NON-LINEAR ISOCHRONES IN PRESSURE-VOLUME LOOPS OF MOUSE VENTRICLES

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Abstract: The concept of linear time-varying elastance E(t) is commonly used to describe the relation between pressure and volume in the left ventricle. Although a number of limitations of this concept have previously been described in literature, it is still frequently applied to determine its peak value (E_{max}), which is a measure of contractile function. The concept is intrinsically based on the linear shape of the isochrones (lines connecting isochronal pressure-volume data) and a common intercept of these isochrones. Because curvilinear end-systolic pressure-volume relationships (ESPVR) have been observed in mouse ventricles and since mice represent the most commonly used species in cardiovascular studies today, we have reevaluated the applicability of the elastance concept for the mouse. More specifically, we analyzed the shape of the isochrones using 6 regression algorithms (2 linear, 2 quadratic and 2 logarithmic, each with a fixed or time-varying intercept) and discussed the consequences for the elastance concept. Our main observations were: (i) the volume intercept varies considerably with time; (ii) isochrones are equally well described using quadratic or logarithmic regression; (iii) linear regression with a fixed intercept poorly fits the pressure-volume data during isovolumic relaxation, and (iv) logarithmic regression is superior in estimating the fixed volume intercept of the ESPVR. In conclusion, the linear time-varying elastance does not provide a sufficiently robust model to account for pressure and volume changes during the cardiac cycle in the mouse ventricle.

Introduction

The concept of linear time-varying elastance E(t) is a relatively simple and hence frequently used framework to describe the mechanics of the left ventricle. It is usually applied to determine the maximum end-systolic elastance, E_{max}. This value corresponds to the slope of the end-systolic pressure-volume relation (ESPVR) and is used as an index for global left ventricular contractility [1].

When calculating E(t), it is assumed that isochrones (lines that connect pressure-volume data acquired at the same instant after the onset of systole) and the ESPVR are linear and intersect the volume axis at a common volume V_0.

The ESPVR is known to be quasi-linear in humans and dogs in normal loading conditions. In small rodents such as mice and rats, however, the ESPVR is virtually always non-linear, even in normal loading conditions [2]. Because of this aspect, problems with defining and interpreting the elastance concept and its derived values, such as E_{max} and V_0, may arise.

Since murine pressure and volume measurements are nowadays extensively used to explore aspects of cardiovascular function, we felt the need to review and discuss the applicability of the conventional linear time-varying elastance in mouse ventricles.

Materials and Methods

Theory: linear time-varying elastance

A pressure-volume loop visualizes the time-course of the relation between left ventricular pressure and volume. It includes isovolumic contraction, ejection, isovolumic relaxation and filling. When decreasing preload (i.e. left atrial pressure), the loops shift to the left, but still fit between the 2 boundaries that describe the intrinsic systolic and diastolic properties of the ventricle, the ESPVR and the end-diastolic pressure-volume relation (EDPVR).

In the early 70ies, Suga and Sugawa discovered that when all isochronal pressure-volume data are linearly fitted, they all converge quite closely to a constant volume intercept V_0 [3]. The time varying slope E(t) of these curves, called the linear time-varying elastance, is mathematically represented as

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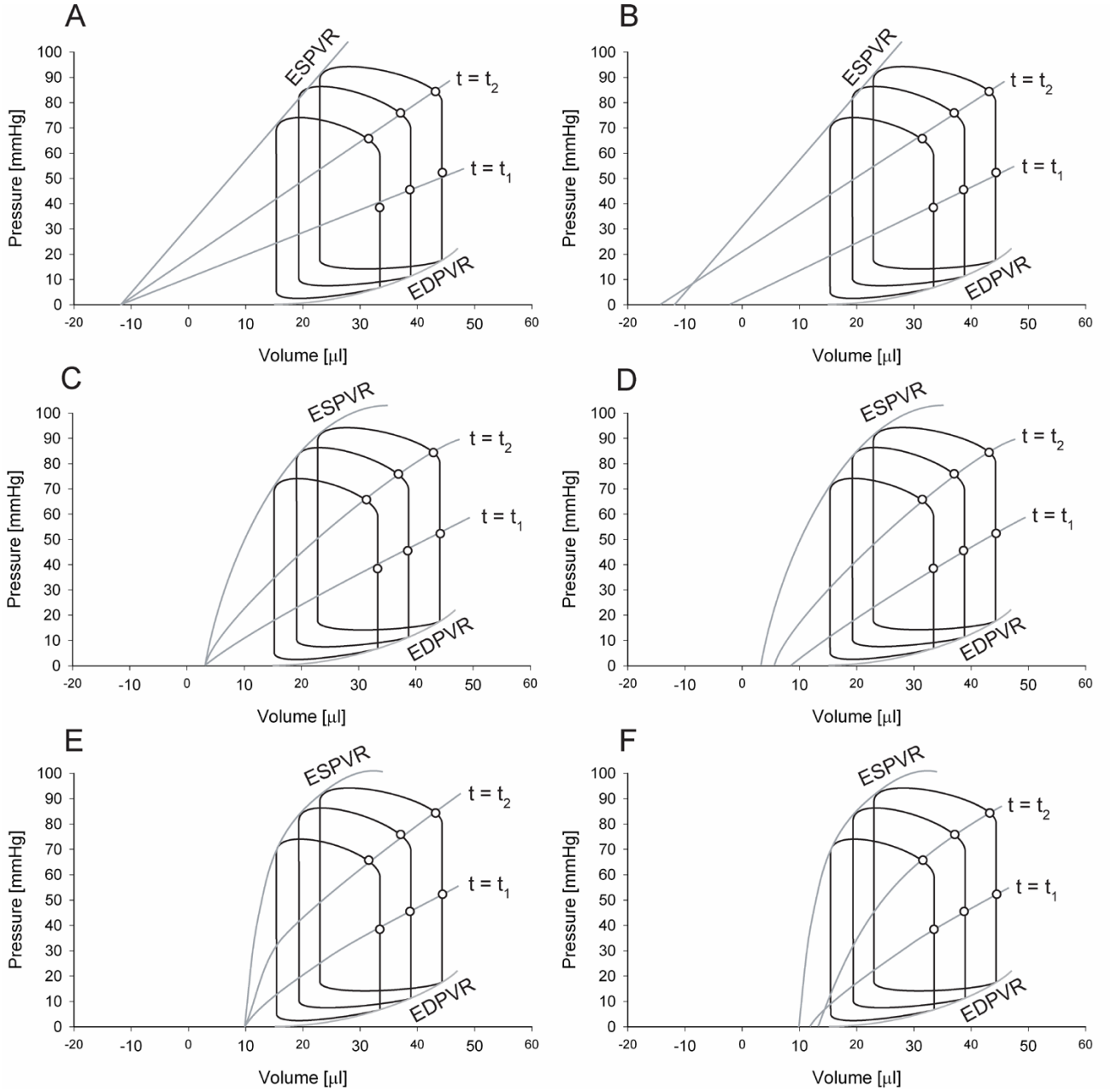

Figure 1: Overview of 6 regressions algorithms (RA): Linear (A-B), Quadratic (C-D) and Logarithmic (E-F), with either a fixed (left column) or a time-varying (right column) intercept with the volume axis. t_1 and t_2 are 2 arbitrarily chosen time points during systole.

$$E(t) = P(t)/(V(t) - V_0) \quad (1)$$

Experimental protocol

For this study, we used 13 anesthetized, open-chest mice (weight 140±18 grams, strains C57BL6 and C57B16/129). The study protocol was approved by the Animal Care and Use committee of the John Hopkins University and conformed with the institutional guidelines.

The animals were ventilated with a constant pressure ventilator with 100% oxygen at 120 breaths/min (tidal volume 200 µl). An anterior thoracotomy was performed to enter the chest. An apical stab using a 26G needle allowed for the placement of a custom-made 4-electrode conductance catheter with a dual pressure sensor (Millar Instruments, Houston, TX). The catheter was advanced retrogradely along the LV long axis with the distal tip (pressure sensor) in the aortic root and such that the proximal electrode was just inside the endocardium. The time-varying intraventricular volume was determined using the formula of Baan et al. [4]. The volume V(t) is calculated as:

$$V(t) = (1/\alpha) \cdot (\rho L^2) \cdot [G(t) - G_p] \quad (2)$$

where α is the gain factor, ρ is the blood resistivity, L the distance between the sensing electrodes, G(t) is the

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instantaneous conductance, and G_p is the instantaneous conductance of the surrounding structures (parallel conductance). The gain factor α used for calibration of the conductance catheter was obtained by matching the conductance derived stroke volume to that measured by a flow probe (1 RB, Transonic, Ithaca, NY) positioned around the thoracic aorta. The offset of the volume signal (parallel conductance G_p) was assessed by an infusion of hypertonic saline (bolus injection 5-10 ml 35% saline). Pressure and volume signals were sampled at 2 kHz.

Data acquisition and treatment

Data were obtained in baseline conditions (BL) and during vena cava occlusion (VCO), which typically lasted for 15-25 cycles of 180 samples each. End-systolic data points were found using an iterative method as previously described [5]. Pressure and volume data were consequently filtered using a 3rd order Savitsky-Golay smoothing filter. Post-processing is performed using a custom-made application in Matlab R14 (The Mathworks, Natick, USA).

Regression of the ESPVR and the isochrones

First, the end-systolic pressure-volume data points were regressed using a linear (2a), quadratic (2b) and a logarithmic (2c) function:

$$P_{es} = \alpha_1 \cdot V_{es} + \alpha_0 \quad (3a)$$

$$P_{es} = \alpha_2 \cdot V_{es}^2 + \alpha_1 \cdot V_{es} + \alpha_0 \quad (3b)$$

$$P_{es} = (\alpha + \beta \cdot V_{es})^{-1} \cdot \ln(V_{es}/V_0) \quad (3c)$$

Secondly, we fitted the isochronal data using 6 regression algorithms (RA): 2 linear (Lin), 2 quadratic (Quad) and 2 logarithmic (Log) algorithms, each with a fixed (Fix) and a variable (Var) time-varying volume intercept (all illustrated in figure 1):

$$RA_{Lin\text{-}Fix}: P = \alpha_1 \cdot V + \alpha_0 \; ; \; P(V_{0,lin}) = 0 \quad (4a)$$

$$RA_{Lin\text{-}Var}: P = \alpha_1 \cdot V + \alpha_0 \quad (4b)$$

$$RA_{Quad\text{-}Fix}: P = \alpha_2 \cdot V^2 + \alpha_1 \cdot V + \alpha_0 \; ; \; P(V_{0,quad}) = 0 \quad (4c)$$

$$RA_{Quad\text{-}Var}: P = \alpha_2 \cdot V^2 + \alpha_1 \cdot V + \alpha_0 \quad (4d)$$

$$RA_{Log\text{-}Fix}: P = (\alpha + \beta \cdot V)^{-1} \cdot \ln(V/V_0) \; ; \; P(V_{0,log}) = 0 \quad (4e)$$

$$RA_{Log\text{-}Var}: P = (\alpha + \beta \cdot V)^{-1} \cdot \ln(V/V_0) \quad (4f)$$

The fixed volume intercepts $V_{0,lin}$, $V_{0,quad}$ and $V_{0,log}$, used as boundary conditions in 4a, 4c and 4e, were obtained from extrapolation of the respective ESPVR.

For the remaining $RA_{Lin\text{-}Var}$, $RA_{Quad\text{-}Var}$ and $RA_{Log\text{-}Var}$, all isochronal pressure-volume data were fitted using linear, quadratic and logarithmic functions, respectively. Every single isochrone was then extrapolated to the volume axis to obtain the time-varying volumes $V_{0,lin}(t)$, $V_{0,quad}(t)$ and $V_{0,log}(t)$, respectively.

In $RA_{Lin\text{-}Fix}$ and $RA_{Lin\text{-}Var}$, coefficient α_1 represents the slope of the linear isochrones. Coefficient α_2 is a measure of curvilinearity. The coefficients α and β used in the logarithmic functions integrate myocardial stiffness, chamber geometry and empiric constants [6].

Statistics

We applied Akaike's Information criterion (AIC), based on the principle of parsimony, to check whether the quadratic and logarithmic regressions offer a better fitting than the linear approach. AIC values are calculated as:

$$AIC = n \cdot \ln\left(\sum_{i=1}^{n}(p_{meas,i} - p_{est,i})^2\right) - n \cdot \ln(n) + 2 \cdot k \quad (5)$$

with n the number of data couples (= number of loops), and $p_{meas,i}$ and $p_{est,i}$ the measured and estimated pressures, respectively. The number of model parameters is represented by k (linear: k = 2; quadratic and logarithmic: k = 3). The k-value that minimizes AIC corresponds to the best model.

For each isochronal regression algorithm, the difference between the estimated (fitted) and the measured pressures was assessed by RMSe (Root Mean Square error) values, defined as:

$$RMSe = \sqrt{\frac{\sum_{i=1}^{n}(p_{meas,i} - p_{est,i})^2}{n}} \quad (6)$$

All time-dependent data were normalized for heart rate and subsequently averaged for all 13 animals. The results are expressed as mean ± SD. Differences between groups were analyzed using paired t-tests. Statistical significance was assumed when $p < 0.05$. Statistics were performed using SPSS 12 (SPSS, Chicago, IL).

Results

Figure 2 shows a representative example of 24 pressure-volume loops obtained under gradual preload decline, showing curvilinear isochrones, and a linear, quadratic and logarithmic extrapolation of the ESPVR, yielding $V_{0,lin}$, $V_{0,quad}$ and $V_{0,log}$, respectively. The onset and the iteratively determined end of systole are also shown.

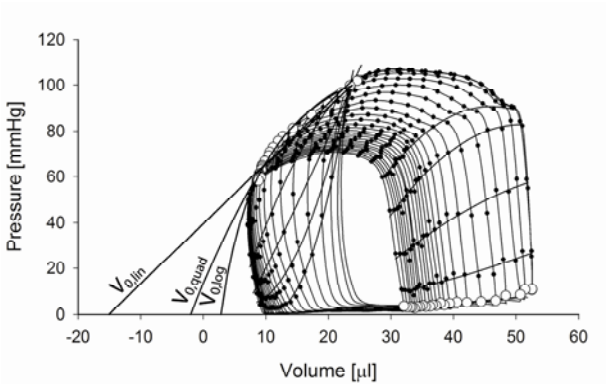

Figure 2: Representative example of a series of 24 pressure-volume loops with non-linear isochrones.

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Table 2: Parameters derived from linear, quadratic and logarithmic fitting of the end-systolic pressure-volume data

	Linear regression				Quadratic regression					Logarithmic regression			
Mouse	**AIC**	α_1	α_0	$V_{0,lin}$	**AIC**	α_2	α_1	α_0	$V_{0,quad}$	**AIC**	α	β	$V_{0,log}$
1	53.5	2.6	39.2	-15.1	31.7	-0.1	6.4	13.2	-2.0	20.73	0.017	1.659	2.8
2	54.9	3.8	65.1	-17.1	37.5	-0.4	11.4	38.0	-3.0	30.45	0.016	3.754	1.1
3	53.9	2.3	40.9	-17.8	34.7	-0.1	5.8	22.8	-4.0	27.82	0.025	2.928	1.5
4	39.7	2.6	61.1	-23.5	27.6	-0.1	5.9	42.3	-6.4	19.59	0.023	1.054	1.1
5	69.5	4.6	69.2	-15.0	53.7	-0.5	11.9	51.6	-3.7	42.46	0.021	5.235	0.5
6	9.8	2.2	59.1	-26.9	-24.9	0.0	2.8	58.0	-17.8	23.97	0.229	-0.003	0
7	55.6	3.8	74.8	-19.7	38.2	-0.1	6.8	64.5	-8.2	18.02	0.037	-2.176	0.2
8	32.8	2.1	69.2	-32.9	7.6	-0.1	5.4	48.8	-7.8	-9.49	0.024	1.800	0.9
9	47.7	2.7	31.8	-11.8	30.3	-0.3	18.5	-143	9.4	25.13	-0.003	3.496	13.9
10	38.9	2.8	64.4	-23.0	20.3	-0.3	11.5	16.4	-1.4	18.19	0.008	4.202	3
11	51.7	5.4	16.7	-3.1	31.3	-1.0	41.5	-297	9.2	22.71	-0.003	3.548	11.8
12	32.8	3.7	66.9	-18.1	-22.1	-0.3	8.6	50.3	-5.0	-8.14	0.030	1.351	0.4
13	17.2	2.9	64.1	-22.1	6.7	-0.1	5.2	54.5	-8.9	6.85	0.041	-1.760	0.2
Mean	**-**	**3.2**	**55.6**	**-18.9**	**-**	**-0.28**	**10.9**	**1.6**	**-3.8**	**-**	**0.036**	**1.930**	**2.9**
SD	**-**	**1.0**	**17.6**	**7.3**	**-**	**0.26**	**10.1**	**104.4**	**7.2**	**-**	**0.060**	**2.252**	**4.6**

Table 2 displays the estimated parameters derived from the ESPVRs and the extrapolated volume intercepts. The lower AIC values in case of the quadratic and logarithmic fitting indicate that the ESPVR is indeed better modeled with a non-linear function.

The quality of fit of the isochrones is quantified by RMSe-values as a function of time (figure 3, panels A and B).

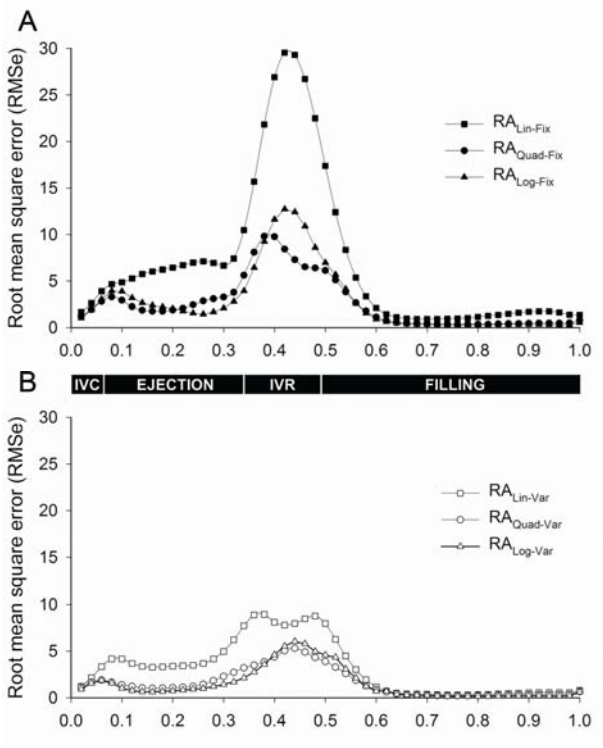

Figure 3: Fitting quality of the regression algorithms quantified by RMSe data (panel A: fixed volume intercept; panel B: variable volume intercept).

As anticipated, non-linear regression yields better results (lower RMSe values) than the more frequently used linear regression. Moreover, when comparing the upper (fixed V_0) with the lower (variable V_0) panels, regression with a fixed V_0 increases the RMSe.
Poor fitting obtained with $RA_{Lin-Fix}$ can be observed particularly during IVR. Quadratic and logarithmic regression algorithms perform similarly during the whole cycle. During filling, when the EDPVR is virtually linear, all algorithms perform comparably.

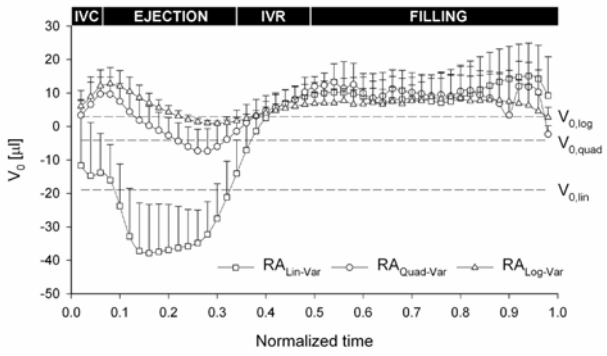

Figure 4: Normalized time-course of the volume intercepts.

The time-varying and the constant volume intercepts are presented in figure 4. From the onset of systole until mid IVR, $V_{0,log(t)} > V_{0,quad(t)} > V_{0,lin(t)}$. $V_{0,lin(t)}$ ranges between -39.1 and 15.5 µl, while $V_{0,quad(t)}$ acts in a smaller interval between -8.3 and 14.2 µl. $V_{0,log(t)}$ changes considerably less during the cardiac cycle, and ranges between 0.7 and 12.8 µl. The SD and the slope of $V_{0,log(t)}$ at end-ejection is considerably smaller than for $V_{0,quad(t)}$ and $V_{0,lin(t)}$, suggesting that logarithmic regression is the most reliable fitting technique to estimate V_0. During the second half of IVR and the

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filling phase, all algorithms result in comparable volume intercepts.

Discussion

The results of the present study showed that the linear time-varying elastance concept is insufficient to describe the relation between pressure and volume in mouse ventricles. More precisely, we found that (i) the shape of the isochrones is best described using a non-linear function (quadratic or logarithmic) with a time-varying volume intercept, while a linear approximation with fixed volume intercept offers the poorest results, particularly during isovolumic relaxation and early filling, (ii) the logarithmic fitting appears superior in estimating the fixed volume intercept of the ESPVR and moreover offers a physiological (i.e. positive) result, and (iii) the intercepts $V_{0,lin}(t)$, $V_{0,quad}(t)$ and $V_{0,log}(t)$ vary considerably with time and differ from each other during IVC and ejection.

Mice represent the most commonly used species in cardiovascular studies today. When curvilinearity is ignored, there is a high risk of making errors when comparing shifts in E_{max} and/or V_0 within individual cases or between different study groups, thus leading to false results and jeopardizing physiologic interpretations of studies at any level of the heart. To avoid these problems, standardized definitions of E_{max} and V_0 need to be developed that are based on a model that best fits the real pressure-volume data.

The intraventricular pressure and volume data were obtained using a single-frequency miniaturized combined pressure conductance system assuming a constant volume signal offset (V_p). The validity of this method has been questioned before [7]. However, it has been shown that this method can certainly be used in mice as nearly all of V_p is due to the ventricle wall [8]. The variation of V_p during the cardiac cycle is therefore very limited. In addition, if V_p was changing during the IVC occlusion, it would cause the pressure-volume loops and the end-systolic points to shift leftwards, and the ESPVR would appear "more linear", which was clearly not observed in our experiments.

In our results, the time varying $V_{0,lin}(t)$, $V_{0,quad}(t)$ and $V_{0,log}(t)$ were markedly different from the constant intercepts, which proves that the assumption of a constant volume intercept is violated, no matter which regression algorithm is used (figure 4). The most accurate and only physiological volume intercept was obtained using the logarithmic regression function, established by Mirsky et al. [6] The relatively large SD observed for all regression algorithms during filling was due to the shallow slope of the EDPVR.

The curve fitting that was subsequently applied using fixed intercepts as boundary conditions, showed to what extent such a mathematical restriction reduces the quality of the fit (figure 3, compare panel A and B). The RMSe values showing the agreement of the measured and fitted data, pointed out that the conventional E(t) concept with fixed $V_{0,lin}$ shows a poor agreement with the data during IVR and early filling.

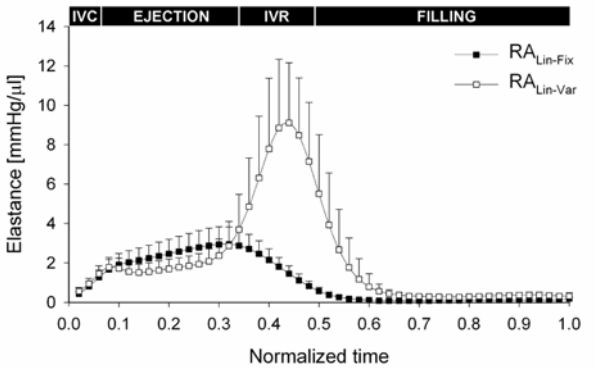

Figure 5: Normalized time-course of the slope of the isochrones fitted with $RA_{Lin-Fix}$ and $RA_{Lin-Var}$.

Figure 5 illustrates the deleterious effects of using the boundary condition $P(V_{0,lin}) = 0$ on the definition of elastance (instantaneous slope of the isochrones). A significant difference between both time-courses is observed. The peak elastance of $RA_{Lin-Var}$ equals E_{max} = 8.41 ± 2.77, while the peak of $RA_{Lin-Fix}$ is E_{max} = 2.8 ± 0.7 mmHg/µl, which is in agreement with data from Reyes et al. (3.3 ± 1.9 mmHg/µl) [9]. The unexpected difference between $RA_{Lin-Fix}$ and $RA_{Lin-Var}$ proves that the linear elastance concept is meaningless during IVR and early filling, although in literature the elastance curve is frequently shown for the whole cardiac cycle.

Finally, it is to be acknowledged that our study also had a few shortcomings: (i) We assumed that the baroreflex activation that may have occurred during the preload reduction did not affect our measurements, because the absolute difference in HR between baseline and vena cava occlusion was very small (620 vs. 624 bpm). (ii) The data shown in figures 3, 4, 5 and 6 were averaged for all animals included in the study. Even though all data has been represented on a normalized time scale, the peaks could be slightly blunted if the peaks of the curves do not occur at the same normalized time for each subject.

Conclusions

We have demonstrated that, in small mammals like the mouse, the conventional linear time-varying elastance concept does not fully describe ventricular performance during the whole cardiac cycle. Even if it always feasible to calculate the time-course of the 'elastance' for any series of pressure-volume loops, once V_0 is determined, the link with the instantaneous stiffness is still unclear.

Therefore, a coherent framework, which accounts for murine cardiac physiology and the variation in time of non-linear isochrones throughout the complete cardiac cycle, still remains to be developed.

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