Variable Unstressed Volume Keeps Normal Distributions of Canine Left Ventricular Contractility and Total Mechanical Energy under Atrial Fibrillation

Satoshi MOHRI*, Juichiro SHIMIZU*[‡], Haruo ITO*[‡], Hiroki YAMAGUCHI[†],
Shunji SANO[†], Miyako TAKAKI[‡], and Hiroyuki SUGA*[§]

Departments of Cardiovascular *Physiology and [†]Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Shikatacho, Okayama, 700-8558 Japan; [‡]Department of Physiology II, Nara Medical University, Shijocho, Kashihara, Nara, 634-8521; and [§]National Cardiovascular Center Research Institute, Fujishirodai, Suita, Osaka, 565-8565 Japan

Abstract: We have reported that the contractility index ($E_{\max}$) and the total mechanical energy (PVA) of arrhythmic beats of the left ventricle (LV) distribute normally in canine hearts under electrically induced atrial fibrillation (AF). Here, $E_{\max}$ is the ventricular elastance as the slope of the end-systolic (ES) pressure-volume (P-V) relation (ESPVR), and PVA is the systolic P-V area as the sum of the external mechanical work within the P-V loop and the elastic potential energy under the ESPVR. To obtain $E_{\max}$ and PVA, we had to assume the systolic unstressed volume (V_0) as the V-axis intercept of the ESPVR to be constant despite the varying $E_{\max}$, since there was no method to obtain V_0 directly in each arrhythmic beat. However, we know that in regular stable beats V_0 decreases by ~7 ml/100 g LV with ~100

times the increases in $E_{\max}$ from ~0.2 mmHg/(ml/100 g LV) of almost arresting weak beats to ~20 mmHg/(ml/100 g LV) of strong beats with a highly enhanced contractility. In the present study, we investigated whether $E_{\max}$ and PVA under AF could still distribute normally, despite such $E_{\max}$ -dependent V_0 changes. The present analyses showed that the $E_{\max}$ changes were only ~3 times at most from the weakest to the strongest arrhythmic beat under AF. These changes were not large enough to affect V_0 enough to distort the frequency distributions of $E_{\max}$ and PVA from normality. We conclude that one could practically ignore the slight $E_{\max}$ and PVA changes with the $E_{\max}$ -dependent V_0 changes under AF. [The Japanese Journal of Physiology 55: 255–264, 2005]

Key words: arrhythmia, cardiac function, $E_{\max}$, PVA, frequency distribution.

Many groups, including ours, have investigated the statistical characteristics of cardiodynamic variables of ventricular arrhythmic beats under atrial fibrillation (AF) in basic and clinical studies [1–17]. We have found that the frequency distributions of the heart rate (HR), beat-to-beat interval (RR), and effective afterload elastance (E_a) (Fig. 1) of the left ventricle (LV) [18, 19] are significantly non-normal or non-Gaussian in canine hearts under AF [9–11]. However, we have recently found that the frequency distributions of both contractility index ($E_{\max}$) and total mechanical energy (PVA) (Fig. 1) are normal or Gaussian in

canine LVs under AF [9–11, 14, 17]. Here, $E_{\max}$ is the ventricular elastance as the slope of the end-systolic pressure-volume (P-V) relation (ESPVR) line, and PVA is the systolic P-V area as the sum of the external mechanical work (EW) within the P-V loop and the elastic potential energy (PE) under the ESPVR (Fig. 1). We have attributed this normal distribution of PVA to the central limit theorem by which the complexity of the PVA formula could attenuate the effect of the considerably non-normal distribution of E_a on PVA [10].

Received on Oct 18, 2005; accepted on Oct 27, 2005; released online on Nov 1, 2005; DOI: 10.2170/jjphysiol.RP000405

Correspondence should be addressed to: Satoshi Mohri, Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Shikatacho, Okayama 700-8558, Japan. Fax: +81-86-235-7114, Tel: +81-86-235-7430, E-mail: smohri@md.okayama-u.ac.jp

Fig. 1. Schematic illustration of cardiac mechanoenergetic variables in the left ventricular (LV) pressure (P)–volume (V) diagram. ESP = end-systolic pressure. ESV = end-systolic volume. ESPVR = end-systolic P-V relation. $E_{\max}$ = end-systolic maximum elastance or P-V ratio as the slope of ESPVR, which was proposed by Suga and has generally been recognized as a reasonably load-independent index of ventricular contractility. EDV = end-diastolic volume. EDPVR = end-diastolic P-V relation. EW (dotted area) = external mechanical work as the area within the P-V loop. PE (hatched area) = elastic potential energy within the ventricular wall generated by each contraction. PVA = total mechanical energy of each contraction as the sum of EW and PE. E_a = effective arterial elastance. For more details of $E_{\max}$, PE, PVA, and E_a , refer to Refs. 18, 21, and 23.

The tacit assumption in our previous study [10], however, was that the V-axis intercept (V_o) of the LV ESPVR was constant despite the arrhythmic beat-by-beat changes in $E_{\max}$ under AF. We had to assume so because V_o could not be determined in each arrhythmic beat [10], unlike in the case of regular stable beats at a given $E_{\max}$ [18, 20, 21]. In fact, our earlier study has shown V_o to decrease by as much as ~ 7 ml/100 g LV, but not constantly, with wide increases in $E_{\max}$ [22].

Therefore, in the present study, we investigated quantitatively the magnitude of the influence of the $E_{\max}$ -dependent V_o changes on the frequency distributions of both $E_{\max}$ and PVA [18, 21, 23] under AF. The LV P-V data that we used in the present study were the same that we used in the previous study [10]. We newly obtained statistical evidence that the $E_{\max}$ -dependent V_o changes under AF little affected both $E_{\max}$ and PVA and kept their frequency distributions normal or Gaussian. The reason for these results was that the range of arrhythmic changes in $E_{\max}$ under AF was too small to shift V_o to the extent that the frequency distributions of both $E_{\max}$ and PVA were deviated from

Fig. 2. Experimental evidence for the $E_{\max}$ dependency of the V-axis intercept of the P-V relation (ESPVR), drawn on the basis of our previous study [22]. Thick solid curves show the actual ESPVR curves obtained in rigor (steepest), at a high $E_{\max}$ (second steepest) and a low $E_{\max}$ (second gentle), and under ischemic arrest (most gentle). Thick dashed lines on or near the actual ESPVR curves show their linearized ESPVRs, apparently converging to a common V_d - P_d point. V_o (ventricular dead volume) = 4.1 ± 1.3 (SD) ml/100 g LV and $P_d = -9.5 \pm 2.0$ (SD) mmHg [22]. The inset square shows the equation of the V-axis intercepts (V_o 's) of these linearized ESPVRs as a function of $E_{\max}$ with V_d and P_d as parameters as proposed previously [22]. Left ventricular (LV) V is labeled in excess of V_d . The horizontal brace shows the full working range of V_o between the maximum $E_{\max}$ in rigor and minimum $E_{\max}$ under arrest. See Figs. 3–9 of Ref. 22 for more details.

normality. This evidently corroborates our previous conclusion [10].

METHODS

Theoretical considerations. Figure 2 shows the $E_{\max}$ -dependent V_o changes that we had obtained experimentally in canine regularly beating LVs in our earlier study [22]. Here, the four thick solid curves are the actual ESPVR curves for different $E_{\max}$ levels varied from a physiologically high contractile level first down to a low contractile level, further down to the zero contractile level under ischemic arrest, and finally up to the maximum contractile level in rigor. All ESPVR curves were drawn within a low P ($< \pm 15$ mmHg) range to see their details around the V axis. These ESPVR curves merged at a common dead volume (V_d) below a negative P of 10–15 mmHg [22].

Figure 2 also shows the four ESPVR (thick dashed) lines fitted to the main linear parts of the four ESPVR curves. Although the ESPVR curves at low $E_{\max}$ levels are obviously convex upward in the further negative P range, these curved parts are not observable

Fig. 3. Schematic illustration of the $E_{\max}$ dependency of V_o (V-axis intercept of ESPVR line or $E_{\max}$ line) and the resultant deviations of V_o and $E_{\max}$ from their virtual values (vV_o and $vE_{\max}$) to actual values (aV_o and $aE_{\max}$). The end-systolic (ES, or es) corners of the three P-V loops are shown in the LV P-V diagram. The three solid ESPVR lines passing these ES corners have the same V intercept at vV_o that has been obtained as the LVV-axis intercept by extrapolating the ESPVR of regular stable contractions. However, the actual ESPVR lines at different $aE_{\max}$ levels rather converge to a single negative P point (V_d , P_d). In other words, all the actual ESPVR lines change their slopes or actual $E_{\max}$ ($aE_{\max}$) values by rotating around the V_d - P_d point as a fulcrum. The three dashed ESPVR lines are the actual ESPVR lines with $aE_{\max}$ and $E_{\max}$ -dependent actual V_o (aV_o). The middle solid and dashed ESPVR lines are drawn to be superimposable on each other, since the extrapolation of the solid ESPVR line passes through the V_d - P_d point with the same $aE_{\max}$ as the $vE_{\max}$ at an intermediate level of $E_{\max}$. The inset square lists 5 equations to describe $vE_{\max}$ (Eq. 1), V_d (Eq. 2), $aE_{\max}$ (Eq. 3), aV_o (Eq. 4), and $aPVA$ (Eq. 5) used in the text. The three resultant aV_o 's are shown on the LVV axis. As the result, the $aE_{\max}$ greater than the middle $aE_{\max}$ ($=vE_{\max}$) is smaller than its corresponding $vE_{\max}$, and decreases PE (elastic potential energy, see Fig. 1) from its vPE to aPE (though not shown) by the triangular area (1). On the contrary, the $aE_{\max}$ smaller than the middle $aE_{\max}$ ($=vE_{\max}$) is greater than its corresponding $vE_{\max}$, and increases PE from its vPE to aPE (though not shown) by the triangular area (2). Although neither are shown, EW (external mechanical work, see Fig. 2) remains unchanged despite the difference between $aE_{\max}$ and $vE_{\max}$.

in normally loaded contractions. These dashed ESPVR lines virtually converged to a common point, the V of which is an LV dead volume (V_d) and the P of which is an LV convergent pressure (P_d). Our earlier study has shown $V_d = 4.1 \pm 1.3$ (SD) ml/100 g LV and $P_d = -9.5 \pm 2.0$ mmHg [22].

Our earlier study [22] has also shown that although $E_{\max}$ decreased from a physiologically high ~ 20 mmHg/(ml/100 g LV) down to a physiologically low ~ 5 mmHg/(ml/100 g LV), V_o increased only by ~ 1 –

ml/100 g LV. However, when $E_{\max}$ further decreased to ~ 1 – 0.5 mmHg/(ml/100 g LV), V_o sensitively increased to as much as ~ 3 – 10 ml/100 g LV. In contrast, when $E_{\max}$ was maximally raised to ~ 50 mmHg/(ml/100 g LV), V_o was as small as ~ 0.5 ml/100 g LV. As a whole, V_o decreased by 11.5 ± 5.0 ml/100 g LV on average from arrest to rigor in the six LVs, as shown by the horizontal brace (Fig. 2).

These nonlinear relations between V_o and $E_{\max}$ once V_d and P_d are given could theoretically be formulated by the following equation:

$$V_o = V_d - P_d / E_{\max}$$

Here, the assumption is that the linearized ESPVR rotates with $E_{\max}$ around the fixed V_d - P_d point as a fulcrum. We assumed these basic concepts of V_o and V_d to hold even in arrhythmic contractions with beat-by-beat variable $E_{\max}$ levels. We then adopted the above equation (see also the inset of Fig. 2) to calculate the varying V_o with $E_{\max}$ of each arrhythmic beat in the present study.

Figure 3 schematically shows three P-V loop segments around the end systole (ES) and two pairs of three ESPVR lines (solid and dashed diagonal). Of these, the solid ESPVR lines have a common fulcrum at the virtual (v) V_o (vV_o) on the V axis. Here, the vV_o stands for the conventional, virtually constant or $E_{\max}$ -independent V_o obtained in control $E_{\max}$ by clamping the inferior vena cava under regular beats as usual [10, 11, 18, 20, 21, 23]. $E_{\max}$ values obtained using the vV_o and virtual ESPVR ($vESPVR$) as usual are hereafter called virtual $E_{\max}$ ($vE_{\max}$). The vV_o has usually been used to calculate both $vE_{\max}$ and virtual PVA ($vPVA$), even in different $E_{\max}$ levels from that used to obtain vV_o , as in the case of our previous study [10].

In contrast to these virtual values, the dashed ESPVR lines have a common fulcrum at the V_d - P_d point below the V axis (Fig. 3). These dashed lines are the actual (a) ESPVR ($aESPVR$) lines, different from the $vESPVR$ lines, and thus the former lines have different $E_{\max}$ values (actual $E_{\max}$, $aE_{\max}$) from the corresponding $vE_{\max}$'s, though they pass virtually the same end-systolic P-V (Pes-Ves) points. The only exceptional case is the identical $E_{\max}$ of the dashed and solid ESPVR lines, both with the same vV_o and aV_o , as illustrated by the middle pair of the dashed and solid ESPVR lines. The $aESPVR$ lines intersect the V axis at different points such as the three vV_o 's in different font sizes (Fig. 3). These intercepts indicate the aV_o which is dependent on $aE_{\max}$.

Figure 3 shows that, as the results, $aE_{\max}$ is smaller than $vE_{\max}$ at a greater $E_{\max}$ level than control and

Table 1. Minor effects of $E_{\max}$ dependency of V_o on $E_{\max}$, PE, and PVA.

Statistical values	% Changes in actual (a) values from virtual (v) values		
	$(aE_{\max} - vE_{\max})/vE_{\max}$	$(aPE - vPE)/vPE$	$(aPVA - vPVA)/vPVA$
Mean			
Mean $\pm$ SD (CV)	-0.38 ± 0.49 (1.3)	-0.72 ± 0.43 (0.6)	-0.12 ± 0.06 (0.5)
p	0.12 (ns)	0.009 (<0.05)	0.003 (<0.05)
Median			
Mean $\pm$ SD (CV)	0.31 ± 1.44 (4.6)	0.12 ± 1.67 (14)	0.05 ± 0.33 (6.6)
p	0.63 (ns)	0.87 (ns)	0.75 (ns)
Mode			
Mean $\pm$ SD (CV)	0.11 ± 2.98 (27)	0.01 ± 2.97 (297)	0.05 ± 1.05 (21)
p	0.93 (ns)	0.99 (ns)	0.98 (ns)
SD			
Mean $\pm$ SD (CV)	-13.6 ± 3.05 (0.2)	-4.8 ± 12.35 (2.6)	2.3 ± 1.15 (0.5)
p	0.0001 (<0.05)	0.38 (ns)	0.005 (<0.05)
Variance			
Mean $\pm$ SD (CV)	-25.2 ± 5.3 (0.2)	-8.1 ± 24.6 (3.0)	4.7 ± 2.4 (0.5)
p	0.0001 (<0.05)	0.46 (ns)	0.005 (<0.05)
Range			
Mean $\pm$ SD (CV)	-13.3 ± 3.35 (0.3)	-3.78 ± 14.85 (3.9)	1.83 ± 1.03 (0.6)
p	0.0002 (<0.05)	0.56 (ns)	0.007 (<0.05)
Minimum			
Mean $\pm$ SD (CV)	12.3 ± 6.35 (0.5)	2.93 ± 10.51 (3.6)	-4.76 ± 4.87 (1.0)
p	0.005 (<0.05)	0.61 (ns)	0.06 (ns)
Maximum			
Mean $\pm$ SD (CV)	-6.42 ± 2.98 (0.5)	-4.58 ± 5.86 (1.3)	0.48 ± 0.20 (0.4)
p	0.003 (<0.05)	0.11 (ns)	0.002 (<0.05)

CV (=SD/Mean): coefficient of variation. p for paired t test with respect to zero. ns: $p > 0.05$.

greater than $vE_{\max}$ at a smaller $E_{\max}$ level than control. Simultaneously, however, $aPVA$ is greater than $vPVA$ by the triangular area labeled (i) at a greater $E_{\max}$ than control and smaller than $vPVA$ by the triangular area (ii) at a smaller $E_{\max}$ level than control (Fig. 3).

These actual (prefix: a) and virtual (prefix: v) parameters could theoretically or geometrically be related to the given V_d and P_d and the measured V_{es} and P_{es} as follows.

$$vE_{\max} = P_{es}/(V_{es} - vV_o) \quad (1)$$

$$V_d = vV_o + P_d/vE_{\max} \quad (2)$$

$$aE_{\max} = (P_{es} - P_d)/(V_{es} - V_d) \quad (3)$$

$$aV_o = V_d - P_d/aE_{\max} \quad (4)$$

$$aPVA = aPE + EW \quad (5)$$

These equations are inset in Fig. 3.

Surgical preparation. The details of the canine heart preparation were described in our previous papers [9–11, 14, 17]. Briefly, we performed the canine experiments in conformity with the guiding

principles for the care and use of animals in the field of physiological sciences of the Japan Physiological Society and the American Physiological Society. We anesthetized six mongrel dogs (6–12 kg) with pentobarbital sodium (25 mg/kg, I.V.) after premedication with ketamine hydrochloride (50 mg/kg, I.M.) and intubated them in each experiment. We maintained the anesthesia by fentanyl (100 μ g/h per dog, I.V.) as usual.

After opening the chest of the dog midsternally, we inserted a 3F catheter-tip micromanometer into the LV from the apex to measure the LV pressure (P). We introduced a 7F eight-electrode conductance catheter (Webster Laboratories, Baldwin Park, CA, USA) into the LV through an apical stab and placed it along the ventricular long axis to measure LV volume (V) continuously. Our previous papers described in detail the method for measuring LVV with this catheter [9–11, 14, 17].

Ventricular volumetry. Briefly, with this catheter we continuously measured the time-varying electrical conductance of the blood in the LV cavity. We used our custom-made signal conditioner-processor (SI

Table 2. Normal frequency distributions of $E_{\max}$, PE, and PVA despite $E_{\max}$ dependency of V_o .

Normality measures (dimensionless)	Actual (a) $E_{\max}$, PE, and PVA data		
	$aE_{\max}$	aPE	aPVA
Kurtosis			
Mean $\pm$ SD	-0.64 ± 0.41	1.35 ± 1.86	-0.41 ± 0.47
p	0.012 (<0.05)	0.14 (ns)	0.09 (ns)
Skewness			
Mean $\pm$ SD	-0.16 ± 0.55	0.97 ± 0.30	0.08 ± 0.49
p	0.52 (ns)	0.0006 (<0.05)	0.71 (ns)
χ^2			
Mean $\pm$ SD	3.00 ± 1.89	2.03 ± 0.90	2.24 ± 1.55
p	0.071–0.77 (ns)	0.36–0.999 (ns)	0.16–0.99 (ns)

For kurtosis and skewness, p for paired t test with respect to 0. For χ^2 test, the full range of p is shown. ns: $p > 0.05$.

Medicotech Co., Ltd., Japan) to convert the blood conductance to LV volume (LVV). For more details, refer to our previous paper [10].

Unstressed volume or volume-axis intercept (V_o). We used $E_{\max}$ as a reliable, load-independent index of LV contractility that was originally proposed by Suga and has been generally accepted [18, 21, 23]. To calculate $E_{\max}$ of every beat, we needed the V-axis intercept (V_o) of the ESPVR line (Figs. 1–3).

We determined an ESPVR and V_o by clamping the inferior vena cava over ~ 10 regular beats before the first AF as usual [9–11, 14, 17]. This clamp gradually decreased both LVP and LVV and shifted the shrinking LV P-V loop toward the origin of the P-V diagram. We drew a straight line tangential to or through the end-systolic, left-upper corners of these P-V loops and extrapolated the line to the V axis to obtain its V_o . These ESPVR and V_o are their virtual values (vESPVR, vV_o) to yield the virtual $E_{\max}$ values ($vE_{\max}$) in the conventional method [10] (Fig. 3).

Contractility index ($E_{\max}$). After we obtained the conventional virtual $E_{\max}$ ($vE_{\max}$) by Eq. 1 in Fig. 3, we newly obtained $aE_{\max}$ by Eq. 3 in Fig. 3. We first calculated V_d from vV_o , $vE_{\max}$, and P_d by Eq. 2 in Fig. 3. Here, we assumed $P_d = -10$ mm Hg as a reasonably representative value close to the mean P_d (9.5 ± 2.0 mmHg) empirically obtained in our previous study [22]. Once $aE_{\max}$ was determined, we obtained aV_o by Eq. 4 in Fig. 3. The aV_o is needed to calculate the aPVA.

Pressure-volume area (PVA). PVA consists of the two areas (Fig. 1): the external mechanical work (EW) (rectangular area) within the P-V loop and the elastic potential energy (PE) (triangular area) between the end-systolic P-V or $E_{\max}$ line and the end-diastolic P-V curve on the origin side of the P-V loop. These two areas of each sampled beat were calculated in a

computer as explained previously in detail [10]. PE had a difference between vPE and aPE by the area marked as (1) or (2) in Fig. 3. In the $E_{\max}$ range higher than the control, $aE_{\max}$ was smaller than $vE_{\max}$ and area (1) had to be added to PE and then to PVA to obtain aPE and aPVA. In the $E_{\max}$ range lower than the control, $aE_{\max}$ was larger than $vE_{\max}$ and area (2) had to be subtracted from PE and then from PVA to obtain aPE and aPVA.

Atrial fibrillation. We attached a pair of stimulation electrodes to the left atrial appendage. Suprathreshold electrical stimulation at 20 Hz via these electrodes induced and maintained AF [9–11, 14, 17]. We produced AF for 2 min and recorded LV P and V during the latter 1 min at sampling intervals of 3 ms in a computer.

Statistics. We performed the basic statistical analyses of $aE_{\max}$, aPE, and aPVA of individual arrhythmic beats sampled in one min under AF in each heart (Table 1). We studied their frequency distributions not only by the two dimensionless measures of departure from normality or Gaussianity: kurtosis and skewness, but also by a χ^2 test of their normality, evaluating this test by its p value (Table 2) [10, 24, 25]. Zero kurtosis and skewness indicate the normality of the frequency distribution [10, 24]. A positive kurtosis indicates a sharper peak and a negative kurtosis a duller peak than the normality [6, 11]. A positive skewness indicates a leftward shift of the peak frequency and a negative skewness its rightward shift [10, 24]. A χ^2 test supports the normality of the frequency distribution when its p value is greater than 0.05 [10, 24]. We compared these statistical results of $aE_{\max}$, aPE, and aPVA with those of $vE_{\max}$, vPE, and vPVA (Table 1). In these statistical analyses, we used both Microsoft Office 2001 Excel and Stat View version 4.5. The data were presented as mean $\pm$ SD.

Fig. 4. Representative examples of the effects of the $E_{\max}$ -dependent changes in V_o on the ESPVR lines and the resultant shifts of the ESPVR lines. Panel A shows a contraction with a relatively high $E_{\max}$. Panel B shows another with a relatively low $E_{\max}$. In Panel A, the hatched area for the elastic potential energy (PE) increases by the white area between the original solid-line ESPVR and the new dashed-line ESPVR. In Panel B, PE decreases by the hatched area between the original solid-line ESPVR and the new dashed-line ESPVR within the PE area. The area (horizontally shaded in Panel A or virtually zero in Panel B) for external mechanical work (EW) remains unchanged in either Panel A or B.

RESULTS

The artificial AF produced LV absolute arrhythmia at heart rates of 223 ± 36 (SD) beats/min in the six LVs, although these were significantly ($p < 0.05$) higher than their regular pacing rates of 169 ± 15 beats/min as previously reported [9]. Under AF, the RR1/RR2 of arrhythmic beats varied from 0.82 ± 0.07 at minimum to 1.91 ± 0.27 at maximum, and the maximum/minimum ratio was 2.35 ± 0.45 . Here, RR1 is the beat interval preceding the beat whose $E_{\max}$ is of interest, and RR2 the beat interval preceding the RR1, which determines proportionally the contractile potentiation, whereas RR2 determines inversely the contractile restitution [17]. Simultaneously, the maximum/minimum ratio of $E_{\max}$ was 3.75 ± 0.90 , though this $E_{\max}$ corresponds to $vE_{\max}$ in the present study.

Figure 4 shows the same representative set of LV P-V loops of 4 consecutive arrhythmic beats under AF in both panels. Panel A illustrates $E_{\max}$, PE, and EW of the arrhythmic beat with the largest $E_{\max}$ and PVA of these four beats. Panel B illustrates those of the arrhythmic beat with the smallest $E_{\max}$ and PVA of these four beats. Of the pair of ESPVRs in each Panel, the solid line shows vESPVR and the dashed one aESPVR. The two vESPVRs in both panels had the same vV_o . In contrast, the two aESPVRs connecting the V_d - P_d point (solid circle) below the V axis had different aV_o 's. In Panel A, aV_o was smaller than vV_o , but in Panel B, aV_o was greater than vV_o , though not labeled as aV_o and vV_o .

In Panel A, PVA consists of the sum of the quasi-rectangular (horizontally shaded) area within the P-V loop and the quasi-triangular (hatched) area between the vESPVR line and the EDPVR curve on the origin side of the P-V loop of this contraction. The rectangular area represents the EW that the LV performed by ejecting blood into the aorta in this contraction. The triangular area represents the PE that this contraction generated in the LV wall, but was not converted into EW [21, 23]. The EW was not influenced by the difference between the vESPVR and aESPVR. However, the PE was greater under the aESPVR than under the vESPVR by the small triangular area, corresponding to area (1) between the vESPVR and aESPVR in Fig. 3.

In Panel B, the EW was almost zero because of virtually zero stroke volume, and not influenced by the difference between vESPVR and aESPVR. However, the PE was smaller under the aESPVR than under the vESPVR by the small triangular area, corresponding to area (2) between the vESPVR and aESPVR in Fig. 3. Thus, not only $aE_{\max}$ but also aPVA, predictably changed with the $E_{\max}$ -dependent aV_o changes.

Figure 5 summarizes the result of our analysis of aV_o . The vV_o of the stable regular contractions (open circle) was 26.1 ± 4.9 ml/100 g LV in the present six hearts. Under the reasonable assumption of P_d of -10 mmHg as described above [22], V_d obtained by extrapolating the vESPVR line to this P_d in the stable regular contractions was 25.3 ± 5.1 ml/100 g LV in these hearts. These V_d values were smaller than the vV_o values of the stable regular contractions in the respective LVs by 0.83 ± 0.40 ml/100 g LV (>0 , $p < 0.0005$), ranging from 0.40 to 1.40 ml/100 g LV. Each aESPVR line connects the thus determined V_d - P_d point (large solid circle) and the end-systolic P-V point of the P-V loop of a given arrhythmic contraction. The intercept of each aESPVR line on the V axis is the aV_o .

Fig. 5. Experimental data of mean $\pm$ SD and range (in parentheses) of $aE_{\max}$ and aV_o (smaller solid circles) shown in a relatively low LVP range of the P-V diagram. aV_o and vV_o (open circle) values are shown in terms of $aV_o - V_d$ and $vV_o - V_d$ as indicated by braces, since both $E_{\max}$ and PVA of our present interest are determined by such corrected aV_o and vV_o . The three dashed lines are aESPVR lines. The larger solid circle is the $V_d - P_d$ point as the fulcrum of aESPVR lines.

of the respective contraction. The aV_o varied with the arrhythmic changes in $E_{\max}$. The aV_o thus obtained as the consequence of its $E_{\max}$ dependency was 26.2 ± 4.9 ml/100 g LV on average (range: 20.0–30.4 ml/100 g LV) in the six hearts. The mean aV_o was insignificantly greater than the vV_o by 0.06 ± 0.09 ml/100 g LV ($p = 0.18$). Figure 5 further shows the magnitudes of aV_o values above V_d at the maximum and minimum $aE_{\max}$ levels in the working range under AF.

We then compared the statistical values, such as mean, SD, variance, range, minimum, and maximum values of $aE_{\max}$, aPE , and $aPVA$ obtained in the present study with those of their virtual values ($vE_{\max}$, vPE , and $vPVA$). Table 1 summarizes the percent changes (mean $\pm$ SD and coefficient of variation: $CV = SD/\text{mean}$) of the individual statistical values of $E_{\max}$, PE , and PVA ($\Delta E_{\max}$, ΔPE , and ΔPVA , where $\Delta = \text{actual} - \text{virtual}$) by the $E_{\max}$ -dependent aV_o changes. Although the mean, median, and mode of $aE_{\max}$ were not significantly different from those of $vE_{\max}$, its SD, variance, range, and maximum values of $aE_{\max}$ were significantly smaller than those of $vE_{\max}$, whereas only the minimum value of $aE_{\max}$ was significantly greater than that of $vE_{\max}$. As the consequence of these $aE_{\max}$ and aV_o changes in individual arrhythmic beats, aPE and $aPVA$ also changed significantly, but only slightly from the corresponding vPE and $vPVA$. For aPE , only the slight decrease in its mean was significant, but the changes in its median, mode, SD, variance, range,

minimum, and maximum were little and insignificant. As for $aPVA$, the changes in its mean, SD, variance, range, and maximum were significant, but the changes in its median, mode, and minimum were only slight, and they were insignificant. These significant changes were opposite in direction between $\Delta E_{\max}$ and ΔPVA .

The SD, variance, range, and maximum of the percent ΔPVA were significant, although those of percent ΔPE were insignificant (Table 1). The significant ΔPVA changes accompanied the CV (coefficient of variation, SD/mean) values smaller than 1, whereas the insignificant ΔPE changes accompanied the CV values greater than 1.

Figure 6 compares a representative pair of the frequency distributions of the $vE_{\max}$ and $vPVA$ using vV_o (Panels A and C) and the $aE_{\max}$ and $aPVA$ using aV_o (Panels B and D) in one LV. The insets list their kurtosis, skewness, and χ^2 ($p > 0.685$ for $aE_{\max}$ and $vE_{\max}$, $p > 0.999$ for $aPVA$ and $vPVA$). These values support the normality or Gaussianity of these four distributions. There were little differences of these statistical results between the respective pairs of the virtual and actual distributions. Although not shown, neither vPE nor aPE were very different. The other 5 LVs had essentially similar results.

Table 2 summarizes the mean $\pm$ SD of kurtosis, skewness, and χ^2 values of $aE_{\max}$, aPE , and $aPVA$ with their p values in the six hearts. All the kurtosis, skewness, and χ^2 values supported the normality of the frequency distribution of $aPVA$.

DISCUSSION

Our present statistical study evidently shows that the normal or Gaussian frequency distribution of not only $E_{\max}$ but also PVA, remained unchanged even if we took the $E_{\max}$ -dependent V_o changes into consideration.

We previously reported the normality of the frequency distributions of both $vE_{\max}$ and $vPVA$ of the absolute arrhythmic beats under AF in the canine hearts despite the significantly non-normal or non-Gaussian frequency distributions of the cardiodynamic variables serving as the determinants of PVA [10]. Of these, the most non-normally distributing variable proved to be E_a and the next to be the RR beat interval [10]. However, both $E_{\max}$ and PVA could remain normal very likely because of the central limit theorem, as we speculated previously [10]. In the present study, E_a remained unchanged despite the $E_{\max}$ dependency of V_o (Fig. 1). Moreover, $E_{\max}$ was only slightly, though significantly, affected by the $E_{\max}$ -dependent V_o , much less than we considered (Table 1, Fig. 6). As the result,

Fig. 6. Comparison of the frequency distribution histograms of vPVA (virtual PVA), using a constant vV_o (virtual V_o) in Panel A, and aPVA (actual PVA), using an $E_{\max}$ -dependent aV_o (actual V_o) in Panel B in one heart as a representative case. A continuous set of 113 beats/30 s were analyzed. The curves are the best-fit normal or Gaussian distribution curves. Although all of

the mean, median, mode, SD, variance, range, maximum, and minimum values of either $aE_{\max}$ or aPVA slightly but significantly changed from those of vPVA as shown in Table 1, their kurtosis, skewness, and χ^2 values ($p > 0.05$) as inset here were significantly different neither between $vE_{\max}$ and $aE_{\max}$ nor between vPVA and aPVA.

the normality of the frequency distribution of $E_{\max}$ in terms of $aE_{\max}$ were little affected.

The slight dependency of $aE_{\max}$ on the $E_{\max}$ -dependent aV_o seems to have led to the slight dependency of aPVA on the aV_o . This is accountable by the little effect of the $E_{\max}$ -dependent aV_o on the aPE part of the aPVA and no effect on the EW part of the aPVA (Figs. 1 and 3).

One may wonder why the SD, variance, range, and maximum of the percent Δ PVA were significant, although those of percent Δ PE were insignificant (Table 1). This seems to have derived from the following two relations: $PVA = EW + PE$ and $\Delta PVA = \Delta PE$ where EW is always independent of V_o changes (Fig. 4). Since PE is always smaller than PVA by a constant EW, the percent Δ PVA ($\Delta PVA/PVA = \Delta PE/PVA$) is always smaller than the percent Δ PE ($\Delta PE/PE = \Delta PE/[(PVA - EW)]$) in each arrhythmic beat. This seems to have caused the present statistical differences of the SD, variance, range, and maximum between the percent Δ PVA and Δ PE.

Figure 7 shows that we could attribute the relatively small changes in not only $aE_{\max}$ but also aPVA, to the hyperbolic aV_o - $aE_{\max}$ relation. This hyperbolic relation shows the $E_{\max}$ dependency of V_o as represented by the inset formula: $aV_o = V_d - P_d/aE_{\max}$ (Eq. 4 in Fig. 3). aV_o increases slightly even with considerable decreases in $aE_{\max}$ from 30 to 10 mmHg/(ml/100 g LV). However, aV_o increases moderately with further decreases in $aE_{\max}$ to below 10 mmHg/(ml/100 g LV), and then markedly with further decreases in $aE_{\max}$ below 5 mmHg/(ml/100 g LV). As described in Results, the maximum/minimum ratio of $vE_{\max}$ was ~ 4 on average in the six hearts. The maximum/minimum ratio of $aE_{\max}$ was $\sim 13\%$ smaller on average than that of $vE_{\max}$, as shown in Table 1 and by the horizontal bi-head arrows and the thick solid line on the abscissa in Fig. 7.

Figure 7 also shows that the same tendency holds whether P_d was set 5 mmHg either higher or lower than -10 mmHg (dashed curves). As a result, the

Fig. 7. The theoretically inverse relations (inset equation) between $aE_{\max}$ (actual $E_{\max}$) and aV_o (actual V_o) that account for the relatively small changes in aV_o (thick part of the ordinate) despite the large changes in $aE_{\max}$ (thick part of the abscissa) among the arrhythmic contractions under atrial fibrillation. The solid curve is for $P_d = -10$ mmHg. The dashed curves on both sides of the solid curve are for $P_d = \pm 5$ mmHg around -10 mmHg. The horizontal bi-head arrows show the working ranges of $aE_{\max}$ in the six individual LVs, where the dashed vertical and horizontal lines show the maximum and minimum values of $aE_{\max}$ and aV_o .

arrhythmic changes in $aE_{\max}$ within the thick part of the abscissa change aV_o only by a few ml/100 g LV, but a further decrease in $aE_{\max}$ below the thick part of the abscissa can sensitively increase aV_o . However, the arrhythmic changes in $aE_{\max}$ during AF did not fall below ~ 5 mmHg/(ml/100 g LV), enough to increase aV_o and thus distort the normal distributions of $aE_{\max}$ and $aPVA$.

We therefore conclude that even the variable aV_o keeps the normal frequency distributions of both $aE_{\max}$ and $aPVA$ of arrhythmic LV contractions under AF because the $E_{\max}$ -dependent V_o changes are too small to affect their distributions. Therefore, we can practically ignore the changes, if any, in $E_{\max}$ and PVA with the $E_{\max}$ -dependent V_o changes under AF in the statistical characterizations of arrhythmic contractions. Moreover, this evidently corroborates our previous conclusion [10].

This work was partly supported by Scientific Research Grants (15650095, 15659186, 15700330, 16659057) from the Ministry of Education, Culture, Sports, Science and Technology, and Cardiovascular Diseases Research Grants (14A-1) from the Ministry of Health, Labour and Welfare, both of Japan.

REFERENCES

1. Atwood JE, Myers J, Sandhu S, Lachterman B, Friis R, Oshita A, Forbes S, Walsh D, and Froelicher V: Optimal sampling interval to estimate heart rate at rest and under exercise in atrial fibrillation. *Am J Cardiol* 63: 45–48, 1989
2. Bootsma BK, Hoelen AJ, Strackerr J, and Meijler FL: Analysis of RR intervals in patients with atrial fibrillation at rest and during exercise. *Circulation* 41: 783–793, 1970
3. Brookes CI, White PA, Staples M, Oldershaw PJ, Redington AN, Collins PD, and Noble MI: Myocardial contractility is not constant during spontaneous atrial fibrillation in patients. *Circulation* 98: 1762–1768, 1998
4. Daoud EG, Weiss R, Bahu M, Knight BP, Bogun F, Govvala R, Harvey M, Strickberger SA, Man KC, and Morady F: Effect of an irregular ventricular rhythm on cardiac output. *Am J Cardiol* 78: 1433–1436, 1996
5. Dubrey SW and Falk RH: Optimal number of beats for the Doppler measurement of cardiac output in atrial fibrillation. *J Am Soc Echocardiogr* 10: 67–71, 1997
6. Hardman SMC, Noble MIM, and Seed WA: Postextrasystolic potentiation and its contribution to the beat-to-beat variation of the pulse during atrial fibrillation. *Circulation* 86: 1223–1232, 1992
7. Hoopen MT and Bongaarts JPM: Probabilistic characterization of R-R intervals. *Cardiovasc Res* 3: 218–226, 1969
8. Horan LG and Kistler JC: Study of ventricular response in atrial fibrillation. *Circ Res* 9: 305–312, 1961
9. Mitani H, Yamaguchi H, Morita T, Oshima Y, Shimizu J, Ito H, Araki J, Takaki M, Sano S, and Suga H: Effective arterial elastance of irregular beats during atrial fibrillation in canine left ventricle. *Jpn J Physiol* 50: 77–89, 2000
10. Mohri S, Shimizu J, Iribe G, Ito H, Morita T, Yamaguchi H, Sano S, Kajiyama F, and Suga H: Normal distribution of ventricular pressure-volume area of arrhythmic beats during atrial fibrillation in canine heart. *Am J Physiol Heart Circ Physiol* 288: H1740–H1746, 2005
11. Morita T, Araki J, Oshima Y, Mitani H, Iribe G, Mohri S, Shimizu J, Sano S, Kajiyama F, and Suga H: Frequency distribution, variance, and moving average of left ventricular rhythm and contractility during atrial fibrillation in dog. *Jpn J Physiol* 52: 41–49, 2002
12. Muntinga HJ, Gosselink AT, Blanksma PK, De Kam PJ, Van Der Wall EE, and Crijns HJ: Left ventricular beat to beat performance in atrial fibrillation: dependence on contractility, preload, and afterload. *Heart* 82: 575–580, 1999
13. Rawles JM and Rowland E: Is the pulse in atrial fibrillation irregularly irregular? *Br Heart J* 56: 4–11, 1986
14. Shimizu J, Mohri S, Iribe G, Ito H, Morita T, Yamaguchi H, Sano S, and Suga H: Predictability of O_2 consumption from contractility and mechanical energy of absolute arrhythmic beats in canine heart. *Jpn J Physiol* 55: 135–142, 2005
15. Suzuki S, Araki J, Morita T, Mohri S, Mikane T, Yamaguchi H, Sano S, Ohe T, Hirakawa M, and Suga H: Ventricular contractility in atrial fibrillation is predictable by me-

- chanical restitution and potentiation. *Am. J. Physiol.* 275: H1513–H1519, 1998
16. Watt JH, Donner AP, McKinney CM, and Klein GJ: Atrial fibrillation: minimal sampling interval to estimate average rate. *J Electrocardiol* 17: 153–156, 1984
17. Yamaguchi H, Takaki M, Ito H, Tachibana H, Lee S, and Suga H: Pressure-interval relationship characterizes left ventricular irregular beat contractilities and their mean level during atrial fibrillation. *Jpn J Physiol* 47: 101–110, 1997
18. Sagawa K, Maughan L, Suga H, and Sunagawa K: *Cardiac Contraction and the Pressure-Volume Relationship*, Oxford Univ Press, New York, pp 232–298, 1988
19. Sunagawa K, Maughan WL, Burkhoff D, and Sagawa K: Left ventricular interaction with arterial load studied in isolated canine heart. *Am J Physiol* 245: H773–H780, 1983
20. Nozawa T, Yasumura Y, Futaki S, Tanaka N, Uenishi M, and Suga H: Efficiency of energy transfer from pressure-volume area to external mechanical work increases with contractile state and decreases with afterload in the left ventricle of the anesthetized closed-chest dog. *Circulation* 77: 1116–1124, 1988
21. Suga H: Ventricular energetics. *Physiol Rev* 70: 247–277, 1990
22. Suga H, Yasumura Y, Nozawa T, Futaki S, and Tanaka N: Pressure-volume relation around zero transmural pressure in excised cross-circulated dog left ventricle. *Circ Res* 63: 361–372, 1988
23. Suga H: Cardiac mechanics and energetics—from Emax to PVA. *Front Med Biol Eng* 2: 3–22, 1990
24. Glantz SA: *Primer of Biostatistics*, McGraw-Hill Book Co., New York, pp 217–221 & 284–292, 1981
25. Snedecor GW and Cochran WG: *Statistical Methods*, 6th ed, Iowa State Univ Press, Ames, Iowa, 1971