

## Basic values of M-mode echocardiographic parameters of the left ventricle in outbreed Wistar rats

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**ABSTRACT:** This paper describes the partial results of an echocardiographic study in sixty outbreed Wistar rats. Animals of parity sex ratio were chosen for the experiment. The animals were grown up during the observation period (the minimum weight was 220 g; the maximum weight was 909 g) and were then sequentially anaesthetised (2–2.5% of isoflurane, 3 l/min O<sub>2</sub>). The second, fourth and fifth examinations were performed under anaesthesia maintained by intramuscular injections with diazepam (2 mg/kg), xylazine (5 mg/kg) and ketamine (35 mg/kg). Transthoracic examination was done using the SonoSite Titan echo system (SonoSite Ltd.) with a microconvex transducer C11 (8–5 MHz). M-mode (according to the leading-edge method of American Society of Echocardiography) echocardiography data were acquired at the papillary muscle: systolic and diastolic interventricular septum (IVSs, d) and left ventricular posterior wall (LVPWs, d) thickness, systolic and diastolic left ventricular dimension (LVDs, d), aorta (Ao) and left atrium (LA) dimensions. According to standard formulas, the following parameters were obtained: ejection fraction (EF), cardiac output (CO), stroke volume (SV), left ventricle end systolic volume (LVESV), left ventricle end diastolic volume (LVEDV), interventricular septum fractional thickening (IVSFT), left ventricular dimension fraction shortening (LVDFS), and left ventricle posterior wall fraction thickening (LVPWFS). In our study we performed 300 examinations both in male and female Wistar rats of various body weights and calculated regression equations to predict expected normal echocardiographic parameters for rats with arbitrary weights. The rats were examined by an echo scan. The first and third examinations were performed during mono-anaesthesia induced by inhalation of isoflurane. Correlations, with one exception (LVDs), were very close, which means that the results of the calculations based on regression equations are very reliable.

**Keywords:** rat; echocardiography; M-mode; Wistar; isoflurane; diazepam; xylazine; ketamine

### List of abbreviations

**ACS** = aortic cusp separation, **Ao** = aortic root diameter, **BSA** = body surface area, **CFD** = colour flow Doppler, **CO** = cardiac output, **CPD** = colour power doppler, **CW** = continuous wave doppler, **EF** = ejection fraction, **EPSS** = E point to septal separation, **IVSFT** = inter ventricular septum fractional thickening, **IVSs, d** = systolic and diastolic interventricular septum, **LA** = left atrium dimensions, **LVDFS** = left ventricular dimension fraction shortening, **LVDs, d** = systolic and diastolic left ventricular dimension, **LVEDV** = left ventricle end diastolic volume, **LVESV** = left ventricle end systolic volume, **LVET** = left ventricle ejection time, **LVPWFS** = left ventricle posterior wall fraction thickening, **LVPWs, d** = left ventricular posterior wall thickness, **PW** = pulse wave doppler, **QRS** = complex QRS, **RPLA** = right parasternal long axis view, **RPSA** = right parasternal short axis view, **SV** = stroke volume

The recent technological advancements in the ultrasound technique allow thorough echocardiographic examination even in small laboratory mammals (Watson et al., 2004). Despite progress in general breed-specific basic echocardiographic standards in each category of laboratory animal and

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for the most common anaesthetic combinations are still missing. Up until now obtained standards are derived from examinations of just one weight category, single gender groups, and are focused only on a small spectrum of parameters, important for the aim of the published work (Hagar et al., 1995; Longobardi et al., 2000; Prunier et al., 2002; Watson et al., 2004; Popovic et al., 2007; Koskenvuo et al., 2010).

The goal of this study is to highlight the suitability of echocardiographic examination for routine experimental practice in rats performed on a common commercial device. The main aim is to describe basic echocardiographic parameters of the left ventricle over the widest possible weight spectrum of individuals using two types of common anaesthetic protocols in Wistar rats.

## MATERIAL AND METHODS

All the animal studies reported in this article were approved by the Institutional animal care and use committee and were in compliance with valid Czech law and international conventions.

Sixty outbred Wistar rats (An Lab Prague) of parity sex ratio were chosen for the experiment. The rats were bought in a range of body weights from 150 to 180 g and acclimation lasted from two to three weeks. The first and the third examination were performed under mono-anaesthesia inhalation with isoflurane. The second, the fourth and the fifth examination were performed under general anaesthesia maintained by intramuscular injections with the anaesthetic agents, diazepam, xylazine and ketamine. The minimal interval between examinations was one week, while the maximum was six weeks.

### Inhalation anaesthesia

Anaesthesia was maintained by mask inhalation of isoflurane vaporized by Dräger Vapor 19.3 (Drägerwerk A.G.) in an open inhalation system. The flow rate of the driving gas – medicinal oxygen – (Convenia Air, Linde a.s.) was 3 l/min. The initial concentration of isoflurane (Isofluran Nicolas Pikamal, Torres Pharma Chiesi) was 1% in the induction phase and was increased according to the effect, usually by up to 2–2.5%. An echo scan was made after reaching the tolerance stadium of anaesthesia and shaving of the chest.

### Injection anaesthesia

Anaesthesia was induced (time 0 min) by intramuscular injection with 2 mg/kg b.w. diazepam (Apaurin, Krka, 5 mg/ml) into the caudal part of the tight muscles. At the same time 5 mg/kg b.w. xylazine (Xylapan, Vétoquinol Biowet Sp. Z o.o., 20 mg/ml) and 35 mg/kg b.w. ketamine (Narketan, Vétoquinol Biowet Sp. Z o.o., 100 mg/ml) were mixed in the same syringe, and applied into the caudal part of the tight muscles of the other pelvic limb. The complete echo scan started in the twentieth minute of the protocol.

### Basic echocardiographic examination

The M-mode measurement was performed using the SonoSite Titan (SonoSite Ltd.) with microconvex transducer C11 (8–5 MHz, 11 mm). The device was fully equipped for cardiologic and vascular modes, including pulsed wave Doppler (PW), continuous wave Doppler (CW), color flow Doppler (CFD) and color power Doppler (CPD) and was connected to the Titan Mini-dock mobile docking station (SonoSite Ltd.). The ECG cable of the echo-system was connected to the patient for M-mode measuring. M-mode accuracy of distance (range 0–26 cm), time measurement (range 0.01–10 s) and heart rate (range 5–923 bpm) are  $< \pm 2\%$ . Measurements were performed in accordance with the leading-edge method of the American Society of Echocardiography. Diastolic dimensions were measured at the onset of the QRS complex. The right ventricle IVS endocardium is added to the IVS dimension and the left ventricle IVS endocardium is a part of the left ventricle diameter. The measured parameters were systolic and diastolic interventricular septum (IVSs, d), systolic and diastolic left ventricular dimension (LVDs, d), systolic and diastolic left ventricular posterior wall (LVPWs, d), aorta (Ao) and left atrium (LA). These parameters were considered as the basic set of parameters. Incomplete sets were discarded. Furthermore, if the quality was sufficient, the following parameters were measured: EF slope of septal leaflet of mitral valve (EF:slope), “E”-point of septal separation (EPSS) and the dimension of aortic valve cusp separation (ACS). According to standard formulas the following parameters were measured – ejection fraction (EF), cardiac output (CO), stroke volume (SV), left ventricle end systolic

volume (LVESV), left ventricle end diastolic volume (LVEDV), interventricular septum fractional thickening (IVSFT), left ventricular dimension fraction shortening (LVDFS), left ventricle posterior wall fraction thickening (LVPWFS) (Boon, 2006). Each rat was examined in dorsal recumbency. Initially, examination was performed from the right parasternal short axis view (RPSA) optimized for the left ventricle and aorta. When all structures were displayed correctly the picture was zoomed in and the M-mode record was acquired. Afterwards, the left ventricle was displayed in RPSA or in right parasternal long axis views (RPLA) and the M-mode record was then made. Complete echo-scans were always performed within 10 minutes.

### Statistical analysis

The basic statistical parameters describing the data selection were evaluated in the data set. The conformity of mean values and variances between the data set of inhalation and injection anaesthesia was checked using the *F*-test and Student's *t*-test. Dependency and regression were evaluated for parameters of the left ventricle, left atrium and aortic dimensions, related to the body weight. The statistical programs SPSS PASW statistic 17 (SPSS inc.) and MS Office 2003 Excel (Microsoft corp.) were used for the statistical analysis.

## RESULTS

The total number of examinations was 300; 120 were performed under inhalation anaesthesia and 180 were carried out using injection anaesthesia. Only complete data sets were included in statistical analyses. Complete data sets were obtained in

61 cases (statistical success rate of 51%) in the inhalation anaesthesia group, and in 119 cases (statistical success rate of 66%) in the injection anaesthesia group. Thus, the total success rate was 60% (180 from 300).

### Body weight

The average body weight of rats was  $0.538 \pm 0.170$  kg, ranging from 0.220–0.905 kg in the injection anaesthesia data set, while in the inhalation anaesthesia group the body weight averaged  $0.469 \pm 0.166$  kg, ranging between 0.232–0.780 kg. There was a significant difference ( $P < 0.01$ ) among mean values as measured using the Student *t*-test (Table 1).

### Heart rate

The mean heart rate of rats was  $312 \pm 56$  bpm, ranging from 194–462 bpm in the injection anaesthesia data set, whereas in the inhalation anaesthesia data set the mean was  $367 \pm 43$  bpm, ranging from 250–462 bpm and the minimum frequency was 250 bpm. There was a significant difference in variations as determined by the *F*-test and also in mean values as determined using the *t*-test (Table 1; *F*-test  $P < 0.05$ ; *t*-test  $P < 0.01$ ).

### Interventricular septum

**Diastolic dimension (IVSd).** The average IVSd thickness was  $0.16 \pm 0.04$  cm, ranging from 0.07–0.29 cm in the injection anaesthesia data set and  $0.15 \pm 0.04$  cm, ranging from 0.07–0.23 cm in the inhalation anaesthesia data set. There was no significant difference in both variations as measured

Table 1. Comparison of *F*-test and Student's *t*-test *P*-values for testing the variance and probability compliance of mean values in inhalation and injection anaesthesia data sets

M-mode	BW	IVSd	IVSs	LVDd	LVDs	LVPWd	LVPWs	EF (%)	CO	SVI	HR
<i>F</i> -test	0.820	0.424	0.002	0.088	0.057	0.012	0.026	0.150	0.216	0.000	0.033
<i>t</i> -test	0.009**	0.131	0.012*	0.524	0.236	0.260	0.086	0.840	0.023*	0.620	0.000**
M-mode	LVESV	LVEDV	IVSFT	LVDFS	LVPWFT	EPSS	EF:slope	LVET	ACS	LA	Ao
<i>F</i> -test	0.004**	0.000**	0.000**	0.297	0.000**	0.630	0.400	0.705	0.389	0.015*	0.020*
<i>t</i> -test	0.540	0.471	0.010**	0.943	0.038*	0.161	0.251	0.600	0.643	0.004**	0.226

\* $P < 0.05$ ; \*\* $P < 0.01$

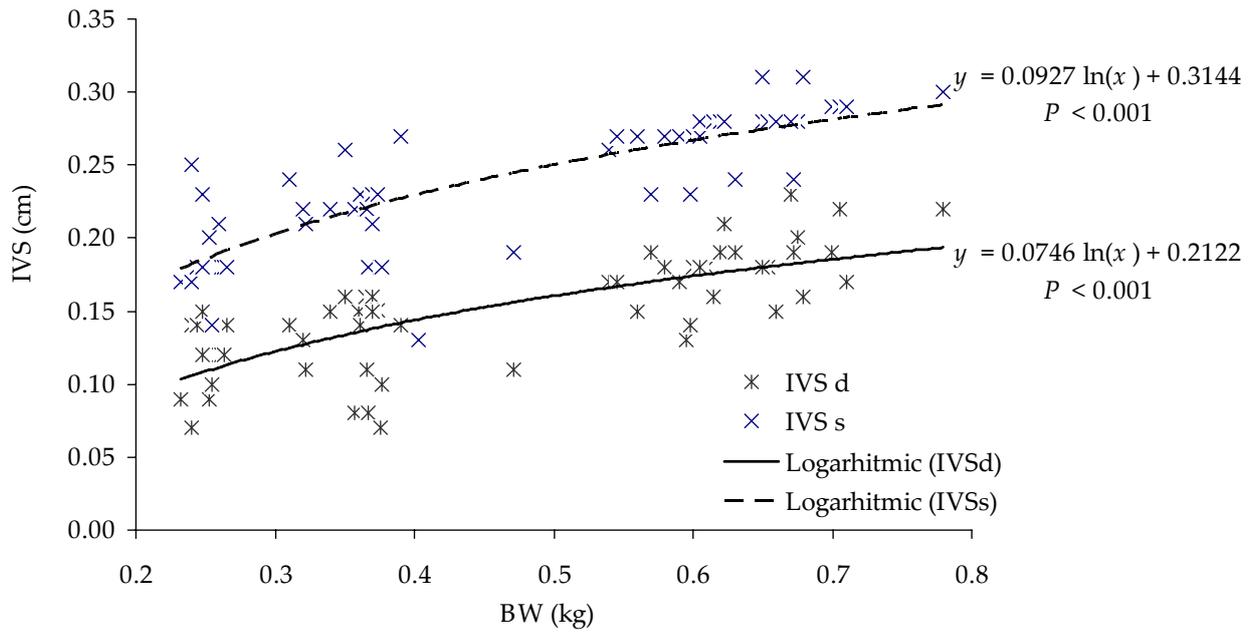


Figure 1. The trend of the dependence IVS on body weight in the rat (inhalation anaesthesia)

by the *F*-test and mean values measured using the *t*-test (Table 1).

Data regression analysis between IVSd and body weight is optimally described using a logarithmic regression curve, in both the inhalation and injection anaesthesia groups (Table 2; Figures 1, 2).

**Systolic dimension (IVSs).** The mean IVSs thickness was  $0.26 \pm 0.06$  cm, ranging from 0.13–0.49 in the injection anaesthesia data set, whereas in the

inhalation anaesthesia data the mean size was  $0.24 \pm 0.04$  cm, ranging between 0.13–0.31 cm. There was a significant difference in variations as determined by the *F*-test and also in mean values measured using the *t*-test (Table 1; *F*-test  $P < 0.01$ ; *t*-test  $P < 0.05$ ).

Data regression analysis between IVSs and body weight is optimally described using a logarithmic regression curve, in both the inhalation and injection anaesthesia groups (Table 1; Figures 1, 2).

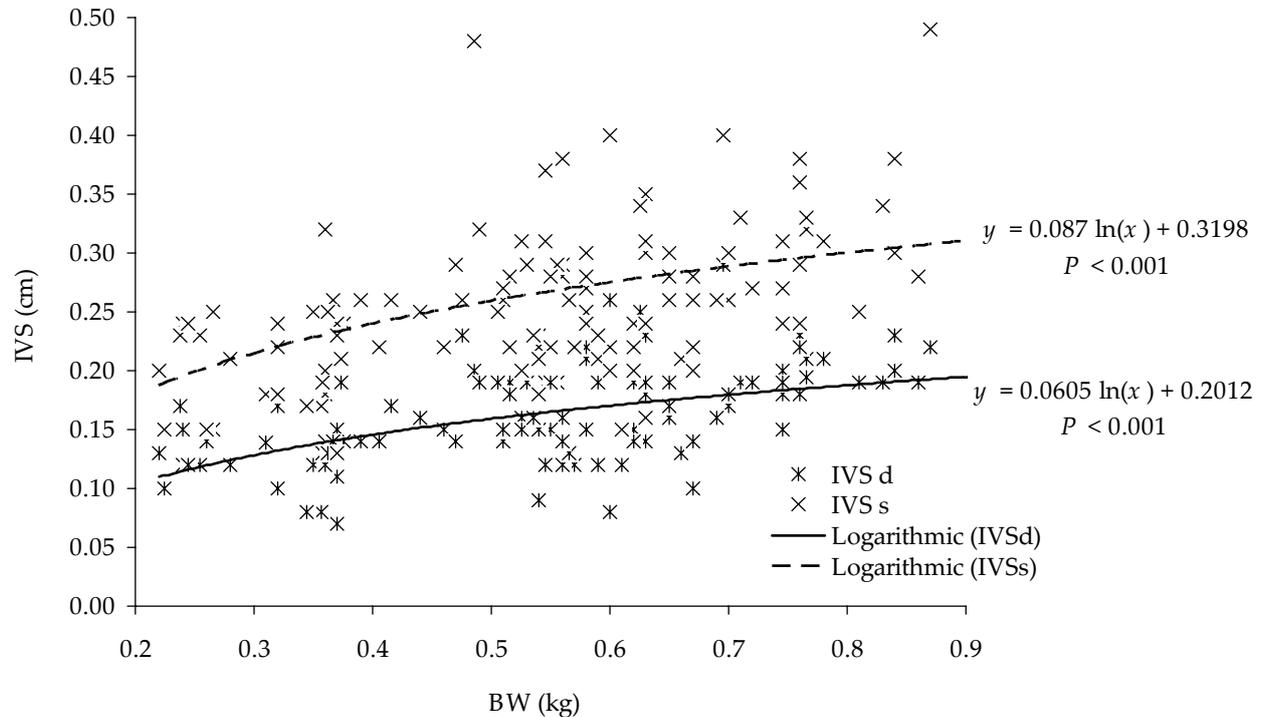


Figure 2. The trend of the dependence IVS on body weight in the rat (injection anaesthesia)

Table 2. Relation between echocardiographic parameters and body weight in the rat

Anaesthesia type	Equation of trend line	R; sign.	95% CI	
			lower bound	upper bound
<b>IVSd</b>				
INH	$y = 0.0746 \ln(x) + 0.2122$	0.764; $P < 0.001$	0.076	1.708
INJ	$y = 0.0605 \ln(x) + 0.2012$	0.536; $P < 0.001$	0.277	1.486
<b>IVSs</b>				
INH	$y = 0.0927 \ln(x) + 0.3144$	0.806; $P < 0.001$	0.088	1.361
INJ	$y = 0.087 \ln(x) + 0.3189$	0.510; $P < 0.001$	0.220	1.134
<b>LVDd</b>				
INH	$y = 0.1372 \ln(x) + 0.832$	0.551; $p < 0.001$	0.032	0.491
INJ	$y = 0.1581 \ln(x) + 0.8365$	0.531; $p < 0.001$	-0.007	0.524
<b>LVDs</b>				
INH	$y = 0.0958 \ln(x) + 0.4952$	0.451; $P < 0.001$	-0.185	0.327
INJ	$y = 0.1066 \ln(x) + 0.5056$	0.394; $P < 0.001$	-0.039	0.542
<b>LVPWd</b>				
INH	$y = 0.0785 \ln(x) + 0.2463$	0.678; $P < 0.001$	0.086	1.094
INJ	$y = 0.0822 \ln(x) + 0.2474$	0.498; $P < 0.001$	-0.299	0.846
<b>LVPWs</b>				
INH	$y = 0.119 \ln(x) + 0.3563$	0.809; $P < 0.001$	0.053	1.023
INJ	$y = 0.1097 \ln(x) + 0.3506$	0.564; $P < 0.001$	-0.126	0.800
<b>LA</b>				
INH	$y = 0.1279 \ln(x) + 0.5014$	0.717; $P < 0.001$	0.189	0.959
INJ	$y = 0.0883 \ln(x) + 0.4885$	0.340; $P < 0.001$	-0.046	0.511
<b>Ao</b>				
INH	$y = 0.1031 \ln(x) + 0.4266$	0.605; $P < 0.001$	-0.063	0.567
INJ	$y = 0.1157 \ln(x) + 0.4334$	0.499; $P < 0.001$	0.318	0.923

INH = inhalation anaesthesia, INJ = injection anaesthesia, R = Pearson test value of reliability, sign. = value of statistical significance between the dependent variables, 95% CI = 95% confidence interval

### Diastolic left ventricular diameter (LVDd)

The mean value of the diastolic left ventricular diameter was  $0.73 \pm 0.11$  cm, ranging from 0.46–1.14 cm in the injection anaesthesia data set, and  $0.72 \pm 0.09$  cm, ranging from 0.35–0.86 cm in the inhalation anaesthesia data. There was no significant difference in both variations as determined by the *F*-test and in mean values determined using the *t*-test (Table 1).

Data regression analysis between LVDd and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia data sets (Table 2; Figures 3, 4).

### Systolic left ventricular diameter (LVDs)

The mean value of the systolic left ventricular diameter was  $0.43 \pm 0.10$  cm, ranging from 0.20–0.72 cm

in the injection anaesthesia data set, and  $0.42 \pm 0.08$ , ranging from 0.21–0.61 cm in the inhalation anaesthesia data set. There was no significant difference in both variations measured using the *F*-test and in mean values measured by the *t*-test (Table 1).

Data regression analysis between LVDs and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia groups (Table 2; Figures 3, 4)

### Diastolic left ventricular posterior wall dimension (LVPWd)

The average thickness of LVPWd was  $0.19 \pm 0.06$  cm, ranging from 0.06–0.44 cm in the injection anaesthesia data set, and  $0.18 \pm 0.05$  cm, ranging from 0.08–0.28 cm, in the inhalation anaesthesia data set. There was no significant difference in both

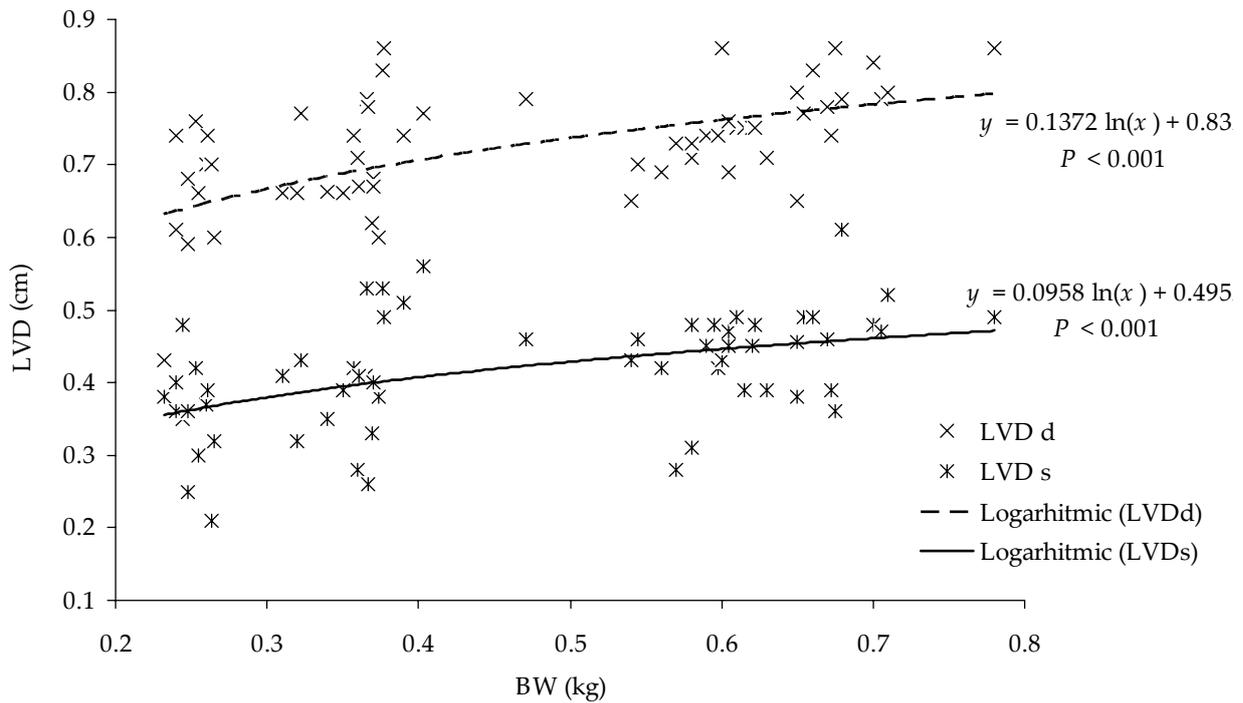


Figure 3. The trend of the dependence LVD on body weight in the rat (inhalation anesthesia)

variations determined by the *F*-test and in mean values determined by the *t*-test (Table 1).

Data regression analysis between LVPWd and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia data sets (Table 2; Figures 5 and 6).

**Systolic left ventricular posterior wall dimension (LVPWs)**

The average thickness of LVPWs was  $0.28 \pm 0.07$  cm, ranging from 0.10–0.53 cm in the injection anaesthesia data set, and  $0.26 \pm 0.06$  cm, ranging from

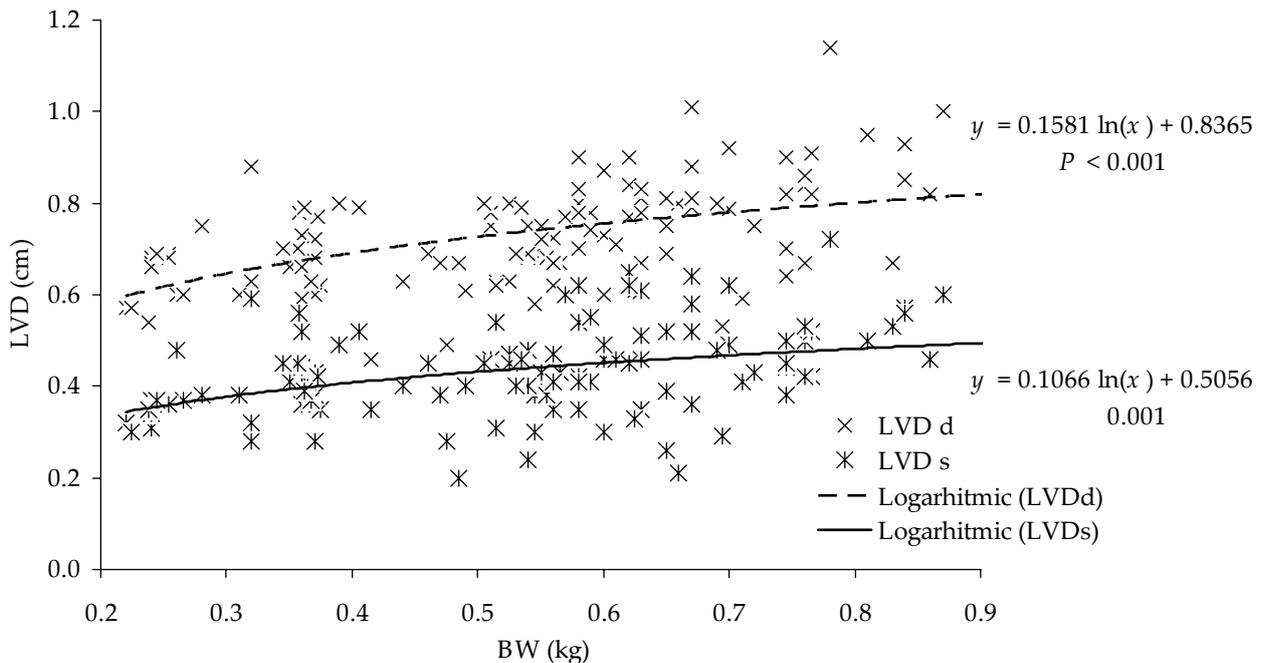


Figure 4. The trend of the dependence LVD on body weight in the rat (injection anesthesia)

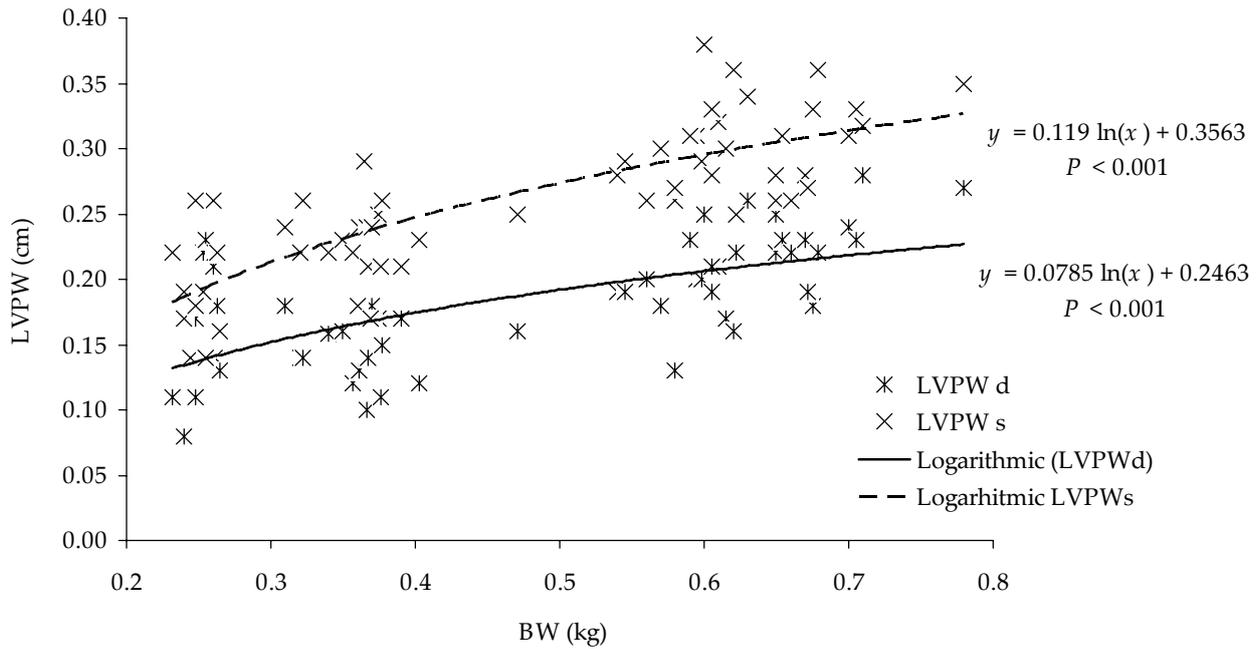


Figure 5. The trend of the dependence LVPW on body weight in the rat (inhalation anesthesia)

0.14–0.38 cm in the inhalation anaesthesia data set. There was no significant difference in both variations determined using the *F*-test and mean values determined by the *t*-test (Table 1).

Data regression analysis between LVPWs and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia groups (Table 2; Figures 5 and 6).

**Left atrium diameter (LA)**

The mean value of the left atrium diameter was  $0.42 \pm 0.09$  cm, ranging from 0.22–0.59 cm in the injection anaesthesia data set, and  $0.43 \pm 0.05$  cm, ranging from 0.32–0.56 cm in the inhalation anaesthesia data set. There was a significant difference in variations measured by the *F*-test and also in mean

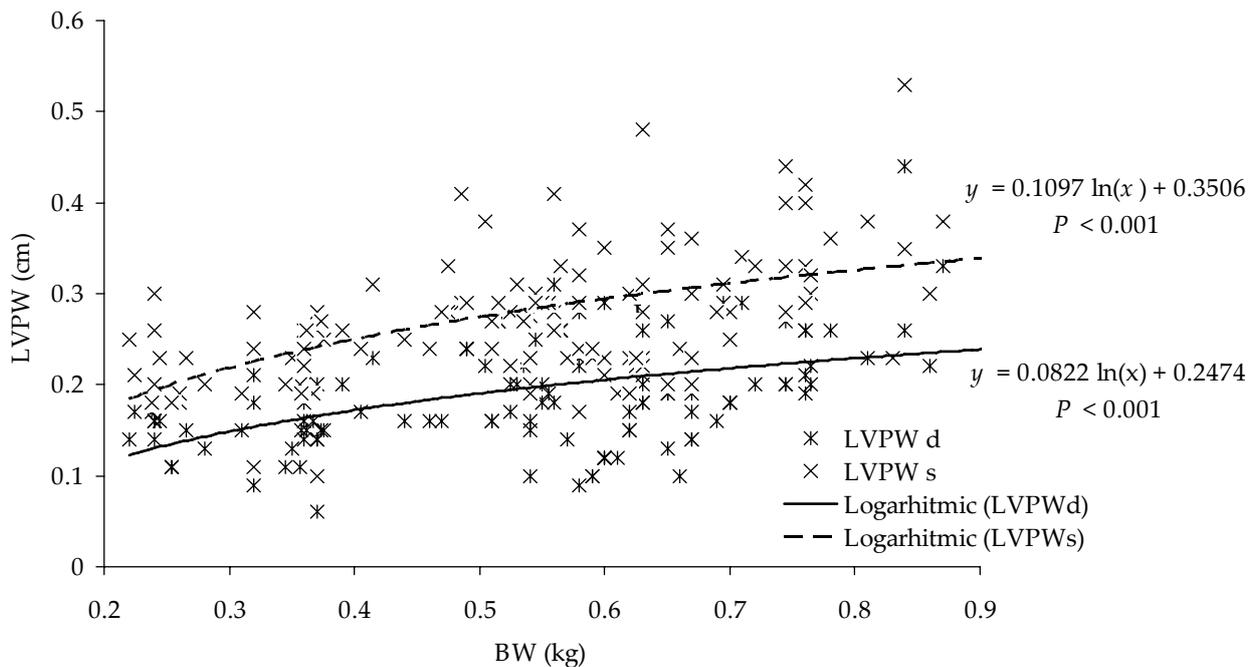


Figure 6. The trend of the dependence LVPW on body weight in the rat (injection anesthesia)

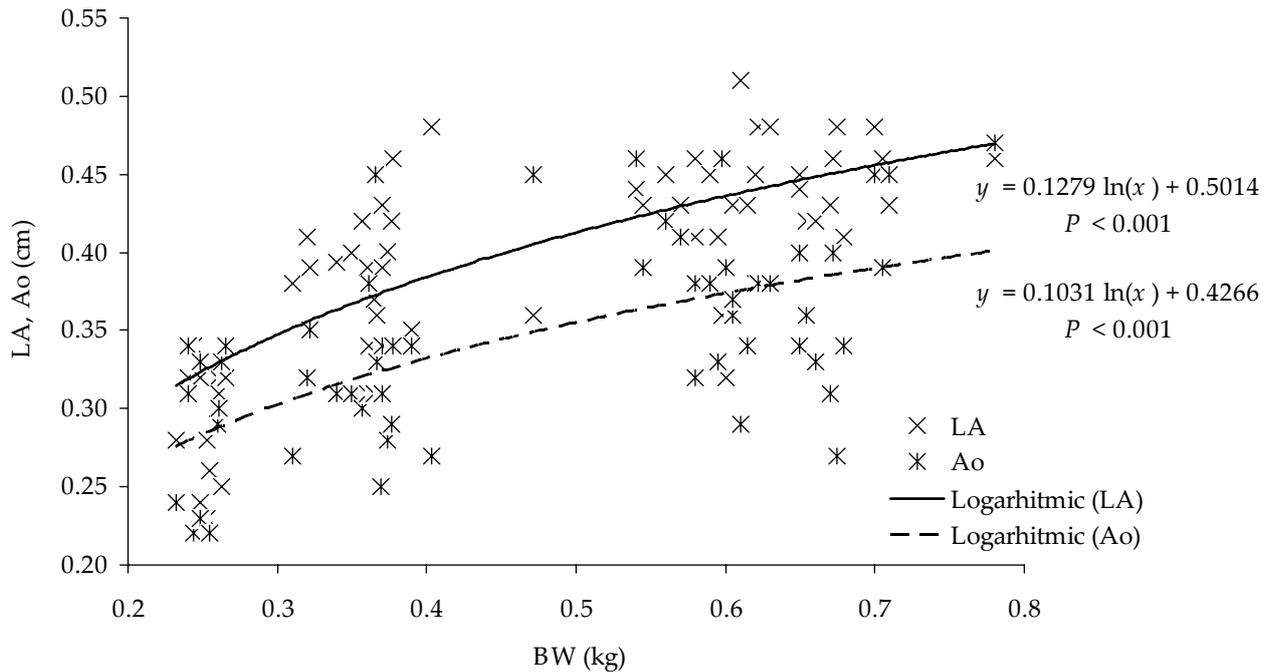


Figure 7. The trend of the dependence LA and Ao on body weight in the rat (inhalation anesthesia)

values measured by the *t*-test (Table 1; *F*-test  $P < 0.05$ ; *t*-test  $P < 0.01$ ). The data regression analysis between LA and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia data sets (Table 2; Figures 7, 8).

**Aortal valve diameter (Ao)**

The mean value of the aortal valve diameter was  $0.36 \pm 0.08$  cm, ranging from 0.20–0.59 cm in the injection anaesthesia data set, and  $0.34 \pm 0.07$  cm, ranging from 0.22–0.47 cm in the inhalation anaes-

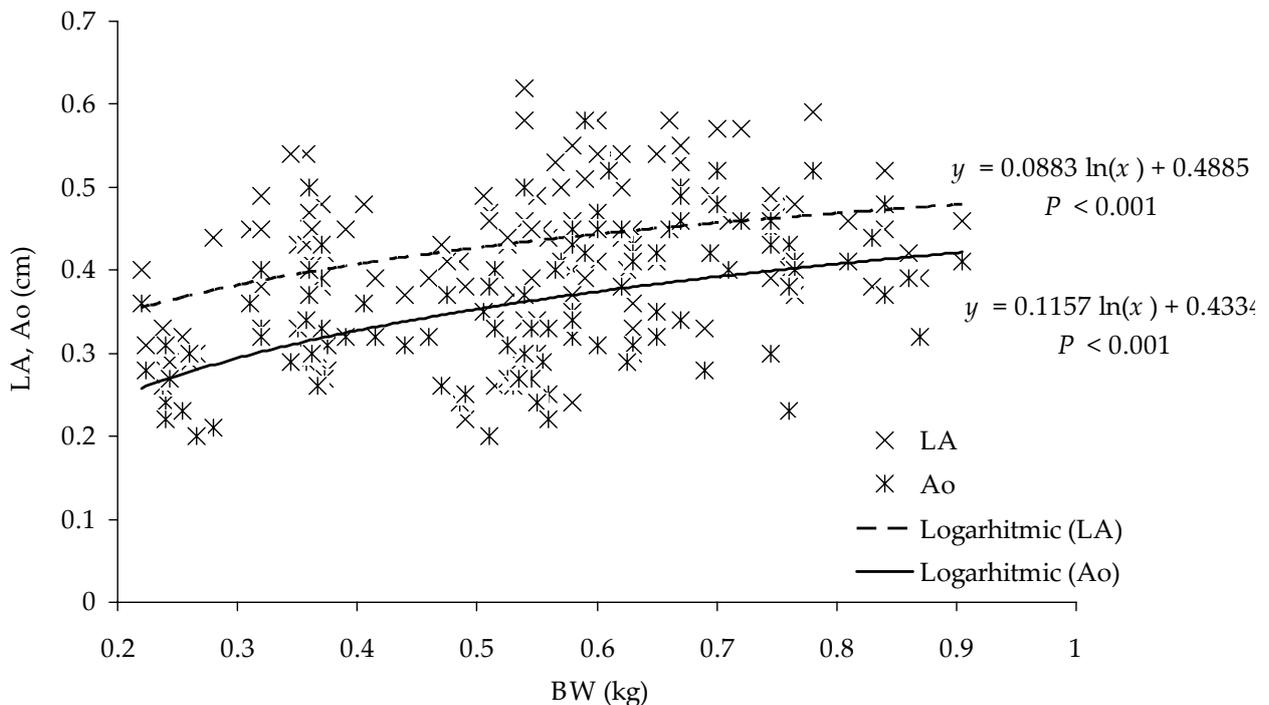


Figure 8. The trend of the dependence LA and Ao on body weight in the rat (injection anesthesia)

Table 3. Summary of the other obtained and calculated parameters from the injection and inhalation anaesthesia data set

Parameter	EF (%)	CO (l/min)	SV (ml)	HR (l/min)	LVESV (ml)	LVEDV (ml)	IVSFT (%)	LVDFS (%)	LVPWFT (%)	EPSS (cm)	EF:slope (cm/s)	LVET (s)	ACS (cm)
<b>Injection anaesthesia</b>													
Mean	75	0.215	0.69	312	0.2	0.92	38	41	30	0.08	3.48	0.09	0.24
SD	11	0.099	0.32	56	0.14	0.41	12	10	14	0.03	1.58	0.02	0.04
Min	98	0.660	2.09	462	0.84	2.93	68	74	61	0.16	8.73	0.17	0.32
Max	23	0.031	0.13	194	0.02	0.24	4	9	–	0.01	1.02	0.05	0.16
<i>n</i>	119	119	119	119	119	119	119	119	119	36	38	47	40
<b>Inhalation anaesthesia</b>													
Mean	76	0.249	0.67	367	0.216	0.888	50.2	40.7	38.2	0.10	2.79	0.10	0.22
SD	10	0.086	0.20	43	0.103	0.228	35.5	8.8	27.0	0.02	0.93	0.02	0.06
Min	93	0.498974	1.25	462	0.527863	1.37	175.0	61.6	140.0	0.13	3.77	0.11	0.27
Max	49	0.129139	0.36	250	0.04	0.48	0.0	21.6	0.0	0.08	1.69	0.08	0.16
<i>n</i>	61	61	61	61	61	61	61	61	61	4	4	3	3

thetia data set. The difference in variations determined using the *F*-test was significant ( $P = 0.05$ ), but there was no significant difference between mean values measured using the *t*-test (Table 1).

Data regression analysis between Ao and body weight is optimally described using a logarithmic regression curve, in both inhalation and injection anaesthesia groups (Table 2; Figure 7, 8).

### Other obtained and calculated parameters

The other obtained and calculated parameters from the injection and inhalation anaesthesia data set was summarised in Table 3.

## DISCUSSION

Both experimental and clinical practice has an absolute requirement for standards, even if these are relatively rough. Clinical practice requires data gained from the widest possible weight spectrum, ideally in both male and female individuals, using a common commercial device. The lack of these data prompted us to embark on this echocardiographic study in rats. In our study we performed 300 examinations in both male and female Wistar rats of various body weights and calculated regression equations to predict expected normal echocardiographic parameters for rats

with arbitrary weights. The correlations were very close, which means that the results of the calculations, based on regression equations are very reliable.

In clinical practice inhalation anaesthesia is preferred, while in experimental practice injection anaesthesia is more commonly employed (Longobardi et al., 2000; Handa et al., 2002; Prunier et al., 2002; Watson et al. 2004; Hacker et al., 2005; Kawahara et al., 2005; Hartmann et al., 2007; Piegari et al., 2007; Popovic et al., 2007; Sung et al., 2009). Thus, we decided to use both of these methods in our study. The obtained results were evaluated separately.

The use of injection anaesthesia with diazepam, xylazine and ketamine is well validated in our experimental practice, in both rats and rabbits, and in a large spectrum of cases (pulmonary embolism, septic shock, myocardial infarction; Dembovska et al., 2008; Klabusay et al., 2009). The combination of diazepam, xylazine, and ketamine reported here is both reasonably priced and readily available worldwide. We chose not to employ pentobarbital, which is still used in non-European countries, due to limited availability. Isoflurane, the most common inhalation anaesthetic and the one most preferred in rodent clinical practice, was chosen to induce anaesthesia through inhalation.

Echocardiographic parameters were deliberately correlated to the most easily obtained parameter – bodyweight. There are, of course, other possibilities: e.g., BSA, the length of the tibia, age, or the circum-

ference of the chest. Nevertheless, body weight is the most easily standardized parameter and can be used in all breeds of rats without the need for correction.

The significant difference among the body weights was expected and was caused by spontaneous increases in the weight of young animals during the time course, which was necessary for repeat examinations. While the first and the third examination were performed under inhalation anaesthesia, the second, fourth and fifth examinations were performed under injection anaesthesia, and were carried out on average later. The interval between the examinations was from one (1<sup>st</sup> and 2<sup>nd</sup>) examination to five weeks (next examinations).

The significant difference among the heartbeat rates was also expected because the anaesthetic combination with xylazine (alpha-agonist) usually leads to bradycardia (Kawahara et al., 2005). Therefore, the results calculated from the heart rate also had to differ (there was a significant difference in cardiac output, but not in stroke volume).

We cannot explain why a significant difference was found only in IVSs and LA values in injection vs. inhalation anaesthesia. Nevertheless, the dimensions of IVSs, IVSd, LVPWs, LVPWd, LA and Ao were lower under inhalation anaesthesia than in injection anaesthesia, whereas the systolic and diastolic diameters of the left ventricle were almost the same, corresponding to the low body weight in the data set from inhalation anaesthesia.

Hyperpnoe either with tachypnoe or with bradypnoe is often observed in response to inhalation anaesthesia. The reasons could lie in the position of the head and the neck during anaesthesia maintained by mask inhalation, which can keep the breathing down. The echoscan was performed as quickly as possible meaning that the percentage of complete measurements was lower for inhalation anaesthesia (55%), compared to the data set obtained under injection anaesthesia (61%). Deep spontaneous breathing has an influence on the venous return and the heart position.

Sometimes when echocardiography is performed in rats, mainly in young ones, the diameter of *aorta descendens*, which can appear behind the left atrium in the short axis view, could be mistakenly added to the dimension of left atrium (measured in M-mode).

In general, injection anaesthesia is more convenient for a precise echo scan. There is no danger of the tongue falling backwards or of the face mask slipping down. However, the length of induction (15–20 min) is a disadvantage of the injection anaesthesia method, which is, moreover, time consuming

in experimental practice. Injection anaesthesia is also not convenient for repeat examinations over a short period of time such as hours or days.

The induction of inhalation anaesthesia is relatively quick and almost all rats were completely anaesthetized within two minutes of induction. Another notable advantage of this method is its safety and also the fact that it can be employed numerous times in one day – for example, if the evaluation of haemodynamics or another dynamic parameter (EF, CO, SV) is the aim of the study.

Comparison with what has been reported in the literature is not possible in this instance, because studies using the same anaesthetic protocol, in the same range of body weights have not yet been carried out. (Longobardi et al., 2000; Handa et al., 2002; Prunier et al., 2002; Watson et al., 2004; Hacker et al., 2005; Kawahara et al., 2005; Piegari et al., 2007; Popovic et al., 2007; Sung et al., 2009).

The main limitation of the proposed study is that it was performed in a single breed of rats. The reliability of regression equations in other breeds is not predictable. Also, a commonly available device was used without previous precise verification of the distance, time and heart rate measurement error. Finally, only one systolic-diastolic cycle was measured, which is close to routine clinical practice, but not ideal, and this could increase the measurement error.

## CONCLUSION

This study attempts to simulate the basic clinical or experimental echocardiography M-mode protocol in rats. Rat echocardiography itself could be used for heart disease screening in clinical rat patients and in rats before inclusion in an experimental study (we scan after pathological auscultation). Precise echocardiographic measurements in a study employing experimental rats could result in a precise description of models with zero-pain and good clinical relevance. We have established reliable regression equations for the calculation of basic echocardiographic parameters to assess expected normal values in rats with arbitrary weights.

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