Introduction

Recently, pulsed Doppler echocardiography has facilitated the recording of blood flow velocity waveforms in specific sites in the cardiac or vascular cavity [1-3], and the clinical significance of individual waveforms has widely been reviewed. In particular, the combined analysis of transmitral flow (TMF) and pulmonary venous flow (PVF) velocities is useful for understanding hemodynamic abnormalities as a pulmonary vein-left atrium (LA)-left ventricle (LV) relationship [4]. When evaluating LV function clinically, the role of the LA with reservoir, conduit, and booster pump functions is very important [4]. It is well known that TMF and PVF velocities in the diseased hearts depend on loading conditions, especially preload [5-7]. Therefore, evaluation of changes in venous and atrioventricular flow velocity patterns during preload alteration may provide valuable information for clarifying...
the contribution of the venous return system to right ventricular or LV filling. In the present study, we performed lower body negative pressure (LBNP) in healthy subjects and examined changes in the venous and atrioventricular flow velocity patterns during preload reduction to investigate the relation of atrial conduit and passive emptying to ventricular filling.

Methods

We evaluated 14 healthy volunteers (all males, mean age 31.7 ± 18 years) in normal sinus rhythm and without physiological valvular regurgitation. The purpose of this study was fully explained to all subjects, and informed consent was obtained. The protocol was approved by the appropriate hospital committee regarding human experimentation of Higashi Tokushima National Hospital.

Lower body negative pressure (LBNP)

A system to apply negative pressure to the lower half of the body (Toshiba Corp., Tokyo, Japan) was used. In this system, the air intake and exhaust of the motor fan providing 2m³/min of air-blasting volume were connected to a stainless steel container via an electromagnetic valve. The lower half of the subject’s body (beneath the iliac crest) was placed in the tightly sealed stainless steel container, and LBNP of -20 and -40mmHg were applied. Loading conditions at atmospheric pressure (0mmHg) defined the control state. All subjects underwent M-mode and pulsed Doppler echocardiography to record LV end-diastolic and end-systolic dimension (LVDd and LVDs, respectively), maximal LA dimension, superior vena cava (SVC) flow, transtricuspid flow (TTF), PVF, and TMF velocities. We initially fixed lower body pressure at atmospheric pressure (0mmHg) defined the control state. All subjects underwent M-mode and pulsed Doppler echocardiography to record LV end-diastolic and end-systolic dimension (LVDd and LVDs, respectively), maximal LA dimension, superior vena cava (SVC) flow, transtricuspid flow (TTF), PVF, and TMF velocities. We initially fixed lower body pressure at atmospheric pressure (0mmHg) and then decreased it to -20 and -40mmHg stepwise, every 3 minutes. During each stage, heart rate and blood pressure were measured, and echo-Doppler recordings were obtained. The equipment used for echocardiography was a commercially available Aloka SSD-870 (Aloka Co., Ltd., Tokyo, Japan) with a 2.5 or 5MHz probe.

Transthoracic echocardiography

The LVDd, LVDs, and maximum LA dimension were determined from parasternal M-mode echocardiogram. With these parameters we calculated the percent fractional shortening of the LV as follows:

Percent fractional shortening of the LV (%) = [(LVDd - LVDs)/LVDd] × 100.

TTF and TMF velocities were recorded with subjects in the left semilateral position after the sample volumes were positioned at the tip of the tricuspid and mitral valves, respectively, in a 4-chamber or LV long-axis view from the apical approach. The peak early diastolic (E) and atrial systolic (A) velocities and their ratio (E/A) were measured from the TTF and TMF velocity patterns (Figure 1). Also, the former time-velocity integral between the start of the atrioventricular flow and the peak of the E wave (IEa), the latter time-velocity integral between the peak and the end of the E wave (IEd), and the time-velocity integral of the A wave (IA) were determined.

SVC flow velocity was recorded from the supraclavicular approach with subjects in the supine position. The sample volume was positioned at the center of the SVC approximately 5 cm under the transducer. From the obtained SVC flow velocity pattern, peak velocities and time-velocity integrals of the first systolic wave (S₁ and IS₁, respectively), second systolic wave (S₂ and IS₂, respectively), and atrial wave (A and IA, respectively) were determined.

Fig. 1. Parameters measured from the transtricuspid flow (TTF) and transmitral flow (TMF) velocity patterns. E, peak early diastolic flow velocity; A, peak atrial systolic flow velocity; IEa, time-velocity integral between the onset and the time of peak flow velocity of the early diastolic filling; IEd, time-velocity integral between the time of peak flow velocity and the end of the early diastolic filling; IA, time-velocity integral of the atrial systolic wave; ECG, electrocardiogram; PCG, phonocardiogram.
respectively), and early diastolic wave (D and ID, respectively) were determined (Figure 2, left).

**Transesophageal echocardiography**

PVF velocity was recorded by determining the sample volume within 1 to 2 cm from the junction of the pulmonary vein and the LA after sections of the LA, including the left superior pulmonary vein, were visualized and the PVF signal to the LA was confirmed by color Doppler imaging. The peak velocities and time-velocity integrals of the first systolic wave (S₁ and IS₁, respectively), second systolic wave (S₂ and IS₂, respectively), and early diastolic wave (D and ID, respectively) were measured from the PVF velocity pattern (Figure 2, right).

**Reproducibility of measurements**

Inter- and intraobserver variabilities of measurements of pulsed Doppler indexes were calculated as the differences in 2 measurements of the same subjects by 2 different observers and by 1 observer divided by the mean value, respectively.

**Statistical analysis**

Values are expressed as the mean ± SD. A 2-factor analysis of variance for repeated measurements was used for comparisons between baseline and LBNP (-20 and -40 mm Hg) values of the clinical, M-mode and pulsed Doppler echocardiographic parameters. The difference of correlations between venous and atrioventricular flow velocity parameters were determined by linear regression analysis. A P value less than 0.05 was considered statistically significant.

**Results**

**Clinical and M-mode echocardiographic parameters**

There were no significant changes in heart rate and mean blood pressure during preload reduction (Table 1). The LVDd was significantly decreased during LBNP of -20 and -40 mm Hg, and LVDs was significantly decreased during LBNP of -40 mm Hg, whereas there were no significant changes in %FS during preload reduction. The maximal LA dimension was
markedly decreased during LBNP of -20 and -40mm Hg.

Atrioventricular flow velocity parameters

The peak E velocities and the time-velocity integrals between the onset and the time of peak flow velocity of the early diastolic filling (IEa) of the TTF and TMF velocity patterns were significantly decreased during LBNP of -20 and -40mm Hg (Table 2). However, there were no significant changes in atrial systolic parameters and the time-velocity integral between the time of peak flow velocity and the end of the early diastolic filling (IEd) of the both atrioventricular flow velocity patterns during LBNP.

Venous flow velocity parameters

The peak second systolic velocities of the SVC flow and PVF velocity patterns were significantly decreased during LBNP of -20 and -40mm Hg (Table 3 and Figure 3). The time-velocity integral of the second systolic wave (IS2) of the PVF velocity pattern also was significantly decreased during LBNP of -40mm Hg, whereas there was no significant change in IS2 of the SVC flow velocity pattern. There were no significant changes in

### Table 1. Comparisons of clinical and M-mode echocardiographic parameters at baseline and during LBNP in normal individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>LBNP (20 mm Hg)</th>
<th>LBNP (40 mm Hg)</th>
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<tbody>
<tr>
<td>HR (/min)</td>
<td>68 ± 12</td>
<td>69 ± 15</td>
<td>70 ± 14</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>90 ± 4</td>
<td>93 ± 4</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>49 ± 3</td>
<td>45 ± 4 *</td>
<td>41 ± 6 *</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>25 ± 4 *</td>
</tr>
<tr>
<td>%FS (%)</td>
<td>42 ± 5</td>
<td>37 ± 4</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>31 ± 4</td>
<td>27 ± 5 **</td>
<td>25 ± 6 *</td>
</tr>
</tbody>
</table>

LBNP, lower body negative pressure; HR, heart rate; MBP, mean blood pressure; LVDd and LVDs, end-diastolic and end-systolic left ventricular dimension, respectively; %FS, percent fractional shortening of the left ventricle; LAD, maximal left atrial dimension.

* p<0.05, ** p<0.01 vs. baseline

### Table 2. Comparisons of transtricuspid and transmitral flow velocity parameters at baseline and during LBNP in normal individuals

<table>
<thead>
<tr>
<th>Velocity Pattern</th>
<th>Baseline</th>
<th>LBNP (-20 mm Hg)</th>
<th>LBNP (-40 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transtricuspid flow velocity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (cm/s)</td>
<td>49 ± 12</td>
<td>39 ± 14 **</td>
<td>35 ± 14 **</td>
</tr>
<tr>
<td>A (cm/s)</td>
<td>32 ± 6</td>
<td>32 ± 7</td>
<td>32 ± 9</td>
</tr>
<tr>
<td>E/A</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.4 *</td>
<td>1.0 ± 0.4 *</td>
</tr>
<tr>
<td>IEa (cm)</td>
<td>3.2 ± 0.7</td>
<td>2.6 ± 0.6 *</td>
<td>2.3 ± 0.5 **</td>
</tr>
<tr>
<td>IEd (cm)</td>
<td>6.2 ± 1.2</td>
<td>6.1 ± 1.6</td>
<td>5.7 ± 1.7</td>
</tr>
<tr>
<td>IE (cm)</td>
<td>9.4 ± 1.9</td>
<td>8.7 ± 2.2 *</td>
<td>8.0 ± 2.2 **</td>
</tr>
<tr>
<td>IA (cm)</td>
<td>3.8 ± 1.8</td>
<td>4.0 ± 1.9</td>
<td>3.8 ± 1.6</td>
</tr>
</tbody>
</table>

| **Transmitral flow velocity** | | | |
| E (cm/s) | 71 ± 9 | 58 ± 13 ** | 53 ± 11 ** |
| A (cm/s) | 46 ± 7 | 44 ± 8 | 43 ± 7 |
| E/A      | 1.5 ± 0.2 | 1.3 ± 0.4 * | 1.2 ± 0.3 ** |
| IEa (cm) | 4.3 ± 1.1 | 3.3 ± 1.3 ** | 2.6 ± 0.8 ** |
| IEd (cm) | 7.5 ± 1.2 | 7.1 ± 2.4 | 6.4 ± 2.0 |
| IE (cm) | 11.8 ± 2.3 | 10.4 ± 3.7 ** | 9.0 ± 2.8 ** |
| IA (cm) | 4.8 ± 1.5 | 4.6 ± 1.1 | 4.6 ± 0.9 |

LBNP, lower body negative pressure; E, peak early diastolic flow velocity; A, peak atrial systolic flow velocity; IEa, time-velocity integral between the onset and the time of peak flow velocity of the early diastolic filling; IEd, time-velocity integral between the time of peak flow velocity and the end of the early diastolic filling; IE, time-velocity integral of the early diastolic filling; IA, time-velocity integral of the atrial systolic wave. * p<0.05, ** p<0.01 vs. baseline

first systolic and early diastolic parameters of the both
venous flow velocity patterns during LBNP.

There was a significant positive correlation between
the changing time-velocity integral in the former-half
of the E wave (ΔIEa) of the TMF velocity pattern and
that in the S 2 wave (ΔIS 2) of the PVF velocity pattern
during LBNP (Figure 4); there was no significant cor-
relation between the ΔIEa of the TTF velocity pattern
and ΔIS 2 of the SVC flow velocity pattern (r=0.51,
p=0.07).

Interobserver and intraobserver variability
The interobserver variability was 1.5% to 2.4%, and
intraobserver variability was 0.8% to 2.0% for all pulsed
Doppler index values.

Discussion
LBNP is known to decrease central venous pressure
(preload) with no significant changes in heart rate and
blood pressure [8, 9]. Ahmad et al. [9] reported that
LBNP of -40mm Hg significantly decreases the LV end-
diastolic volume without influencing the heart rate or
blood pressure, while there were no significant
changes in LV end-systolic volume or ejection fraction,
which was consistent with the results of the present
study. In addition, Tsukamoto et al. [10] indicated
that LBNP decreases pulmonary capillary wedge and
right atrial pressures.

LA function is classified into 4 categories based on

Table 3. Comparisons of superior vena cava and pulmonary venous flow velocity parameters at base-
line and during LBNP in normal individuals

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>LBNP (-20 mm Hg)</th>
<th>LBNP (-40 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superior vena cava flow velocity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S 1 (cm/s)</td>
<td>21 ± 6</td>
<td>21 ± 7</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>S 2 (cm/s)</td>
<td>54 ± 21</td>
<td>41 ± 15 *</td>
<td>40 ± 17 *</td>
</tr>
<tr>
<td>D (cm/s)</td>
<td>34 ± 7</td>
<td>29 ± 9</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>IS 1 (cm)</td>
<td>2.6 ± 0.8</td>
<td>3.0 ± 1.5</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>IS 2 (cm)</td>
<td>13 ± 5</td>
<td>12 ± 6</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>ID (cm)</td>
<td>8.9 ± 3.0</td>
<td>9.2 ± 3.3</td>
<td>9.2 ± 3.5</td>
</tr>
<tr>
<td><strong>Pulmonary venous flow velocity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S 1 (cm/s)</td>
<td>38 ± 10</td>
<td>39 ± 10</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>S 2 (cm/s)</td>
<td>56 ± 9</td>
<td>47 ± 10 **</td>
<td>41 ± 7 **</td>
</tr>
<tr>
<td>D (cm/s)</td>
<td>50 ± 11</td>
<td>51 ± 13</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>IS 1 (cm)</td>
<td>6.0 ± 2.1</td>
<td>6.6 ± 1.9</td>
<td>6.9 ± 2.5</td>
</tr>
<tr>
<td>IS 2 (cm)</td>
<td>12 ± 4</td>
<td>10 ± 4</td>
<td>8 ± 3 **</td>
</tr>
<tr>
<td>ID (cm)</td>
<td>11 ± 3</td>
<td>12 ± 3</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

LBNP; lower body negative pressure ; S 1, peak first systolic flow velocity ; S 2, peak second
systolic flow velocity ; D, peak early diastolic flow velocity ; IS 1, time-velocity integral of the first
systolic wave ; IS 2, time-velocity integral of the second systolic wave ; ID, time-velocity integral
of the early diastolic wave. *p<0.05, **p<0.01 vs. baseline

Fig. 3. Changes in superior vena cava flow (SVCF) and pulmo-
nary venous flow (PVF) velocity patterns during lower body negative pressure (LBNP) of -20 and
-40mmHg. Although the peak first systolic (S 1) and early diastolic (D) velocities have no significant
changes, peak second systolic (S 2) velocity is sig-
nificantly decreased during LBNP. Other abbrevia-
tions are as for Fig. 2.
hemodynamic characteristics: relaxation, filling, conduit, and booster pump functions. PVF velocity waveform accurately reflects these individual functions [4]. Therefore, the combined analysis of TMF and PVF velocities provides important information for evaluating the functional role of the LA in LV filling [2, 5, 11]. In clinical practice, the TMF and PVF velocities are often used to clarify the pathogenesis of pulmonary congestion in the presence of left heart failure [12, 13]. However, these waveforms depend on loading conditions. In particular, many studies emphasized that TMF velocity changes easily from the pseudonormalized (E/A>1) pattern to the relaxation failure (E/A<1) pattern during preload reduction in the presence of elevated LV end-diastolic pressure, and PVF decreases peak early diastolic and atrial systolic velocities [5-7, 12, 13].

Some studies indicated that preload reduction decreases the peak velocity and time-velocity integral of the E wave in TMF velocity, but there is no significant change in peak velocity and time-velocity integral of the A wave in normal hearts [5, 7, 10, 12]. In the present study, preload reduction decreased the time-velocity integral of the E wave in TMF velocity, and its decreasing rate depended on that in IEa. In addition, the decreasing rate in IEa (ΔIEa) correlated positively with that in the time-velocity integral of the S2 wave of the PVF velocity (ΔIS2), whereas there was no significant correlation between ΔIEd of the TMF velocity and ΔID of the PVF velocity during preload reduction.

It is well known that early diastolic TMF is determined by pressure differences between LA and LV, and that LA pressure and LV relaxation [14], and LV myocardial active suction effects [15] are important influencing factors. The nadir of LV pressure curve is approximately consistent with the peak of the E wave in the TMF velocity. Therefore, IEa and IEd of the E wave are produced by different factors: the former is affected by LA pressure, LV relaxation, and LV myocardial suction effects, and the latter is affected by LV relaxation and LV compliance. Keren et al. [2] speculated that early diastolic TMF volume depends on the LA passive emptying and conduit, and changes in E wave of the TMF velocity during preload alteration mainly reflect those in S2 wave of the PVF velocity, since changes in S2 wave of the PVF velocity are relatively more marked than those in D wave of the PVF velocity.

Based on these findings, decreases in E wave parameters during preload reduction in the present study were possibly associated with a decrease in the early diastolic pressure gradient between LA and LV related to a decrease in venous return. The main mechanism may involve the utilization of blood reserved in the LA (S2 wave of the PVF velocity), that is, LA passive emptying, but not conduit function (D wave of the PVF velocity) reflecting direct inflow from the pulmonary veins to the LV. This is supported by the following previous studies: there was a positive correlation between peak S2 velocity of the PVF and cardiac output, and there was a close relationship between deceleration times of the early diastolic waves of the TMF and PVF velocities [5]; the peak of the E wave of the TMF velocity was delayed by approximately 50 msec compared to that of the D wave of the PVF velocity [2]; and peak E and D velocities were closely related to LV myocardial restoring force (recoil) [16].

In diseased hearts, peak E velocity of the TMF increases with an increase in peak S2 velocity of the PVF during increase in preload in patients with relaxation failure (E/A<1) pattern and no elevation in LA pressure [12]. In patients with pseudonormalized or restrictive (E/A>1) pattern and elevated LV end-diastolic pressure, there are no marked changes in early diastolic LV filling during increase in preload. As a result, LA active contraction acts as regurgitation from the LA to the pulmonary veins (increase in peak A
velocity of the PVF) rather than that from the LA to the LV (decrease in peak A velocity of the TMF), resulting in pulmonary congestion [12, 13].

Changes in TTF and SVC flow velocities during preload reduction resembled those in TMF and PVF velocities in the present study. However, there was no significant correlation in the changing rate between IEa of the TTF velocity and IS2 of the SVC flow velocity. Concerning the SVC flow velocity, peak systolic velocity is usually higher than peak diastolic velocity regardless of age [3]. However, there are age-related changes in the PVF velocity: peak S<peak D in young subjects vs. peak S>peak D in elderly subjects [3, 17]. The age-related differences in the 2 venous flow velocity waveforms may be associated with differences in the relaxation rate [18] and filling pattern [19] between the right and left ventricles. Furthermore, the reproducibility of TTF velocity recording is lower than that of TMF velocity recording, which may have contributed to the difference in changes in the vein-atrium-ventricle relationship between the right and left heart sides during preload reduction.

References