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## Scaling of diastolic intraventricular pressure gradients is related to filling time duration

Zoran B. Popović,<sup>1</sup> Kathryn E. Richards,<sup>1</sup> Neil L. Greenberg,<sup>1</sup> Aleksandr Rovner,<sup>1</sup> Jeannie Drinko,<sup>1</sup> Yuanna Cheng,<sup>1</sup> Marc S. Penn,<sup>1</sup> Kiyotaka Fukamachi,<sup>1</sup> Niladri Mal,<sup>1</sup> Benjamin D. Levine,<sup>2</sup> Mario J. Garcia,<sup>1</sup> and James D. Thomas<sup>1</sup>

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**Popović, Zoran B., Kathryn E. Richards, Neil L. Greenberg, Aleksandr Rovner, Jeannie Drinko, Yuanna Cheng, Marc S. Penn, Kiyotaka Fukamachi, Niladri Mal, Benjamin D. Levine, Mario J. Garcia, and James D. Thomas.** Scaling of diastolic intraventricular pressure gradients is related to filling time duration. *Am J Physiol Heart Circ Physiol* 291: H762–H769, 2006; doi:10.1152/ajpheart.00081.2006.—In early diastole, pressure is lower in the apex than in the base of the left ventricle (LV). This early intraventricular pressure difference (IVPD) facilitates LV filling. We assessed how LV diastolic IVPD and intraventricular pressure gradient (IVPG), defined as IVPD divided by length, scale to the heart size and other physiological variables. We studied 10 mice, 10 rats, 5 rabbits, 12 dogs, and 21 humans by echocardiography. Color Doppler M-mode data were postprocessed to reconstruct IVPD and IVPG. Normalized LV filling time was calculated by dividing filling time by RR interval. The relationship between IVPD, IVPG, normalized LV filling time, and LV end-diastolic volume (or mass) as fit to the general scaling equation  $Y = kM^\beta$ , where  $M$  is LV heart size parameter,  $Y$  is a dependent variable,  $k$  is a constant, and  $\beta$  is the power of the scaling exponent. LV mass varied from 0.049 to 194 g, whereas end-diastolic volume varied from 0.011 to 149 ml. The  $\beta$  values relating normalized LV filling time with LV mass and end-diastolic volume were 0.091 (SD 0.011) and 0.083 (SD 0.009), respectively ( $P < 0.0001$  vs. 0 for both). The  $\beta$  values relating IVPD with LV mass and end-diastolic volume were similarly significant at 0.271 (SD 0.039) and 0.243 (SD 0.0361), respectively ( $P < 0.0001$  vs. 0 for both). Finally,  $\beta$  values relating IVPG with LV mass and end-diastolic volume were  $-0.118$  (SD 0.013) and  $-0.104$  (SD 0.011), respectively ( $P < 0.0001$  vs. 0 for both). As a result, there was an inverse relationship between IVPG and normalized LV filling time ( $r = -0.65$ ,  $P < 0.001$ ). We conclude that IVPD decrease, while IVPG increase with decreasing animal size. High IVPG in small mammals may be an adaptive mechanism to short filling times.

hemodynamics; diastolic function; comparative physiology

THE CONCEPT OF SCALING IS an important idea in both comparative and applied physiology. Analysis of scaling patterns may uncover the facts on the way the organ structure and function interact. When crossing species, scaling is usually analyzed through an allometric relationship. According to this concept, throughout various species, the size and function of the organ can be linked by a power function to the size of the mammal. Furthermore, it is frequently hypothesized that a size of the

scaling exponent close to 0.25 indicates fractallike branching structure of underlying entities, such as the arterial tree (20) or a Purkinje system (13).

As the heart weight keeps a constant proportion with mammal weight, it can be assumed that the functional properties of the heart could be scaled allometrically to its size (15, 21). It has indeed been proven that cardiac output (21), heart rate (21), PR interval (13), and myocardial tissue velocities (15) follow allometric scaling. However, no previous study addressed the way diastolic phenomena scale to the heart size.

During early diastole, left ventricular (LV) filling is facilitated by the development of low pressure within the apical part of the LV that results in intraventricular (base-to-apical) pressure difference (IVPD) (6). This negative pressure gradient results from restoring forces during ventricular relaxation, which restore the shape and increase the volume of the ventricle that was squeezed out during the preceding contraction (3, 12). The presence of this early diastolic IVPD is well documented in large mammals, such as dogs (5) and humans (7). However, it is unknown if it is present in smaller mammals, and, if present, whether it is of the same magnitude. This is important as one may argue that IVPD should be similar between the species, since it is well known that, despite huge differences in cardiac cycle, pressures within the cardiovascular system are size independent. On the other hand, maintaining similar pressure differences would mean that the changes of LV pressure over a unit base-apical distance would be much larger in a mouse than in a human. Identifying the parameters that are constant, in contrast to the parameters that scale with the heart size, could point to the fundamental physiological properties that have to be conserved, independent of heart size.

In this study, our primary aim is to determine whether IVPD and gradient scale allometrically to the heart size. We examined two mutually exclusive conjectures. The first was that the IVPD remains constant between the species, whereas the second was that the intraventricular pressure gradient (IVPG) remain constant, leading to scaling of pressure differences to ventricular size. Our secondary aim was to determine the relationship between IVPD and gradient and LV filling time (LVFT), a major determinant of total LV filling.

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## METHODS

### Background

We first propose working definitions of IVPD and IVPG (19). IVPD is defined as the pressure difference between two points; for the present paper, the two points will be the mitral annulus and the LV apex. An IVPG is defined as the change in pressure over a certain distance  $L$ , in this case the base-to-apex distance. The relationship between IVPD and IVPG is described by Eq. 1.

$$\text{IVPG} = \frac{\text{IVPD}}{L} \quad (1)$$

In other words, IVPG is the derivative of IVPD with respect to location. As previously noted, pressures within the cardiovascular system remain relatively constant throughout the mammalian class. However, it is unknown if this proposition pertains to the distribution of pressures within the LV cavity during early diastole. Here we will examine two mutually exclusive conjectures. The first is that the IVPD remains constant between the species, thus leading to early diastolic LV pressures lower in the apex than in the base of the ventricle by  $\sim 2$ – $4$  mmHg throughout the mammalian class. The second hypothesis is that the IVPG remains constant, leading to scaling of IVPD to ventricular size.

### Study Samples and Procedures

For the human subjects sample, we used data collected in normal volunteers that participated in two previously published studies (1, 16). The written, informed consent has been obtained from all subjects, while the studies conformed to the standards set by the Declaration of Helsinki. The Institutional Review Board approved those studies, as well as the data use for this study, and all participants gave written, informed consent. The volunteers were selected if they satisfied the criteria that included normal physical examination, no history of cardioactive drug use except for aspirin, normal 12-lead ECG, normal echocardiography, and no preexisting cardiac illness (14, 16). Out of these subjects, we selected the ones whose age at the time of the study was between 21 and 45 yr. A total of 21 subjects [average age 34 yr (SD 9), 6 women] satisfied this criterion.

Animal data used in this study were collected during control (normal) echocardiographic evaluations used in several different studies in the period of 2001 to the present. Although these studies may be viewed technically as retrospective to our current report, the data collection in each of them was prospective and specified by the respective study protocols.

The animal studies were approved by the Institutional Animal Care and Use Committee and were in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

Human and animal subjects were studied in left lateral decubitus position. Procedures slightly differed among the species due to the mammal's specific behaviors. Twenty-one human subjects were assessed in a standard manner. Twelve mongrel dogs were trained to lie calmly for echocardiography. Five New Zealand White rabbits were sedated with 85 mg/kg ketamine intramuscularly, after which a soft cloth cover was placed over their heads, and examination was performed in a dark room. Ketamine (85 mg/kg) was given intraperitoneally to sedate 10 Lewis rats. Lastly, 11 C57/BL6 mice were assessed in a conscious state (15).

To assess the impact of the fusion of early and late wave of mitral inflow on the IVPD, we anesthetized an additional group of eight Lewis rats with the combination of 85 mg/kg of ketamine and 5 mg/kg of xylazine intramuscularly. Anesthesia slowed down the heart rate to  $< 300$  beats/min in all rats.

### Data Collection

Echocardiography was performed by either Vivid 7 (GE Medical, Milwaukee, WI) (in mice, rats, and rabbits), Acuson (Siemens Medical Solutions USA, Malvern, PA) (in dogs), or ATL HDI 5500 (Philips, Eindhoven, The Netherlands) (in humans) echocardiography machines. Gray-scale two-dimensional (2D) and color M-mode echocardiography data were collected using a dual-harmonic 1.7/3.4-MHz or 3.5/5.0-MHz sector transducer (humans, dogs), 6-MHz or 11.5-MHz pediatric sector transducer (rabbits, rats), or a 14-MHz epicardial linear transducer (mice). Data were digitized in both proprietary and DICOM-compatible format and stored for further analysis.

### Data Analysis

Pro-Solv Cardiovascular (Problem Solving Concepts, Indianapolis, IN) and Echopac PC (GE Medical, Milwaukee, WI) analysis software were used to derive all of the measurements, except for IVPD. LVFT, LV diastasis time, and RR values were measured using pulse-wave Doppler recordings obtained at the mitral leaflets from the four-chamber apical view. LVFT was measured as the time between mitral valve opening and closing clicks. Since LVFT measurements in larger animals are influenced by diastasis, a nonfunctional part of diastole during which no flow is detected by pulse Doppler across the mitral valve during the middle period of diastole, we also calculated LVFT corrected for diastasis duration by subtracting diastasis time from the total LVFT. For that purpose, LV diastasis time was measured as an interval between the end of the E wave and the beginning of the A wave.

LV end-diastolic volume (EDV) and end-systolic volume were measured from the long-axis view by the single-plane Simpson equation. LV mass was calculated from 2D echocardiography data by the bullet equation (15), except in 14 humans in whom magnetic resonance imaging was used. Measuring LV volume and mass from 2D echocardiography and magnetic resonance echocardiography data was previously validated (10, 15).

*IVPD measurements.* The data were analyzed on a per-species basis using custom software previously described in detail (8). In brief, if we simplify 3D Navier-Stokes equations for incompressible fluid flow by assuming that the viscous term can be neglected and that gravitational force is balanced by hydrostatic buoyancy, then the flow along a streamline conforms to the Euler equation, which has the form:

$$\frac{\partial p}{\partial s} = -\rho \left( \frac{\partial v}{\partial t} + v \frac{\partial v}{\partial s} \right) \quad (2)$$

where  $p$  is pressure,  $\rho$  is a constant,  $v$  is velocity, and  $t$  is time. We applied this equation to color M-mode data, since they closely approximate the spatiotemporal velocity distribution,  $v(s,t)$ , along an inflow streamline from the LV base toward the apex. To obtain spatiotemporal velocity distribution, digital images of the color M-mode data were calibrated by custom software developed in the LabView environment (National Instruments, Houston, TX), and the resulting 2D matrix was used to calculate time-varying intraventricular pressure drop over a fixed length. The peak pressure drop during early diastole was defined as IVPD. The sensitivity of this method to angle misalignment of the color M mode has been previously tested (8, 23).

For the humans, as previously described, the intraventricular length over which the IVPD value was measured was 4 cm (14), with the signal filtered with the low-pass cutoff frequency of 30 Hz. For the dogs, the length over which the IVPD value was measured was 3.8 cm, with a cutoff frequency of 30 Hz, and at least two beats were measured in dogs. In the rabbits, rats, and mice, we used the low-pass cutoff frequency of 90 Hz, whereas the lengths over which IVPD was measured in rabbits, rats, and mice were 1.2, 0.5, and 0.25 cm, respectively. We chose the different cutoff frequencies since smaller animals have higher frequency content of their pressure signal. It

should be emphasized that use of higher frequencies by default increases IVPD measurements; however, in our samples, these changes were small (in the range of <0.1 mmHg). At least three beats were measured and averaged in all of the species, except in mice, in which at least four beats were used. Figure 1 displays an example of color M modes and derived IVPD measurements in the five species studied.

**Intra- and interobserver variability.** Intra- and interobserver variability of color M-mode data has been studied in humans (17). Here we present intra- and interobserver variability of color M-mode measurements in small rodents. Four data sets measured in rats and four in mice that were not included in this report were measured twice after a time interval of >1 mo by the same observer, and at that time also by a second observer blinded to the measurements of the first. Inter- and intraobserver variability was then quantitated as absolute as well as relative difference between the two measurements, and with correlation (quantified by correlation coefficient  $r$  of the simple linear regression) between corresponding sets of measurements.

#### Statistical Methods

Allometric scaling is assumed to follow the power function (24):

$$Y = kM^\beta \quad (3)$$

where  $M$  is LV heart size parameter (i.e., LV EDV or LV mass),  $Y$  is a dependent variable (i.e., RR interval, LVFT, IVPG, or IVPD),  $k$  is a constant, and  $\beta$  is the power of the scaling exponent.

To fit the data, we used nonlinear regression method with the Levenberg-Marquardt algorithm (SPSS 10.0, SPSS, Chicago, IL). Ninety-five percent confidence intervals for exponent  $\beta$  were calculated using asymptotic error of estimate. A single-sample  $t$ -statistic was used to compare the exponent  $\beta$  with a priori values of 0 and 0.25.  $t$ -Test statistics were also used to compare the values of exponents  $\beta$ . Finally, Mann-Whitney  $U$ -test was performed to compare the echocardiographic values obtained in anesthetized and sedated rats. Data are presented as average (SD), or as exponent  $\beta \pm 95\%$  confidence interval. A  $P$  value <0.05 was considered significant.

## RESULTS

Basic morphometric and echocardiography parameters of different species are presented in Table 1. In our subjects, LV mass varied from 0.049 to 194 g, whereas EDV varied from 0.011 to 149 ml. Thus body weight and heart size varied by up to four orders of magnitude among our subjects. EDV and LV filling volume scaled to LV mass with the scaling exponents ( $\beta_{LV\ mass}$ ) of  $1.093 \pm 0.14$  and  $1.11 \pm 0.19$ , meaning that they both kept a constant proportion to LV mass throughout all the species assessed (Fig. 2).

#### Scaling of Heart Cycle Intervals

The scaling of the LV ejection time interval to LV mass and LV EDV showed a power exponent of  $\beta_{LV\ mass} = 0.249 \pm 0.044$  and EDV scaling exponent ( $\beta_{EDV}$ ) =  $0.234 \pm 0.042$ , respectively ( $P > 0.05$  compared with the value of 0.25 for both). Similarly, scaling of the LV isovolumic relaxation time to LV mass and LV EDV showed a power exponent of  $\beta_{LV\ mass} = 0.299 \pm 0.070$  and  $\beta_{EDV} = 0.271 \pm 0.068$ , respectively ( $P > 0.05$  compared with the value of 0.25). However, LVFT showed a very high exponent for both LV mass and EDV ( $\beta_{LV\ mass} = 0.586 \pm 0.180$ ,  $\beta_{EDV} = 0.606 \pm 0.180$ ,  $P < 0.001$  for both compared with 0.25). Interestingly, despite high  $r$  values ( $r = 0.93$  for both regressions), the fitted line showed a large deviation from mouse and rat data. In contrast, LVFT corrected for diastasis showed a good fit

throughout its values. However, even after correcting for the diastasis duration, LVFT scaled to both LV mass and LV EDV with unexpectedly large exponents ( $\beta_{LV\ mass} = 0.393 \pm 0.092$  and  $\beta_{EDV} = 0.389 \pm 0.094$ ,  $P < 0.01$  compared with 0.25 for both). This clearly shows that, in contrast to LV ejection time (15), filling time even after correcting for diastasis duration deviated from the value of 0.25 that was previously assumed to be universal for the scaling of all heart cycle intervals and subintervals (Fig. 3A). Finally, LVFT normalized by dividing filling time by the RR interval duration showed a  $\beta_{LV\ mass} = 0.091 \pm 0.022$  and  $\beta_{EDV} = 0.083 \pm 0.019$  ( $P < 0.0001$  compared with the value of 0 for both) (Fig. 3B).

#### Scaling of IVPD and Gradients

Figure 3, C and D, relate IVPD and IVPG with LV size. IVPD scaled to LV mass and LV EDV with exponents  $\beta_{LV\ mass} = 0.271 \pm 0.078$  and  $\beta_{EDV} = 0.243 \pm 0.072$ , respectively ( $P > 0.5$  compared with value of 0.25 for both) (Fig. 3C). Interestingly, IVPG showed negative scaling exponents for LV size parameters with  $\beta_{LV\ mass} = -0.118 \pm 0.026$  and  $\beta_{EDV} = -0.104 \pm 0.022$  ( $P < 0.0001$  compared with 0 for both) (Fig. 3D). These data show that there are significant between-species differences in both the pressure gradients and pressure differences. They also show that, as mammals get smaller, IVPG becomes larger; in contrast, as mammals get larger, the IVPD becomes larger, in good correlation with the power rule.

Interestingly, there was a linear inverse relationship between IVPG and the normalized LVFT with an  $r = -0.65$  ( $P = 0.0001$ ). It shows that, as the normalized filling time becomes smaller, the IVPG becomes larger (Fig. 4A). As a result, no scaling was observed between the product of IVPG and LVFT/RR ( $\beta_{LV\ mass} = -0.023 \pm 0.025$ ,  $P =$  not significant vs. 0).

#### Impact of the Fusion of Early and Late Wave of Mitral Inflow on the IVPD

The sedated rats group had an average RR value of 146.00 (SD 20.56), and the anesthetized rats group had an average RR value of 163.58 (SD 12.50),  $P < 0.05$ . The LVFT of sedated rats was 59.5 ms (SD 9.44), whereas, for the anesthetized rats, it was 101.55 ms (SD 15.39),  $P < 0.001$ . Longer LVFT in anesthetized mammals resulted in clear separation of E and A waves in all mammals. Despite this, the average IVPD value of the sedated rats and for the anesthetized rats was similar [0.461 (SD 0.181) vs. 0.545 mmHg (SD 0.142),  $P > 0.1$ ]. This implies that the fusion of E and A waves did not dramatically affect IVPD measurements.

#### Intra- and Interobserver Variability

Table 2 displays intra- and interobserver variability of IVPD in mice and rodents. As might be expected, relative variability was higher in mice compared with both rats and humans, but still within acceptable limits. Correlation coefficients for intra- and interobserver pairs of measurements were  $r = 0.98$  and  $r = 0.97$ , respectively ( $P < 0.01$ ).

## DISCUSSION

The purpose of this study was to determine the nature of the relationship between IVPD and animal size. We have shown

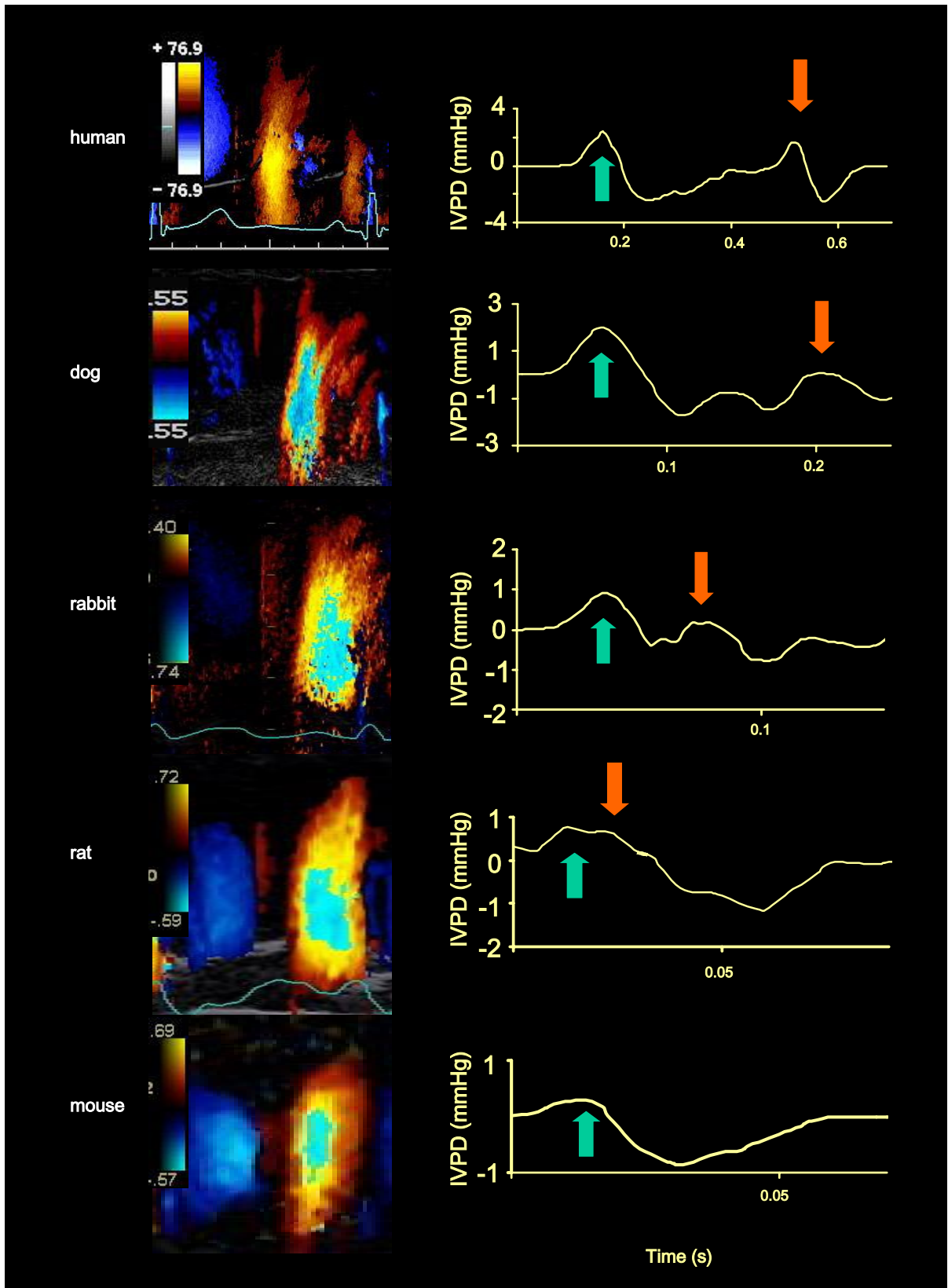


Fig. 1. Examples of color M modes of the mitral inflow recorded in the 5 species studied (left), along with the corresponding intraventricular pressure differences (IVPD; right). Note a no-flow diastasis period on the color-M mode that precedes the P wave of the electrocardiogram in the human data. Red and green arrows point to the maximum of the intraventricular pressure gradient (IVPG) occurring in early diastole (green) and after atrial contraction (red).

Table 1. Basic morphometric and echocardiographic data

	Body Weight, kg	RR Interval, ms	EDV, ml	ESV, ml	EF	LV Mass, g
Mouse	0.024 (0.004)	93 (12)	0.023 (0.002)	0.012 (0.002)	0.55 (0.07)	0.07 (0.01)
Rat	0.28 (0.28)	154 (18)	0.19 (0.05)	0.08 (0.02)	0.61 (0.08)	0.57 (0.12)
Rabbit	3.01 (0.44)	307 (2)	3.1 (0.71)	1.22 (0.48)	0.61 (0.09)	3.57 (0.58)
Dog	25.7 (1.9)	518 (79)	59.9 (12.9)	23.3 (6.0)	0.61 (0.07)	82.3 (11.1)
Human	76.3 (15.2)	932 (177)	110.9 (20.0)	39.1 (8.2)	0.64 (0.07)	151.1 (27.3)

Values are means (SD). LV, left ventricular; EDV, LV end-diastolic volume; ESV, LV end-systolic volume; EF, ejection fraction.

that IVPD scaling follows our second conjecture: that is, they vary with the size of the mammal in that, as a mammal gets smaller, the IVPD also becomes smaller. Surprisingly, we have not found that the IVPG are constant; instead they increase as the heart size became smaller. These findings were associated with a very high scaling exponent for LVFT, the result of which was that, with the decrease of the heart size, filling time duration proportionally decreases more than any other heart cycle interval.

### The Role of Diastole in the Cardiac Cycle

Diastole has two fundamental roles. Its primary role is to enable the filling of the LV. It should be pointed out that, in large mammals, such as humans, a prominent diastasis period may occur in the middle of diastole. This period, from the viewpoint of LV filling, is nonfunctional, and, because of this, the teleological role of diastole, as solely providing a period for LV filling, is debatable. A secondary role of the diastole is that it enables the perfusion of the coronary arteries, since during diastole, the pressure of the coronary capillary bed, which closely follows LV cavity pressure, becomes significantly lower than aortic root pressure. In contrast to filling, coronary perfusion occurs throughout diastole. Westerhof and Elzinga (22) have even postulated that the scaling of total diastole duration is determined by its role in coronary perfusion, noting that the scaling exponent of the total diastole time is identical to the exponent of aortic compliance.

### Factors That Enable LV Filling

LV inflow is a function of the period during which the ventricle is relaxed and the pressure differs between the body of left atrial chamber and LV apical region during that same period. This pressure difference can be compartmentalized into atrio-LV basal pressure difference and IVPD. The total atrioventricular pressure difference is not static and may be modulated by several mechanisms. During early diastole, the increase in restoring forces (through, for example, contractility increase) (2) increases these differences. This increase, in turn, may be blunted by impaired relaxation (brought, for example, by aging or LV hypertrophy). During late diastole, the atrio-LV basal pressure difference may be increased by atrial contraction, or eliminated if atrial fibrillation is present.

It follows by necessity that, in a physiological setting, any shortening of diastole must be accompanied by the physiological mechanism that increases pressure differences. This indeed occurs. It has been shown that the shortening of diastole during exercise leads to an increase of both early and late total atrioventricular pressure difference, an increase that is severely blunted in heart failure (4). Our findings are congruent with this, showing that short relative duration of diastole (assessed here as LVFT/RR) is associated with increased IVPG.

The differences in IVPG are possibly due to the fact that the smaller mammals have shorter filling times, and the IVPG must be larger to produce a sufficient EDV. Also, the greater IVPDs associated with larger mammals may be due to the larger LV length and volume, for there is more space to have a greater pressure differences from the valves to the apex.

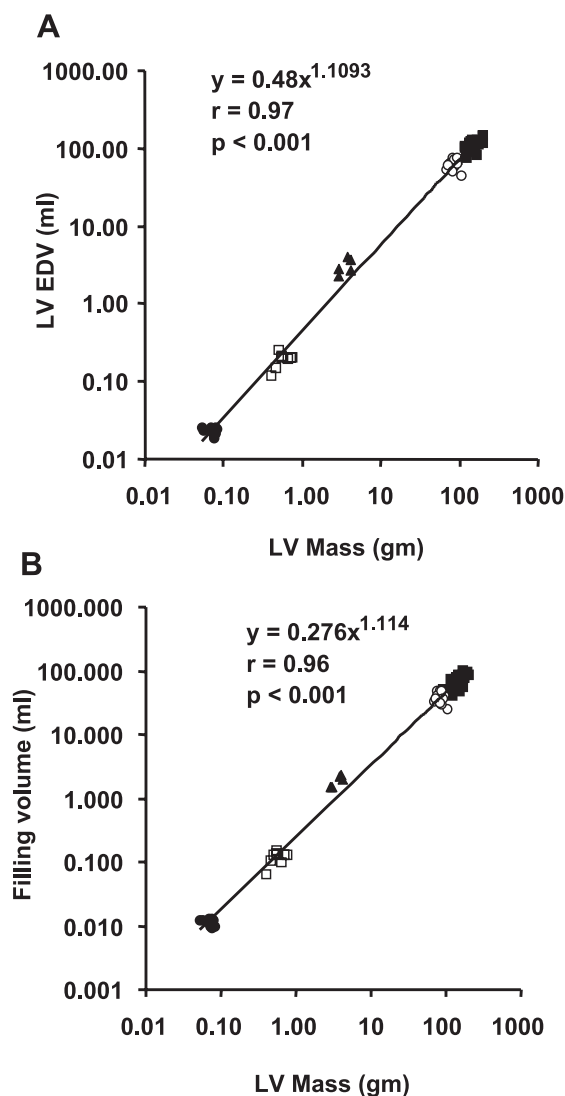


Fig. 2. Scaling of left ventricular (LV) end-diastolic volume (EDV; A) and LV filling volume (FV; B) vs. mass. ●, Mice; □, rats; ▲, rabbits; ○, dogs; ■, humans.

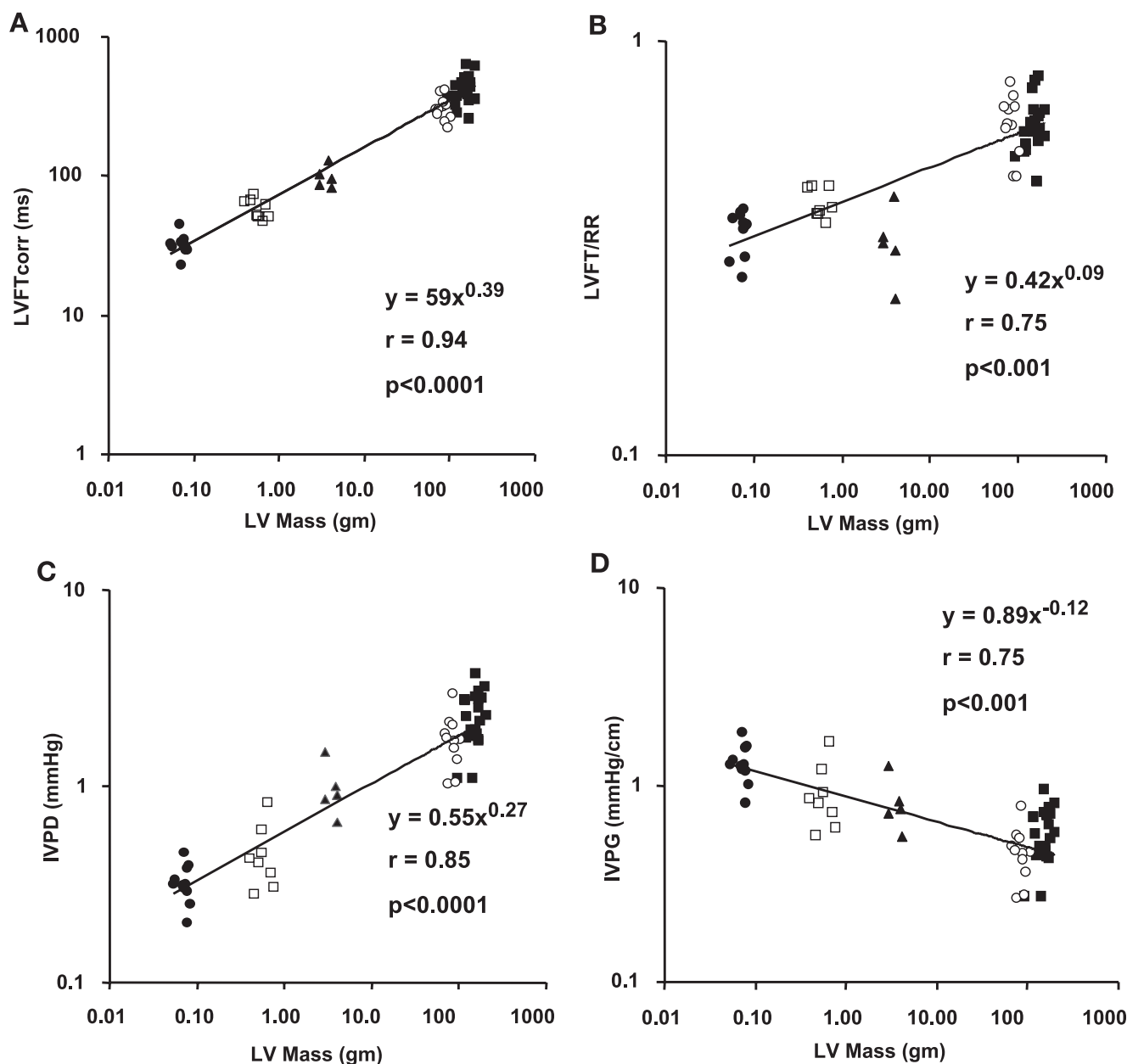


Fig. 3. A: scaling of LV filling time corrected for diastasis duration (LVFT<sub>corr</sub>) vs. LV mass. B: scaling of LVFT (not corrected by diastasis duration) normalized by RR interval duration (LVFT/RR) vs. LV mass. C: scaling of IVPD vs. LV mass. D: scaling of IVPG vs. LV mass. Symbols are as described in Fig. 2 legend.

*Scaling of the RR Interval and Its Subintervals*

Until now, scaling of heart cycle intervals to body (or heart) size has been shown to have a scaling exponent close to 0.25, demonstrated for the RR interval (21), LV ejection time (15), and PR interval (13). For the PR interval, Noujaim et al. (13) have nicely shown that this is the logical consequence of the fractal structure of the conduction tree. In contrast, in this paper, we show that both total LVFT, as well as a filling time corrected for diastasis period, have a scaling exponent that is significantly larger than 0.25. Interestingly, although it is well known in the research community that diastole is very short in small rodents [causing fusion of E and A waves and thus needing special interventions to discern between them (18)], no data exist on the normal duration of the LVFT in mice and rats.

This may stem from the fact that research is biased toward accentuating similarities, and not differences, between human and rodent physiology.

Importantly, interaction of different scaling parameters results in a filling volume that is a constant fraction of LV mass. It should be pointed out that, as mitral annulus diameter scales in perfect proportion to LV length (11), in the presence of relatively shorter diastolic times, increased IVPG may work as an additional mechanism needed to fill the ventricle with proportionally the same amount of blood.

*Why Small Mammals Have Larger IVPG*

To our knowledge, no study ever addressed this question. One of the possibilities is that smaller mammals have higher

baseline contractility, as evidenced by a smaller ratio of maximum heart rate to resting heart rate. On the other hand, Kass et al. (9) have shown that preload recruitable stroke work, a contractility measure independent of subjects' size, is similar in mice and humans. This implies that larger IVPG is not induced by higher resting contractility in small mammals. If so, this may point to some alternative unidentified mechanism.

One can surmise that, in small animals, because IVPD is less, filling pressures are less also, making them less likely to suffer less from pulmonary congestion. On the other hand, as larger IVPG are necessary to maintain filling during the smaller proportion of RR intervals, their loss in pathological states makes smaller animals more likely to suffer from low cardiac output. Although no data exist to support these hypotheses, they constitute a fertile ground for future studies.

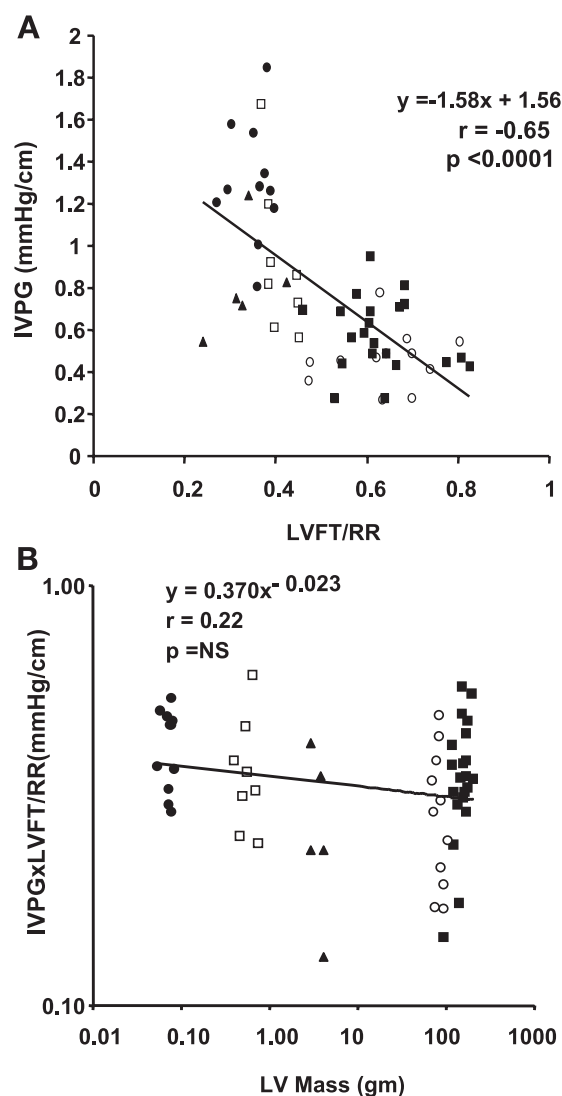


Fig. 4. A: relationship of LVFT/RR and IVPG. There was moderately strong inverse relationship signifying that higher IVPGs develop in the ventricles with short filling intervals. B: no scaling was observed between the product of IVPG and LVFT/RR (IVPG  $\times$  LVFT/RR) and LV mass. Symbols are as described in Fig. 2 legend.

Table 2. Inter- and intraobserver variability data for intraventricular pressure difference measurement in small rodents

	Mouse		Rat	
	Absolute, mmHg	Relative, %	Absolute, mmHg	Relative, %
Intraobserver	0.04 (0.01)	15.3 (6.3)	0.05 (0.03)	10.1 (7.9)
Interobserver	0.06 (0.02)	17.8 (3.0)	0.02 (0.02)	4.4 (5.8)

Values are means (SD).

### Practical Implications

As research is frequently performed initially in small mammals, scaling relationships are important when it comes to translating the findings to the clinical arena. Smaller mammals are used in research due to their size, short life spans, and affordability with a supposed correlation between their metabolic and organ function to that of humans. However, identifying the appropriate extrapolations of heart function data from small mammals to humans is still incomplete. This is particularly important, as newer inroads into small-mammal noninvasive imaging allow us to gather data available only in large mammals until recently (15).

Demonstrating that IVPG changes with size may also help assess the diastolic function of pediatric patients. From what we have found, we would expect pediatric patients to have smaller IVPD but larger IVPG than adults.

### Limitations

The subjects were studied in comparable, but not identical conditions, as the rabbits and rats were pharmacologically sedated. While the effects of anesthesia or sedation on IVPD are unknown, IVPD are strongly affected by contractility and catecholamine stimulation (8). Thus any change of sympathetic level induced by anesthesia could have influenced our data. However, we were careful so as to keep the sedation level at a minimum (15). Also, in a group of rats that were anesthetized with the combination of ketamine and xylazine to slow down the heart rate, this procedure may have affected IVPG. Also, we have not compared noninvasive IVPG with a gold-standard multisensor high-fidelity pressure measurements. However, this approach to IVPG has been well validated in dogs (14) and humans (15), whereas in small animals, "gold-standard" should be taken cautiously, as the pressure differences are very small, likely within the noise range of the pressure sensors. Finally, as the IVPD waveforms seem different among species, previous validation studies of noninvasive IVPD may not be necessarily valid in small-animal studies. However, as underlying laws of the physics are the same, this factor probably contributes little to a measurement error.

In conclusion, we have shown that IVPG and IVPD scale to animal size in opposite directions. Furthermore, we have shown that this is associated with shorter relative duration of LVFT in very small mammals. This indicates that increase of IVPG in very small mammals may be a necessary adaptive mechanism to both absolute and relative shortening of filling time duration.

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