.6	380.1 ± 9.5

Values are means ± SE. NC, normal control; MI-NT, myocardial infarction with no treatment; MI-VS, myocardial infarction treated with vagal stimulation. *$P < 0.05$, †$P < 0.01$ vs. NC by Tukey test following one-way ANOVA.

in the MI-NT and MI-VS groups compared with the NC group. Central venous pressure, measured just after inserting arterial and venous catheters and before isolating baroreceptor regions, was higher in the MI-NT and MI-VS groups compared with the NC group. Mean AP and HR under the baseline conditions did not differ among the three groups.

Typical time series obtained from the NC, MI-NT, and MI-VS rats are shown in Fig. 1. The gray lines in the AP and HR signals represent 200-Hz resampled data. The gray lines in the SNA signals represent 10-Hz resampled data. The black lines in the SNA, AP, and HR signals indicate 2-s moving averaged data. The increase in CSP decreased SNA, AP, and HR in all groups, but the baroreflex responses in the MI-NT rat were smaller than those in the NC and MI-VS rats.

Static characteristics of the baroreflex neural arc obtained from the NC, MI-NT, and MI-VS groups are illustrated in Fig. 2, *A–C*. The response range of percent SNA was significantly narrower in the MI-NT group than in the NC group and was significantly wider in the MI-VS than in the MI-NT group (Fig. 2*D*). Differences in the maximum slope among the three groups were not statistically significant (Fig. 2*E*). The midpoint pressure was significantly lower in the MI-NT group than in the NC group, but the difference was not observed between the NC and MI-VS group (Fig. 2*F*). Changes in the lower plateau of SNA were reciprocal to those in the response range of SNA because the maximum SNA was assigned to 100% in each animal (Fig. 2*G*).

Static characteristics of the baroreflex peripheral arc approximated a straight line in all of the NC, MI-NT, and MI-VS groups (Fig. 3, *A–C*). The slope was significantly smaller in the MI-NT and MI-VS groups than in the NC group (Fig. 3*D*). The slope did not differ significantly between the MI-NT and MI-VS groups. The intercept tended to be higher in the MI-VS than in the NC group, but the difference in the intercept was not statistically significant among the three groups (Fig. 3*E*).

Static characteristics of the baroreflex total loop obtained from the NC, MI-NT, and MI-VS groups are summarized in Fig. 4, *A–C*. The response range of AP was significantly narrower in the MI-NT and MI-VS groups than in the NC group (Fig. 4*D*). The maximum gain was significantly smaller in the MI-NT group than in the NC group, but was not significantly different between the NC and MI-VS groups (Fig. 4*E*). The midpoint pressure and the lower plateau did not differ significantly among the three groups (Fig. 4, *F* and *G*).

Static characteristics of the baroreflex control of HR obtained from the NC, MI-NT, and MI-VS groups are depicted in Fig. 5, *A–C*. The response range of HR was significantly narrower in the MI-NT group than in the NC and MI-VS groups (Fig. 5*D*). The maximum slope of the HR response was significantly greater in the MI-VS group than in the MI-NT group (Fig. 5*E*). The midpoint pressure and the lower plateau did not differ significantly among the three groups (Fig. 5, *F* and *G*).

DISCUSSION

The present results demonstrated that the 6-wk VS treatment improved the ability of the carotid sinus baroreflex to suppress

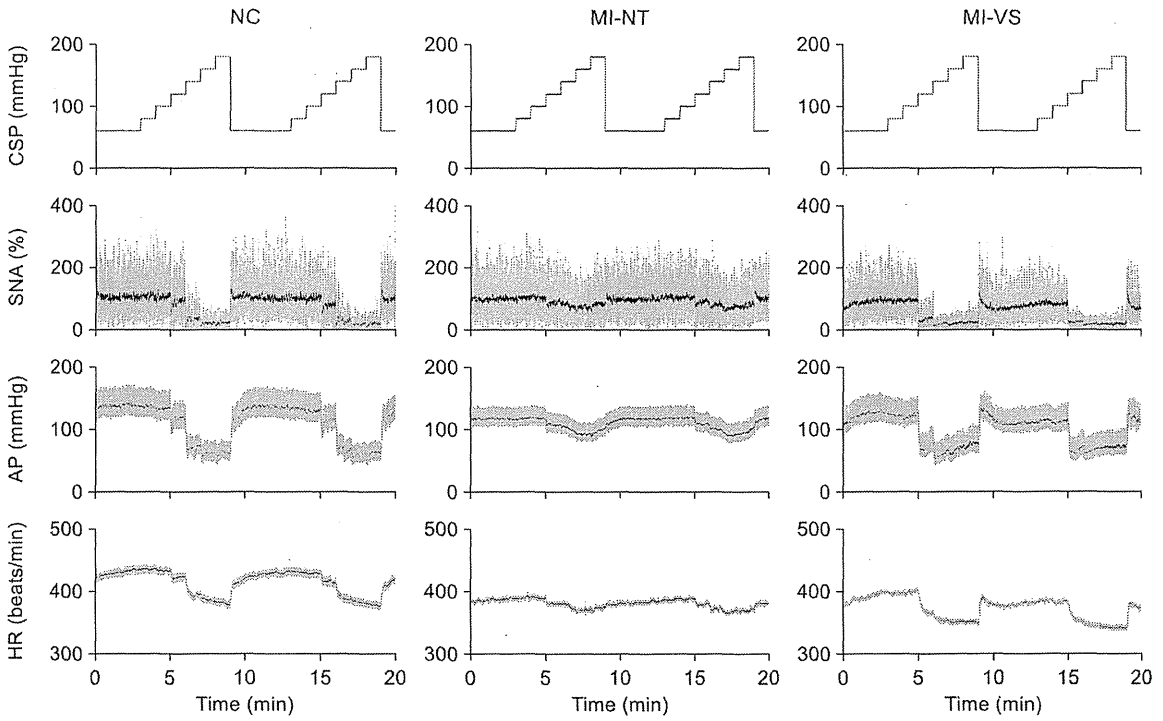

Fig. 1. Typical experimental recordings of carotid sinus pressure (CSP), sympathetic nerve activity (SNA), arterial pressure (AP), and heart rate (HR) obtained from rats of normal control (NC), myocardial infarction with no treatment (MI-NT), and myocardial infarction with vagal stimulation (MI-VS). The gray lines in the AP and HR plots represent 200-Hz resampled signals. The gray lines in the SNA plots represent 10-Hz resampled signals. The black lines in the SNA, AP, and HR plots represent 2-s moving averaged signals. CSP was changed from 60 to 180 mmHg with an increment of 20 mmHg every minute. SNA, AP, and HR were decreased in response to the increase in CSP. The baroreflex responses were smaller in the MI-NT rat than in the NC and MI-VS rats.

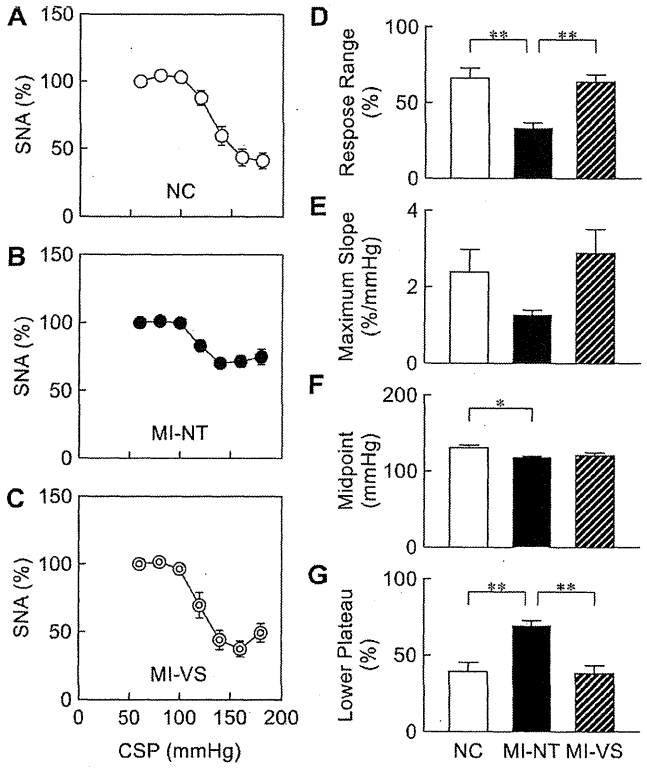

Fig. 2. Open-loop static characteristics of the baroreflex neural arc (SNA vs. CSP) obtained from the NC (*A*), MI-NT (*B*), and MI-VS (*C*) groups. The response range of percent SNA (*D*), maximum slope (*E*), midpoint (*F*), and lower plateau (*G*) derived from fitted sigmoid curves. Data are mean and SE values. $^{**}P < 0.01$ and $^{*}P < 0.05$ by Tukey test following one-way ANOVA.

percent SNA in the neural arc (Fig. 2*D*). On the other hand, the VS treatment did not affect the slope of the AP response to percent SNA in the peripheral arc (Fig. 3*D*). As a result, the VS treatment did not reveal statistically significant improvement in the response range of AP in the baroreflex total loop (Fig. 4*D*). No significant difference in the maximum gain between the NC and MI-VS groups may partly reflect an improvement of the baroreflex total-loop function (Fig. 4*E*).

Effects of VS treatment on the baroreflex neural arc. The response range of percent SNA was narrowed in the MI-NT group compared with the NC group (Fig. 2*D*), being consistent with our previous study that was performed in rats with longer duration of heart failure (100–200 days after MI) (8). The response range of percent SNA in the MI-VS group was nearly the same as that in the NC group. The improved neural arc function to suppress percent SNA may partly contribute to the sympathetic suppression induced by the VS treatment such as reduced plasma norepinephrine levels (14, 29). Because percent SNA is influenced by basal sympathetic discharge, the improved response range of percent SNA does not necessarily indicate the improved sympathetic control has physiological significance. However, because the VS treatment also increased the response range of HR in the MI-VS group compared with the MI-NT group (Fig. 5D), we speculate that VS might have actually improved the sympathetic control of target organs.

Several mechanisms are considered for the impaired baroreflex control of SNA in heart failure. Plasma angiotensin II levels are increased in heart failure, and VS can suppress the increased plasma angiotensin II levels (29). Angiotensin II can increase the maximum SNA without significantly affecting the response range of SNA in the baroreflex neural arc (7). Cardiac sympathetic afferent activation inhibits the baroreflex control of SNA via a mechanism associated with central angiotensin II (3). On the other hand, vagal afferent stimulation by phenylbiguanide can decrease the maximum SNA without significantly affecting the response range of SNA in the baroreflex neural arc (6). Because VS might have activated not only efferent but also afferent pathways in our study, the vagal afferent stimulation probably antagonized the sympathoexcitation such as that induced by angiotensin II. In addition, pharmacological vagal activation (13) or electrical VS (29) can reduce systemic inflammatory responses in heart failure, which may contribute to sympathetic suppression by reducing the pathological afferent signals from the heart. Aside from the systemic inflammation, increases in proinflammatory cytokines in the brain may also produce sympathoexcitation in heart failure (28). Although a link between the VS treatment and central anti-inflammation seems elusive, the presence of a cholinergic anti-inflammatory pathway (22) suggests a possibility that VS suppresses sympathetic overactivity through the anti-inflammatory mechanism in the central nervous system as well as in the heart.

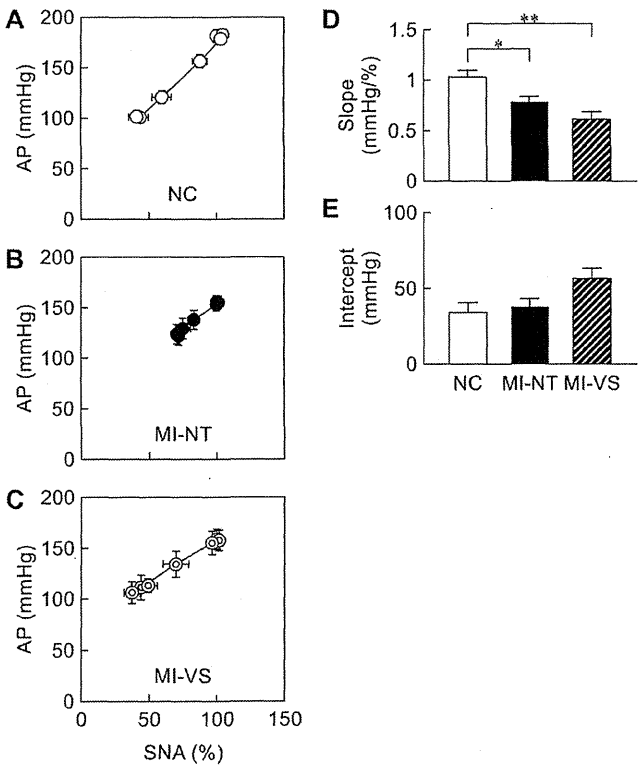

Fig. 3. Open-loop static characteristics of the baroreflex peripheral arc (AP vs. SNA) obtained from the NC (*A*), MI-NT (*B*), and MI-VS (*C*) groups. The slope (*D*) and intercept (*E*) derived from regression lines. Data are means and SE values. $^{**}P < 0.01$ and $^{*}P < 0.05$ by Tukey test following one-way ANOVA.

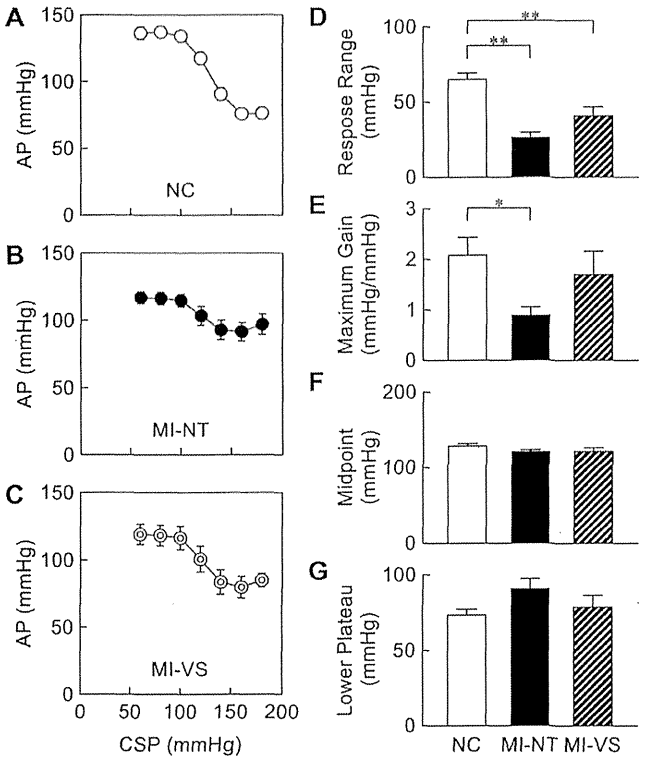

Fig. 4. Open-loop static characteristics of the baroreflex total loop (AP vs. CSP) obtained from the NC (*A*), MI-NT (*B*), and MI-VS (*C*) groups. The response range of AP (*D*), maximum gain (*E*), midpoint (*F*), and lower plateau (*G*) derived from fitted sigmoid curves. Data are means and SE values. $^{**}P < 0.01$ and $^{*}P < 0.05$ by Tukey test following one-way ANOVA.

Effects of VS treatment on the baroreflex peripheral arc. Open-loop static characteristics of the baroreflex peripheral arc approximated a straight line (Fig. 3, *A–C*). As has been reported in our previous study (8), the slope of the peripheral arc was significantly reduced in the MI-NT group compared with the NC group. Pump failure associated with MI may blunt the AP response to percent SNA. Regarding this point, Feng et al. (2) examined the AP response to preganglionic sympathetic nerve stimulation using pithed rats. In that study, the AP response is decreased in MI rats compared with control rats. They discussed that the pressor response in pithed rats mainly represented the vascular response because cardiac output did not change significantly in response to preganglionic sympathetic nerve stimulation. However, when cardiac output is reduced in the MI rats compared with the control rats, the same amount of changes in the peripheral vascular resistance would yield smaller AP response in the MI rats than in the control rats. In this sense, the contribution of pump failure to the decreased AP response may be inevitable.

The slope of the peripheral arc in the MI-VS group was not increased compared with the MI-NT group, indicating that the VS treatment had little effect on the sympathetic control of AP. While these results seem to be consistent with no significant effect of the VS treatment on the normalized biventricular weight (Table 2), we need to be cautious about the fact that the slope of the peripheral arc can be differently estimated depending on how SNA was expressed. For instance, if the absolute maximum SNA is decreased in the MI-VS group compared with in the MI-NT group, the slope of the peripheral arc assessed by the AP response to the absolute change in SNA could be increased.

Effects of VS on the sympathetic HR control. Because the vagal nerves were sectioned bilaterally in the acute baroreflex study, the CSP-HR relationship represents the sympathetically mediated HR response (Fig. 5). The response range of HR was narrowed in the MI-NT group compared with the NC group, but it was nearly the same between the MI-VS and NC groups. In a previous study using canine heart failure induced by high-rate pacing, Zhang et al. (29) demonstrated that chronic VS improves the baroreflex sensitivity assessed by the RR interval response to a phenylephrine-induced AP increase. In that study, both the sympathetic and vagal limbs might have contributed to the RR interval response. In contrast, the present result indicates that the VS treatment may improve the HR response even in the absence of the vagal control of HR. Sustained sympathetic activation in heart failure results in downregulation and/or desensitization of β-adrenergic receptors (15). In addition to the improved SNA response to the CSP input, the VS treatment may correct the functionality of β-adrenergic signaling through sympathetic suppression and improve the sympathetically-mediated HR response in the MI-VS group compared with the MI-NT group.

Consideration of MI models. Our previous study indicates that the VS treatment prevents cardiac remodeling and ventricular

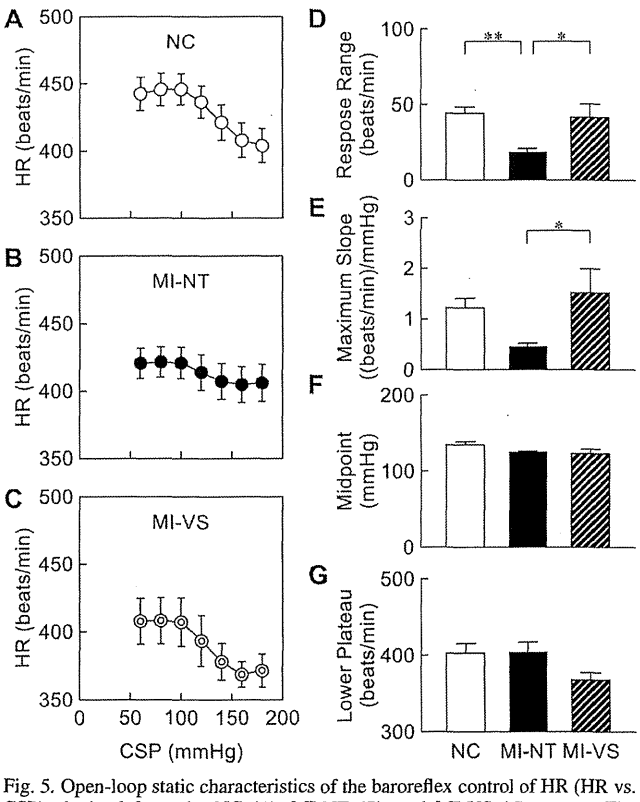

Fig. 5. Open-loop static characteristics of the baroreflex control of HR (HR vs. CSP) obtained from the NC (*A*), MI-NT (*B*), and MI-VS (*C*) groups. The response range of HR (*D*), maximum slope (*E*), midpoint (*F*), and lower plateau (*G*) derived from fitted sigmoid curves. Data are means and SE values. $^{**}P < 0.01$ and $^{*}P < 0.05$ by Tukey test following one-way ANOVA.

dysfunction in MI rats (14). In that study, normalized biventricular weight was 2.75 ± 0.25 g/kg in a treated group (CHF-VS) and 3.14 ± 0.22 g/kg in an untreated group (CHF-SS). In contrast, normalized biventricular weight was nearly the same between the MI-NT and MI-VS groups (Table 2), and both groups showed normalized biventricular weight similar to that in the CHF-VS group. One possible explanation for the discrepancy between the previous and present studies is the difference in the MI-NT and CHF-SS group characteristics as follows. In the previous study (14), a second surgery was performed for telemeter implantation and dummy stimulator implantation in the CHF-SS group. The second surgery and the weight burden of the implanted devices might have aggravated heart failure and promoted cardiac remodeling. In the present study, only MI was induced in the MI-NT group without the second surgery. Accordingly, heart failure might have been less severe in the MI-NT group than in the previous CHF-SS group.

Although performing the second surgery in the untreated MI group might have been a better protocol, it is confounded by another problem that the rat with too severe heart failure could not survive the acute baroreflex study (see APPENDIX for details). In this sense, there was unintentional bias as to the severity of heart failure in the MI-NT group. It may be worth noting that two rats in the MI-NT group but no rats in the MI-VS group were lost during the acute baroreflex study (Table 1).

Limitations. First, the baroreflex static characteristics were examined in anesthetized conditions. Because anesthesia affects autonomic nervous activities, the present results may not be directly extrapolated to interpret the baroreflex function in heart failure under conscious conditions. Second, we were unable to determine whether the VS treatment prevented worsening of the baroreflex neural arc function or restored the baroreflex neural arc function that had been worsened by heart failure before the initiation of the VS treatment. Further studies are required to elucidate the time course of the effects of the VS treatment on the baroreflex function. Finally, basal sympathetic discharge might have been increased in the MI-NT group compared with the NC group, and VS might have ameliorated the elevated sympathetic discharge in the MI-VS group. Our previous study (14) has demonstrated that chronic VS treatment reduces plasma norepinephrine concentration in MI-induced heart failure rats. Thus, if SNA was evaluated in absolute amplitude and/or frequency, the response range of SNA in the MI-NT group might become larger. In other words, the response range of the neural arc in the MI-NT group might be underestimated in Fig. 2, and the slope of the peripheral arc in the MI-NT group might be overestimated in Fig. 3. These points need to be taken into account when interpreting the data.

Conclusions. The 6-wk VS treatment significantly improved the ability of the carotid sinus baroreflex to reduce percent SNA. Because sympathetic overactivity promotes the progression of heart failure, the suppression of SNA may be beneficial for the improved prognosis of heart failure. On the other hand, the treatment effect of VS on the baroreflex total-loop function was limited due to the lack of significant improvement in the AP response to percent SNA.

APPENDIX

To address the effect of a second surgery in untreated MI rats, we performed an additional experiment, which included a sham operation on the right vagus and implantation of dummy devices in MI rats (MI-SS). Eight rats survived until the day of the acute baroreflex study. One rat showed small normalized biventricular weight (<2.2 g/kg) and was excluded, and the remaining seven rats exhibited normalized biventricular weight of 3.05 ± 0.12 g/kg (mean ± SE), confirming that a second surgery promoted cardiac remodeling compared with the MI-NT group. Among the seven rats, three rats died during the acute baroreflex study, and the remaining four rats exhibited normalized biventricular weight of 2.93 ± 0.13 g/kg. The problem with the MI-SS group was that rats with severe heart failure died during the acute baroreflex study, and we could not create a group of severe heart failure compared with the MI-NT group any further. As a reference, data obtained from the MI-SS group combined with other groups are depicted in Fig. 6. Statistical analyses were not performed due to the small number of successful baroreflex study in the MI-SS group ($n = 4$). The mean parameter values of the baroreflex total loop and the neural arc indicate that the baroreflex function in the MI-SS group was not worse than that in the MI-NT group.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.K. and M.S. conception and design of research; T.K., M.L., C.Z., and S.S. performed experiments; T.K. analyzed data; T.K., M.L., C.Z., S.S., K.U., M.J.T., and H.Y. interpreted results of experiments; T.K. prepared figures; T.K. and M.S. drafted manuscript; T.K. and M.S. edited and revised manuscript; T.K., M.L., C.Z., S.S., K.U., M.J.T., H.Y., and M.S. approved final version of manuscript.

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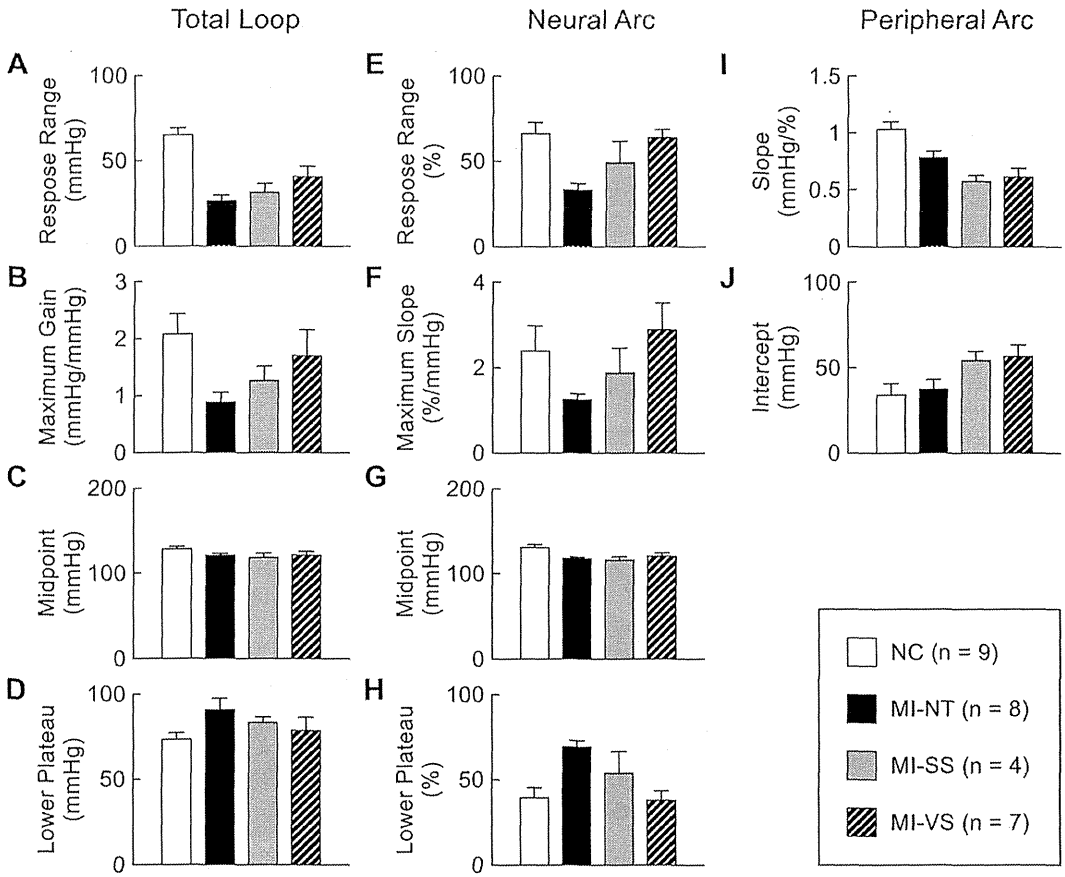

Fig. 6. Parameters of the baroreflex total loop (*A–D*), neural arc (*E–H*), and peripheral arc (*I* and *J*), including data from a group of myocardial infarction with second surgery (MI-SS, $n = 4$). Statistical analyses were not performed due to the small number of successful acute baroreflex studies in the MI-SS group. Judging from the mean parameter values of the total loop and the neural arc, the baroreflex function of the MI-SS group was not worse than that of the MI-NT group. See APPENDIX for details.

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Heart Vessels
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ORIGINAL ARTICLE

A novel technique to predict pulmonary capillary wedge pressure utilizing central venous pressure and tissue Doppler tricuspid/mitral annular velocities

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Abstract Assessing left ventricular (LV) filling pressure (pulmonary capillary wedge pressure, PCWP) is an important aspect in the care of patients with heart failure (HF). Physicians rely on right ventricular (RV) filling pressures such as central venous pressure (CVP) to predict PCWP, assuming concordance between CVP and PCWP. However, the use of this method is limited because discordance between CVP and PCWP is observed. We hypothesized that PCWP can be reliably predicted by CVP corrected by the relationship between RV and LV function, provided by the ratio of tissue Doppler peak systolic velocity of tricuspid annulus (S_T) to that of mitral annulus (S_M) (corrected CVP:CVP·S_T/S_M). In 16 anesthetized closed-chest dogs, S_T and S_M were measured by transthoracic tissue Doppler echocardiography. PCWP was varied over a wide range (1.8–40.0 mmHg) under normal condition and various types of acute and chronic HF. A significantly stronger linear correlation was observed between CVP·S_T/S_M and PCWP ($R^2 = 0.78$) than between CVP and PCWP ($R^2 = 0.22$) ($P < 0.01$). Receiver-operating characteristic (ROC) analysis indicated that CVP·S_T/S_M >10.5 mmHg predicted PCWP >18 mmHg with 85 % sensitivity and 88 % specificity. Area under ROC curve for CVP·S_T/S_M to predict PCWP >18 mmHg was 0.93, which was significantly larger than that for CVP (0.66) ($P < 0.01$). Peripheral venous pressure (PVP) corrected by S_T/S_M (PVP·S_T/S_M) also predicted PCWP reasonably well, suggesting that PVP·S_T/S_M may be a minimally invasive alternative to CVP·S_T/S_M. In conclusion, our technique is potentially useful for the reliable prediction of PCWP in HF patients.

Keywords Heart failure · Hemodynamics · Echocardiography

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Introduction

Assessing left ventricular (LV) filling pressure is an important aspect in the care of patients with heart failure (HF) [1]. The clinical gold standard for evaluating LV filling pressure is pulmonary capillary wedge pressure (PCWP) measured using pulmonary artery catheter. However, the invasive nature of catheterization limits its routine clinical use [2]. Noninvasive estimations of LV filling pressure by echocardiography have been developed [3–5]. However, the reliability of these methods in HF patients has been inconsistent [6, 7]. A minimally invasive and reliable method to estimate LV filling pressure has been keenly awaited.

In clinical practice, physicians rely on right ventricular (RV) filling pressures such as central venous pressure (CVP) to predict LV filling pressure, assuming concordance between the two filling pressures [8, 9]. However, the use of this method is limited because discordance between the two filling pressures has been observed in a substantial proportion of acute [10] or chronic [11, 12] HF patients. Physiologically, equality of stroke volumes (SV) of RV and LV is maintained by shifting of blood between the systemic and pulmonary vascular beds in accordance with the relation between RV and LV functions, which adjusts the two filling pressures [10, 13, 14]. If LV function is selectively impaired while RV function is spared, blood shifts from the systemic to pulmonary circuit to maintain

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the equality of RV and LV SV, but discordance between the two filling pressures occurs (increased LV and decreased RV filling pressures) [10, 13, 14]. The relation between RV and LV functions may determine the relation between RV and LV filling pressures, i.e., the relation between CVP and PCWP.

Peak systolic tricuspid (S_T) [15–17] and mitral (S_M) [18–21] annular velocities measured by tissue Doppler echocardiography have been shown to sensitively reflect RV and LV systolic functions, respectively. Our theoretical analysis (see "Materials and methods") indicates that PCWP, CVP, S_T and S_M are related as follows:

$$\mathrm{PCWP} = \alpha \cdot \mathrm{CVP} \cdot S_T/S_M \quad (1)$$

where α is a constant. Based on Eq. 1, we hypothesized that CVP corrected by the relationship between RV and LV functions ($\mathrm{CVP}\cdot S_T/S_M$) reliably predicts PCWP. The primary purpose of this study was to validate this hypothesis. CVP is invasively measured by central venous catheterization, while peripheral venous pressure (PVP) is easily and safely measured [22]. PVP tightly correlates with CVP [23]. Hence, the secondary purpose of this study was to examine whether $\mathrm{PVP}\cdot S_T/S_M$ can be used as a minimally invasive alternative to $\mathrm{CVP}\cdot S_T/S_M$ in predicting PCWP. For these purposes, we conducted rigidly controlled experiments in canine models of acute and chronic HF over a wide preload range, which is not possible in humans.

Materials and methods

Theoretical analysis of the relation between PCWP, CVP, S_T and S_M

Assuming RV and LV SV are equal, ejection fraction of RV (RVEF) and LV (LVEF) are related to end-diastolic volumes of RV (RVEDV) and LV (LVEDV), respectively, as follows:

$$\mathrm{RVEF} = \mathrm{SV}/\mathrm{RVEDV} \quad (2)$$

$$\mathrm{LVEF} = \mathrm{SV}/\mathrm{LVEDV} \quad (3)$$

Dividing Eq. 2 by Eq. 3 and rearranging yields:

$$\mathrm{RVEF} \times \mathrm{RVEDV} = \mathrm{LVEF} \times \mathrm{LVEDV} \quad (4)$$

Based on previous studies [24, 25], we assume that RV filling pressure (CVP) and LV filling pressure (PCWP) are related to RVEDV and LVEDV, respectively, as follows:

$$\mathrm{CVP} = k_1 \cdot \mathrm{RVEDV} \quad (5)$$

$$\mathrm{PCWP} = k_2 \cdot \mathrm{LVEDV} \quad (6)$$

where k_1 and k_2 are constants.

S_T and S_M measured by tissue Doppler echocardiography have been shown to correlate well with RVEF [15, 16] and LVEF [18, 19], respectively, as follows,

$$S_T = k_3 \cdot \mathrm{RVEF} \quad (7)$$

$$S_M = k_4 \cdot \mathrm{LVEF} \quad (8)$$

where k_3 and k_4 are constants.

Substituting the four variables in Eq. 4 by Eqs. 5–8 and rearranging yields:

$$\mathrm{PCWP} = \frac{k_2 \cdot k_4}{k_1 \cdot k_3} \cdot \mathrm{CVP} \cdot S_T/S_M \quad (9)$$

If we define constant α as follows:

$$\alpha = \frac{k_2 \cdot k_4}{k_1 \cdot k_3} \quad (10)$$

PCWP, CVP, S_T and S_M are related as shown in Eq. 1.

Animals

We used 18 adult mongrel dogs (9 male and 9 female, 22 ± 2 kg). The investigation conformed 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). All protocols were approved by the Animal Subjects Committee of the National Cerebral and Cardiovascular Center.

Preparation

After anesthesia was induced with thiamylal sodium (25 mg kg^{-1}), the animals were intubated endotracheally, and their lungs were ventilated artificially. An appropriate level of anesthesia was maintained by continuous inhalation of 2.0 % isoflurane. A 6-F pulmonary artery catheter (T173HF6, Edwards Lifesciences, Irvine, CA) was positioned in the pulmonary artery through a 9-F sheath introducer placed in the right jugular vein. The pulmonary artery catheter and the side-port of the introducer were connected to pressure transducers (DX-200, Nihon Kohden, Tokyo, Japan) to measure PCWP and CVP, respectively. An 8-F sheath introducer was placed in the left carotid artery and the side-port was connected to a pressure transducer to measure systemic arterial pressure (AP). A 16-gauge catheter was placed in the right cephalic vein (a superficial vein of the right upper limb), and was connected to a pressure transducer to measure PVP. The animals were held in a standing position using a sling on a fluoroscopy table to allow continuous echocardiographic monitoring during cardiac catheterization and hemodynamic interventions [21, 26]. After being