Effects of Myosin Isozyme Shift on Curvilinearity of the Left Ventricular End-Systolic Pressure-Volume Relation of In Situ Rat Hearts

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Abstract: Recently we have shown that the left ventricular end-systolic pressure-volume relation (ESPVR) of in situ rat hearts is an upward convex curve in contrast to the linear left ventricular ESPVR in dog and human hearts. Within the smaller left ventricular volume range, the left ventricular end-systolic pressure rose steeply with increases in left ventricular volume, but it gradually reached a plateau at the larger left ventricular volumes. In adult rat hearts, the myosin isozyme is V₁, unlike V₃ in dog and human hearts. To investigate whether myosin isozyme affects the curvilinearity of the left ventricular ESPVR, we evaluated the left ventricular ESPVR in hypothyroid rats in which the left ventricular myosin isozyme had been shifted to V₃. In the hypothyroid rats, the left ventricular contractility was depressed and the ESPVR became closer to linear. However, after dobutamine administration the ESPVR returned to curvilinear. In normal rats the curvilinearity of the left ventricular ESPVR was decreased by negative inotropic agents such as adrenergic blockers. These results indicate that the depressed left ventricular contractility in the hypothyroidism make ESPVR linear and that the enhanced left ventricular contractility from dobutamine make it curvilinear. We concluded that the curvilinearity of the rat left ventricular ESPVR is not determined by myosin isozyme per se, but by the left ventricular contractility. [Japanese Journal of Physiology, 48, 445–455, 1998]

Key words: pressure-volume relationship, isomyosin, hypothyroidism, contractility.

The left ventricular (LV) end-systolic (ES) pressure (P)-volume (V) relation (ESPVR) has been well studied in the isolated cross-circulated canine heart preparation [1–7], and its relation is relatively linear within the physiological P-V range [1, 3–7]. Therefore a linearized slope of the ESPVR has been used as an index of LV contractility within the physiological P-V range. However, our recent studies revealed that the ESPVRs of the isolated cross-circulated [8, 9] and in situ ejecting adult rat hearts [10] are upward convex curves even within the physiological range. Within the smaller LVV range, LV ESP steeply increased with an increase in LVV, but it gradually reached a plateau at the larger LVVs. This curvilinearity suggests the possibility that the LV in rat hearts normally has a relatively high contractility [2] and thus a smaller contractile reserve than in canine hearts. But we have shown that even in the rat, the mechanoenergetic evaluation of cardiac function can be assessed as well as it can be in the canine heart by using the ESPVR-PVA framework [8,10]. Furthermore, rats have some advantages over dogs; rats can be more easily used in genetic engineering experiments and pathological experiments without the size limitation of mice.

The purpose of the present study was to investigate the LV ESPVR in hypothyroid adult rat hearts. In canine and human hearts, LV myocardial myosin isozyme is V₃ in contrast to V₁ in normal adult rat
hearts [11]. It is well known that ATPase activity of V1 myosin isozyme is higher than that of V3 myosin isozyme, and that the shortening velocity of V1-domi-
nant myocardium is faster than that of V3-dominant myocardium [12, 13]. The isometric tension of glyc-
erinated trabecular preparations decreased but slightly in the hypothyroid rat (V3-dominant myocardium) [14]. However, the isometric tension of the native tra-
becular preparations sizably decreased [14], and the peak developed tension of LV papillary muscle prepar-
ations significantly decreased [15] in the hypothyroid rat (V3-dominant myocardium). Therefore we hypothe-
sized that the difference of myosin isozyme would affect the curvilinearity of the LV ESPVR in the rat in
situ whole heart.

At first we reconfirmed the curvilinearity of the LV
ESPVR of in situ ejecting normal rat hearts. We then
transformed the isozyme pattern from V1 in adult nor-
mal rats to V3 by hypothyroidism and evaluated the
LV ESPVR in the hypothyroid rat hearts. To deter-
mine whether the myosin isozyme per se affects the
curvilinearity of the ESPVR, we investigated the ef-
ects of inotropic agents on the LV ESPVRs in normal
and hypothyroid rat hearts. After each experiment,
we confirmed the transformation of LV myocardial
myosin isozyme from V1 to V3 by polycrylamide gel
electrophoresis. Although myosin isozyme per se
proved not to determine the curvilinearity of the rat
LV ESPVR, the curvilinearity was decreased in the
hypothyroid rats, indicating a decrease in LV contrac-
tility.

METHODS

LV volumetric conductance catheter system.
We used the previously developed type of miniatur-
ized 3F conductance catheter for rats [16]. It is
equipped with six electrodes, of which the four inner
electrodes (3 mm apart) are for sensing three segmental
conductance signals. The two outermost electrodes
electrode distance of 1.2 cm) deliver a constant high-
frequency driving current (0.02 mA root-mean-square,
20 kHz) from a conductance catheter signal process-
ing apparatus (custom-made for rats by S-l Medicotech
Co., Ltd., Osaka, Japan) [16]. This apparatus
works on the same principle as Sigma 5 (Lecycom, The
Netherlands) for canine hearts. The circuit was de-
signed to measure the segmental conductances (G1,
G2, and G3) with the sensing electrodes. The total con-
ductance G(t) [= G1(t) + G2(t) + G3(t)] is theoretically
proportional to the LV blood volume in rat hearts, as it
is in canine hearts [17, 18].

The conductance catheter method of measuring
LVV has been described in detail by Baan et al. [17,
18] and others [10, 16, 19]. Briefly, instantaneous
intraventricular conductance volume V(t) [= V1(t) +
V2(t) + V3(t)] is obtained from the measured conduc-
tance G(t) by this equation:

\[
V(t) = \frac{1}{\alpha} \times \rho \times L^2 \times G(t),
\]

where \(\alpha\) is a dimensionless empirical constant for the
V(t)-G(t) relationship. This value was reasonably as-
sumed to be 1.0 [10, 16–19]. L is the distance between
two adjacent sensing electrodes, and \(\rho\) is the specific
resistivity of blood. The absolute blood volume is ob-
tainable by subtracting a constant offset volume \(V_c\)
calculated by this equation from \(V(t)\):

\[
V_c = \frac{1}{\alpha} \times \rho \times L^2 \times G_p,
\]

where \(G_p\) is the parallel conductance that partially
conducts the driving current, causing an overestima-
tion of intraventricular conductance G(t) [10, 16–19]. G_p and thus
\(V_c\) were obtained by the hypertonic saline dilution
method [18].

Animal preparations. Twelve age-matched
breeder male Wistar rats weighing 390 to 450 g (16 to
17 weeks) consisted of two groups (6 normal and 6
hypothyroid). Hypothyroidism was induced by adding
propylthiouracil (PTU) to the drinking water (0.8
mg/ml) for 4 to 5 weeks [15].

Surgical preparation. The investigation con-
formed with the Guiding Principles for the Care and
Use of Animals approved by the Council of the Physi-
ological Society of Japan.

Normal rats were anesthetized with pentobarbital
sodium (50 mg/kg I.P.), and hypothyroid rats were
anesthetized with pentobarbital sodium (40 mg/kg
I.P.). This dose difference was due to the high suscep-
tibility of hypothyroid rats to pentobarbital. Further
doses of pentobarbital sodium were administered as
needed. The trachea was intubated, and each rat was
ventilated with room air mixed with oxygen to main-
tain \(P_{O_2}, P_{CO_2}\), and pH within their normal ranges. The
chest was opened via a midline sternotomy and the
pericardium was dissected to expose the heart. A con-
ductance catheter (custom-made by Inter Medical Co.,
Ltd., Tokyo, Japan, see below) was introduced into the
LV through an apical stab with a purse string suture.
The distal driving electrode of the conductance
catheter was positioned carefully at the level of the
aortic valve and the proximal electrode near the apex.
This positioning is critical to obtain reliable LVV data.
Throughout each experiment we confirmed that all
segmental conductance volume changes were syn-
chronous. A 2.5 F catheter-tip micromanometer (Mil-
lar Instruments, Houston, Texas, USA) was also in-
served through the apex into the LV. A polyethylene tube (3F) was cannulated into the external jugular vein for an intravenous injection of drugs. A string occluder was placed loosely around the ascending aorta. The respirator was stopped during data acquisition to avoid respiratory fluctuation of cardiac signals.

**Experimental protocols.** Experiments were performed in 6 adult normal and 6 hypothyroid rat hearts. At first, the specific resistivity of sampled blood was measured in a specially designed small cuvette (volume content, 0.2 ml), which was connected to the processing apparatus. When cardiac hemodynamics were stable, a series of LV P-V loops was obtained during increasing afterload by a gradually ascending aortic occlusion. The occlusion was performed until end-diastolic volume (EDV) slightly increased, and thus the right ventricular volume almost unchanged. The duration of the occlusion was limited within a few seconds to avoid any reflex interferences. This intervention was repeated after a few minutes when arrhythmias occurred during the first aortic occlusion.

After collecting the baseline P-V data in normal rat, we injected the following drugs via the polyethylene tube cannulated in the external jugular vein. The sequence of drug administration was first propranolol 0.5 mg/kg i.v., then phenolamine 2 mg/kg i.v., then guanethidine 4 mg/kg i.v. Within a few minutes after each administration of propranolol and phenolamine, and also 30 min after the additional administration of guanethidine for complete catecholamine depletion from sympathetic nerves, a series of LV P-V loops was obtained during an aortic occlusion. After propranolol injection, we fixed the heart rate at 250 beats/min by right atrial pacing.

Similarly, a series of LV P-V loops of hypothyroid rat was obtained during a gradual aortic occlusion, and the heart rate was fixed at 250 beats/min by right atrial pacing. After collecting the baseline P-V data, we injected dobutamine (0.5 mg/kg). A few minutes later, a series of LV P-V loops was obtained during an aortic occlusion.

In the final part of each experiment, Gp and thus \( V_c \) were measured by injecting hypertonic saline (5% NaCl solution; 0.020–0.025 ml) into the pulmonary artery to transiently change the resistivity of the blood in the LV and calculated \( V_c \) value [10, 16, 18]. The calculated \( V_c \) value was subtracted from the measured LV conductance volume to obtain LV absolute blood volume.

At the end of each experiment, all 12 rats were killed by injecting a lethal dose of pentobarbital sodium. The LV including the interventricular septum was excised and weighed after the atria and the right ventricular free wall were trimmed off. The LV of the normal rats weighed 0.76±0.04 g, ranging from 0.69 to 0.80 g. The LV of the hypothyroid rats weighed 0.65±0.07 g, ranging from 0.57 to 0.75 g (\( p<0.005 \)). These weights were used to normalize LVV by 1 g LV mass in individual hearts.

After experiments, myocardial myosin isozymes in the LV of normal and hypothyroid rats could be identified by using polyacrylamide gel electrophoresis.

**Polyacrylamide gel electrophoresis.** The LV myocardium from each heart was frozen and stored at −20°C. Myosin was extracted from 50 mg of the LV myocardium with Hasselbach-Schneider solution (pH 6.4) containing 0.6 M KCl, 0.1 M potassium phosphate, 10 mM Na₂HPO₄, and 1 mM MgCl₂ (0 to 4°C). Three myosin isozymes (V₁, V₂, and V₃) were separated by 3.7% polyacrylamide gel electrophoresis in the presence of pyrophosphate, which was carried out at 80 V for 24 h (0 to 3°C) according to a slightly modified procedure of Hoh et al. [13]. The gels were stained with 0.5% Coomassie Brilliant Blue R 250. We photographed the gels, scanned them with a scanner, and calculated \( V₁ \):\( V₂ \):\( V₃ \) ratio by NIH image analysis. We then added the half-amount of \( V₂ \) (a heterodimer of \( \alpha \) and \( \beta \) heavy chain) to the amount of \( V₁ \) and \( V₂ \) and obtained the resultant \( V₁ \) and \( V₂ \) percent values to total myosin.

**Data collection.** LV pressure (LVP) and the three individual segmental conductance volume signals were digitized in a 12-bit accuracy at a sampling frequency of 500 Hz and stored on a floppy disk in a computer system (NEC, PC-9801 RA, Japan) for later analyses.

**Data analysis.** In in situ normal rat LV, a curvilinear ESPVR was obtained by drawing an upper enveloping curve on a series of P-V loops in a manner similar to our previous method [10]. The LV end-systolic P-V data on the left-upper shoulder of all the P-V loops were plotted and fitted by the method of the least squares by using the following equation (Eq. 3) proposed by Suga et al. [20] in puppies.

\[
\text{LVP}=A\{1-\exp[-B(LVV-V₀)]\},
\]

where LVP is LV pressure, LVV is LV volume, and \( A \), \( B \), and \( V₀ \) are fitting parameters. If the ESPVR is an upward convex curve, \( B \) will be larger. The larger \( B \) is, the more curved ESPVR is. We then obtained the best-fit ESPVR curve in each of the 12 hearts.

LV systolic pressure-volume area (PVA) is a measure of the total mechanical energy generated by an LV contraction [7, 21–23]. It is quantified by the area in the P-V diagram bounded by the ESPVR, end-diastolic P-V relation, and the systolic P-V trajectory [7, 24].
After obtaining the LV ESPVR curve of ejection contractions mentioned above, we assumed the LV ESPVR curve of ejection contractions to be that of isovolumic contractions [24]. This assumption was validated by our previous paper [10]; the ESP-V point obtained by an isovolumic one-beat clamp was on or near the ESPVR curve of ejection contractions. The PVA of an isovolumic contraction represents the maximal capability of external mechanical work of the LV at a given preload [22]. In the present study, PVA was defined as the area in the P-V relation diagram surrounded by the already determined best-fit ESPVR curve, the volume axis (instead of negligibly small end-diastolic P-V relation curve), and the vertical isovolumic P-V line at any preloaded LVV. PVA as a function of LVV was obtained by integrating Eq. 3 from the extrapolated \( V_o \) along the volume axis. The following Eq. 4 is the obtained function [23].

\[
PVA = A \left[1 - \exp\left(-B(LVV - V_o)\right)\right]/\left[1 - \exp\left(-B(LVV - V_o)\right)\right] - A \left[1 - \exp\left(-B(LVV - V_o)\right)\right]/B,
\]

where LVV ranged from \( V_o \) to 0.3 ml/g.

In the present study, we used ESP at an LVV 0.15 ml/g (ESP_{0,15}) and PVA at an LVV such as 0.15 ml/g (PVA_{0,15}) to practically assess LV contractility instead of Ees (the slope of the linear ESPVR) (Table 1). We had proposed that in in situ hearts, PVA at an appropriate LVV can be used to evaluate LV mechanoenergetics despite the curvilinear LV ESPVR [10], for the following reasons. Positive and negative inotropic drugs obviously shift the baseline ESPVR curve upward and downward at any volume [10]. Therefore ESP at a midrange LVV on the curvilinear ESPVR is preferable for evaluating the shift of the ESPVR. Positive and negative inotropic drugs obviously shift the concave baseline PVA-V relation curve upward and downward [10]. Therefore PVA at an appropriate LVV on the curvilinear PVA-V relations is preferable to evaluate the upward and downward shifts of the PVA-V relations and thus the increase and decrease in LV work capability [10]. As an appropriate common LVV, we chose 0.15 ml/g to compare LV ESPVR between normal and hypothyroid rat hearts. This particular volume was chosen because it was approximately midway between the maximum and minimum ESV values in normal and hypothyroid rats.

**Statistics.** All data were expressed as mean±SD (standard deviation). A comparison of paired mean values was performed by a paired t-test. A comparison of unpaired mean values was performed by a pooled t-test. A multiple comparison was performed by an analysis of variance and the least significant difference method. In all statistical tests, \( p \) values <0.05 were considered statistically significant.

**RESULTS**

**Curvilinear ESPVR in normal rat**

Figure 1A shows a representative series of LV P-V loops of an in situ normal rat heart while changing afterload by a gradual aortic occlusion. We drew an enveloping curve on the series of P-V loops without the preset \( V_o \) in contrast to our previous study [10]. We obtained similar results in the other 5 normal rats.

**Linear ESPVR in hypothyroid rat**

Figure 1B shows a representative series of LV P-V loops of an in situ hypothyroid rat heart while changing afterload by a gradual aortic occlusion. We drew an almost straight line on the series of P-V loops. We obtained similar results in the 5 other hypothyroid rats.

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Fig. 1. Representative left ventricular (LV) pressure (P)-volume (V) loops in in situ ejecting rat hearts during an aortic occlusion. A: normal rat; B: hypothyroid rat. Volume axes indicate normalized absolute LV volume for 1 g LV mass.
Fig. 2. ESPVR curves best fitted to the ESP-V data points by an exponential function [Eq. 3: left ventricular pressure (LVP) = A(1 - exp[-B(LV - Vc)])] and PVA-V relations obtained by integrating the best-fit ESPVR curves. A: The baseline ESPVR curve in the same normal rat (end-systolic P-V point • and solid curve), as shown in Fig. 1A. B: The baseline PVA-V relation curve in this normal rat (solid curve). C: The baseline ESPVR curve (though it appeared to be linear) in the same hypothyroid rat (• and solid curve), as shown in Fig. 1B. D: The baseline PVA-V relation curve in this hypothyroid rat (solid curve). Dotted vertical lines (0.15 ml/g) indicate a midrange LV. End-systolic pressure (ESP) at a midrange left ventricular volume (LV = 0.15 ml/g) in this normal rat (A) was larger than in this hypothyroid rat (C). PVA at a midrange LVV (= 0.15 ml/g) in this normal rat (B) was larger than in this hypothyroid rat (D).

Table 1. Fitting parameters of the best-fit Eqs. 3 and 4 (see text) in normal and hypothyroid rats.

<table>
<thead>
<tr>
<th></th>
<th>A ± SE</th>
<th>B ± SE</th>
<th>R ± SE</th>
<th>Vc (ml/g) ± SE</th>
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</thead>
<tbody>
<tr>
<td>Normal rat (n=6)</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>205 ± 28</td>
<td>24 ± 17</td>
<td>0.995 ± 0.009</td>
<td>0.016 ± 0.012</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg</td>
<td>199 ± 24</td>
<td>9.5 ± 3.4* (p&lt;0.01)</td>
<td>0.996 ± 0.001</td>
<td>0.016 ± 0.010</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg + phentolamine 2 mg/kg</td>
<td>239 ± 67</td>
<td>7.2 ± 5.5* (p&lt;0.001)</td>
<td>0.998 ± 0.001</td>
<td>0.018 ± 0.017</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg + phentolamine 2 mg/kg + guanethidine 4 mg/kg</td>
<td>230 ± 101</td>
<td>8.3 ± 4.9* (p&lt;0.001)</td>
<td>0.999 ± 0.002</td>
<td>0.010 ± 0.019</td>
</tr>
<tr>
<td>Hypothyroid rat (n=6)</td>
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<tr>
<td>Baseline</td>
<td>549 ± 317* (p&lt;0.01)</td>
<td>2.1 ± 1.9* (p&lt;0.01)</td>
<td>0.999 ± 0.000</td>
<td>0.033 ± 0.016</td>
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<tr>
<td>Dobutamine 0.5 mg/kg</td>
<td>239 ± 355 (p&lt;0.05)</td>
<td>12.9 ± 6.15 (p&lt;0.01)</td>
<td>0.998 ± 0.002</td>
<td>0.017 ± 0.018</td>
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*Significantly different vs. baseline of normal rat. †Significantly different vs. baseline of hypothyroid rat.

Comparison between normal and hypothyroid rats

Figure 2A and C show two representative ESPVRs of the normal and hypothyroid rats determined for the data shown in Fig. 1. Each ESPVR is the best-fit curve of a series of experimentally obtained ESP-V data by an exponential function (Eq. 3). We obtained similar results in the other 5 normal and 5 hypothyroid rats. The averaged best-fit A, B, and Vc in the normal and hypothyroid rats were listed in Table 1. These B
Table 2. Variables of cardiac mechanoenergetics in normal and hypothyroid rats.

<table>
<thead>
<tr>
<th></th>
<th>ESP&lt;sub&gt;0.15&lt;/sub&gt; (mmHg)</th>
<th>PVA&lt;sub&gt;0.15&lt;/sub&gt; (mmHg·ml·beat&lt;sup&gt;-1&lt;/sup&gt;·g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>HR (beats/min)</th>
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<tr>
<td>Normal rat (n=6)</td>
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<tr>
<td>Baseline</td>
<td>180±31</td>
<td>17.3±5.5</td>
<td>320±33</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg</td>
<td>137±19</td>
<td>11.1±2.6&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.05)</td>
<td>250±0&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg + phentolamine 2 mg/kg</td>
<td>106±33&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.05)</td>
<td>9.0±4.1&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.01)</td>
<td>250±0&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
</tr>
<tr>
<td>Propranolol 0.5 mg/kg + phentolamine 2 mg/kg + guanethidine 4 mg/kg</td>
<td>128±31</td>
<td>10.8±4.1&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.05)</td>
<td>250±0&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
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<tr>
<td>Hypothyroid rat (n=6)</td>
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<tr>
<td>Baseline</td>
<td>77±15&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.01)</td>
<td>4.7±1.2&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
<td>253±5&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
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<tr>
<td>Dobutamine 0.5 mg/kg</td>
<td>181±15&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
<td>15.6±4.3&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.001)</td>
<td>295±33&lt;sup&gt;∗&lt;/sup&gt; (p&lt;0.01)</td>
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</table>

<sup>∗</sup> Significantly different vs. baseline of normal rat. <sup>§</sup> Significantly different vs. baseline of hypothyroid rat. ESP<sub>0.15</sub>: end-systolic pressure (ESP) at a midrange end-systolic volume (ESV) of 0.15 ml/g. PVA<sub>0.15</sub>: pressure-volume area (PVA) at a midrange ESV of 0.15 ml/g. HR, heart rate.

Fig. 3. A: Comparison of end-systolic pressure (ESP) at a midrange left ventricular volume (LVV) of 0.15 ml/g (ESP<sub>0.15</sub>) between 6 normal and 6 hypothyroid rat hearts. There is a significant (p<0.001) decrease in ESP<sub>0.15</sub> from normal rats to hypothyroid rats. B: Comparison of pressure-volume areas (PVA) at a midrange LVV of 0.15 ml/g (PVA<sub>0.15</sub>) between 6 normal and 6 hypothyroid rat hearts. There is a significant (p<0.001) decrease in PVA<sub>0.15</sub> from normal rats to hypothyroid rats. Each solid circle with vertical bar indicates mean±standard deviation.

values indicate that the ESPVRs in the normal rats were consistently curvilinear and that the ESPVRs in the hypothyroid rats were almost linear. Although the ESPVR described in a low-pressure range appears to be linear even in normal rats, the ESPVR is virtually curvilinear in a wide pressure range. If one describes the linear ESPVR in a low-pressure range, the <i>v</i><sub>0</sub> value would be negative as reported in our previous paper [10].

We used ESP at a midrange LVV at 0.15 ml/g (ESP<sub>0.15</sub>) on the curvilinear ESPVR to evaluate the shift of the ESPVR. The mean ESP<sub>0.15</sub> in the normal rats was 180 mmHg; the mean ESP<sub>0.15</sub> in the hypothyroid rats was 77 mmHg (43% of the normal rats) (Table 2). The mean±SD values of ESP<sub>0.15</sub> were compared in Fig. 3A. Pooled t-test showed that ESP<sub>0.15</sub> in the hypothyroid rats was significantly (p<0.001) smaller than in the normal rats.

Figure 2B and D show two representative pairs of upward concave curvilinear PVA-V (preload) relations in the normal and hypothyroid rats by integrating the respective best-fit ESPVR curves (Eq. 3) in Fig. 2A and C (i.e., Eq. 4).

PVA at a certain LVV on the curvilinear PVA-V relations is preferable for evaluating the upward and downward shifts of the PVA-V relation and thus the increase and decrease in LV work capability [10]. We used a midrange PVA such as 0.15 ml/g (PVA<sub>0.15</sub>). The mean±SD values of PVA<sub>0.15</sub> in the normal and hypothyroid rats were listed in Table 2 and compared in Fig. 3B. A pooled t-test showed that PVA<sub>0.15</sub> in the hypothyroid rats was significantly (p<0.001) smaller than in the normal rats.

Effects of propranolol, phentolamine, and guanethidine on normal rat ESPVR

In normal rats, the curvilinear LV ESPVR shifted right-downward with decreased LV contractility by propranolol (Fig. 4B). Additional phentolamine further decreased ESP but the shape of the LV ESPVR did not change (Fig. 4C). Thirty minutes after additional guanethidine, the curvilinearity of the LV ESPVR appeared to decrease (Fig. 4D). The mean baseline heart rate of the normal rats was 320
Curvilinearity of ESPVR of *In Situ* Rat Left Ventricle

![Graphs of pressure-volume loops](image)

**Fig. 4.** Representative left ventricular (LV) pressure (P)-volume (V) loops in an *in situ* ejecting normal rat heart during an aortic occlusion. A: baseline; B: after propranolol injection; C: after propranolol+phenolamine injection; D: after propranolol+phenolamine+guanethidine injection. Volume axes indicate normalized absolute LV for 1 g LV mass. The curvilinear ESPVR shifted right-downward with decreased LV contractility by propranolol. Even if we added either phenolamine or guanethidine, ESPVR did not further change.

beats/min. After propranolol, heart rate was fixed at 250 beats/min (about 80% of baseline; *p*<0.001) by right atrial pacing (Table 2).

Respective calculated ESP0.15, PVA0.15 and best-fit parameters *A*, *B*, and *V₀* after propranolol, phenolamine, and guanethidine in the 6 normal rats are listed in Tables 1 and 2. Respective best-fit *B* after propranolol, phenolamine, or guanethidine were much smaller than baseline, but larger than in hypothyroid rats. This indicates that none of the ESP-PVRs is linear. ESP0.15 was significantly (*p*<0.05) decreased by propranolol and phenolamine to approximately 59% of baseline. An additional guanethidine showed no further depressive effects on ESP0.15 in the normal rats. PVA0.15 was significantly (*p*<0.05) decreased by propranolol to approximately 64% of baseline. Neither additional phenolamine nor guanethidine had further depressive effects on PVA0.15 in the normal rats.

**Effect of dobutamine on hypothyroid rat ESPVR**

ESP0.15 was significantly (*p*<0.001) increased by dobutamine to approximately 235% of baseline. PVA0.15 was also significantly (*p*<0.001) increased by dobutamine to 332% of baseline (Table 2). The linear LV ESPVR in the hypothyroid rats shifted left-upward with increased LV contractility by dobutamine and became curvilinear (Fig. 5B). The mean baseline heart rate of the hypothyroid rats was 253 beats/min (about 80% of normal rats), but after dobutamine the heart rate increased to 295 beats/min (about 90% of baseline in the normal rats).

The best-fit parameters *A*, *B*, and *V₀* after dobutamine in the 6 hypothyroid rats are listed in Table 1. The best-fit *B* in the baseline significantly increased to 12.9 after dobutamine on average. This indicates that LV ESPVR became curvilinear after dobutamine.

**Myosin isozyme**

Myosin isozyme in LV myocardium showed a predominance of *V₃* (approximately 98% of total myosin)
Hypothyroid rat

Fig. 5. Representative left ventricular (LV) pressure (P)-volume (V) loops in an in situ ejecting hypothyroid rat heart during an aortic occlusion. A: baseline; B: after dobutamine injection. Volume axes indicate normalized absolute LVV for 1 g LV mass. The linear ESPVR shifted leftward with increased LV contractility from dobutamine, and ESPVR became curvilinear.

Fig. 6. Comparison of percent V₁ and V₂ myosin isozymes of the left ventricular rat myocardium between normal and hypothyroid rat hearts (16 to 17 weeks). Each column indicates mean±SD of percent V₁ and V₂ (n=6).

in the hypothyroid rats (16 to 17 weeks old, n=6), whereas in the normal rats (16 to 17 week old, n=6) only V₁ was observed (Fig. 6).

DISCUSSION

The present study has for the first time revealed the relationship between myocardial myosin isozyme and curvilinearity of the rat LV ESPVR in in situ ejecting heart. The curvilinearity of rat LV ESPVR was not determined by myosin isozyme pattern per se, but was more closely related to LV contractility.

The ESPVR in the excised cross-circulated canine heart has been fully studied and clarified to be almost linear within physiological P-V ranges [1, 3–7]. The ESPVR in human hearts is also almost linear [25]. However, the curvilinearity of LV ESPVR even in the adult cross-circulated canine heart has been reported to be dependent on the LV contractility [2, 19]; the convexity is upward in supernormal contractilities and downward in subnormal contractilities. Furthermore, the puppy LV ESPVR was upward convex curvilinear [20, 23]. Therefore the present result showing that the LV ESPVR is curvilinear in rat hearts is not surprising.

The main cardiovascular effects of thyroid hormone are an acceleration of the activity of Ca²⁺ channel on myocardial cellular membrane [26], an acceleration of Ca²⁺ release from the sarcoplastic reticulum (SR) [26], an acceleration of Ca²⁺ ATPase of SR [26], an increase in the number of myocardium β receptors [27], and a transformation of cardiac myosin isozyme from V₃ to V₁ [26]. Conversely, hypothyroidism [13], diabetes [28], aging [29], and chronic pressure over-load [30, 31] lead to an increase in V₃ myosin isozyme in the rat ventricle.

We transformed V₁ to V₃ by making rats hypothyroid to investigate whether myocardial myosin isozyme pattern is related to the curvilinearity of the in situ rat LV ESPVR. Rat myocardial myosin is V₃ in the viviparity period. After birth it is transformed to V₁, and to V₃ again in the advanced age. Thus we used age-matched rats to make hypothyroid rats. We compared LV ESPVRs in in situ ejecting hearts, of which myosin isozymes were V₁ in adult normal rats and V₃ in hypothyroid rats.

The shape of ESPVR in the normal rat was an upward convex curve. This seems to indicate that the normal heart works under a higher LV contractility condition and has a less contractile reserve than the hypothyroid rat heart does. Pharmacological or chemical sympathectomy did not make the ESPVR linear, indicating that LV higher contractility is not due to catecholamines released from sympathetic nerves or adrenal glands. In consideration of our unpublished observation, which shows that Langendorff-perfused
rat hearts easily become \( \text{Ca}^{2+} \) overload [32], the higher LV contractility in normal rats may be due to a high intracellular \( \text{Ca}^{2+} \) concentration. Therefore the ESPVR may become linear in normal rats when we use much more potent negative inotropic drugs to decrease intracellular \( \text{Ca}^{2+} \) concentration.

The LV ESPVR in the hypothyroid rat was linear. However, the ESPVR became curvilinear associated with increased LV contractility by dobutamine as a positive inotropic agent. Therefore we consider that myosin isozyme per se does not determine the curvilinearity of the ESPVR.

**Evaluation by the curvilinear ESPVR and the linear ESPVR**

We cannot use \( \text{Ees} \) to evaluate LV contractility; instead, we used \( \text{ESP}_{0.15} \) and \( \text{PVA}_{0.15} \) in rat hearts. In the present study, \( V_o \) values in the normal and hypothyroid rats were not significantly different; thus the evaluation method for the LV contractility by using \( \text{ESP}_{0.15} \) is reasonable. However, we should use \( \text{PVA}_{0.15} \) to compare LV contractility between dilated failing hearts and normal hearts with markedly different \( V_o \) values.

Moreover, it is PVA that closely and linearly correlates with cardiac oxygen consumption per beat under a variety of loading conditions in a stable contractility in the cross-circulated rat hearts [8, 9]. Therefore the \( \text{PVA}_{0.15} \) can reasonably be used as a measure of total mechanical energy and of work capability as a function of preload of a ventricular contraction even under a nonlinear ESPVR in a rat LV.

Results have been reported indicating that the isotropic tension sizably decreased in the native trabecular preparations [14] and that peak developed tension significantly decreased in LV papillary muscle preparations [15] of the hypothyroid rat (\( V_3 \) myocardium). In contrast is a different result showing that in *vitro* motility assay \( V_1 \) myosin produces only half the average cross-bridge force of \( V_3 \) myosin [33]. Furthermore, in skinned rat right ventricular myocardium, it has been reported that the myosin isozyme shift does not affect the force-free [\( \text{Ca}^{2+} \)] relationship [34]. In the present rat whole heart preparation, LV \( \text{ESP}_{0.15} \) and LV \( \text{PVA}_{0.15} \) were significantly smaller in the \( V_3 \) myocardium than in the \( V_1 \) one, indicating that the LV contractility decreased. However, the ESPVR became curvilinear after increasing LV contractility with dobutamine in the \( V_3 \) myocardium. As shown in Fig. 7, the best-fit parameter \( B \) (shape parameter) linearly correlated with \( \text{PVA}_{0.15} \) \((r=0.8610)\) under various LV contractile conditions in normal and hypothyroid rats. Therefore myosin isozyme per se does not decide the curvilinearity of ESPVR, which is decided by the LV contractility.

**Limitations of the present study**

We studied *in situ* ejecting rat hearts, which are much more physiological than the excised cross-circulated isovolumically contracting hearts, but the myocardium suffered from some injuries by LV catheterization. High-dose pentobarbital anesthesia had negative inotropic effects [5]. Therefore the possibility that LV contractility had already been depressed by pentobarbital anesthesia and some injuries cannot be excluded.

P-V loops obtained by our present method showed a tendency to lean toward the left side, rarely the right side. The leaned loop might be caused by the dysfunction (regurgitation) of the aortic and mitral valves or by LV deformation resulting from the inserted catheters. In this study we assumed that any volume changes during the otherwise normally isovolumic phase did not significantly affect the ESPVR.

No dependence of the linear ESPVR on heart rate has been reported in the canine heart within the physiological heart rate range [35]. In the present study, the heart rate was kept within 250 to 320 beats/min. We confirmed that the LV ESPVR in *in situ* rat hearts did not change significantly within this physiological heart rate range.
CONCLUSION

We revealed that the LV ESPVR in *in situ* hypothyroid rat hearts with V$_1$ myosin isozyme was almost linear in contrast to the curvilinear ESPVR in normal rat hearts with V$_1$ myosin isozyme. However, the convexity of the LV ESPVR in the normal rat became weak with decreased LV contractility by various negative inotropic agents. Furthermore, the convexity of the LV ESPVR in the hypothyroid rat became strong with increased LV contractility by positive inotropism. Although the underlying mechanism for the higher LV contractility in normal rats remains to be investigated, we concluded that the shape of ESPVR is decided by the LV contractility.

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