



## Echocardiographic evaluation including tissue Doppler imaging in New Zealand white rabbits sedated with ketamine and midazolam <sup>☆</sup>

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Accepted 26 February 2008

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### Abstract

Limited data are available on the use of more recent echocardiographic parameters in the rabbit. Echocardiographic examination, including conventional echocardiography and tissue Doppler imaging (TDI), was performed on 26 male New Zealand white rabbits under ketamine–midazolam sedation. Particular emphasis was placed on the more recent systolic and diastolic parameters, such as myocardial performance index (Tei index) and mitral annular motion (from septal and lateral sides of the left ventricle) obtained using pulsed TDI.

Parameters that assessed systolic and diastolic function (fractional shortening, Tei index, and maximal mitral *E*- and *A*-wave velocities) were comparable to those reported in the literature for rabbits in the awake state. The less cardiodepressive anaesthetic protocol could offer a good alternative in performing echocardiographic evaluation whenever such caution is necessary. TDI is feasible in healthy rabbits and potentially suitable for the investigation of left ventricle systolic and diastolic function.

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**Keywords:** Doppler echocardiography; Tissue Doppler Imaging; Reference value; Rabbit; Anaesthesia

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### Introduction

Cardiac disease has been described in pet rabbits (Martin et al., 1987) and the species is widely used in cardiovascular research (Bras-Silva et al., 2006; Lange et al., 2006; Barraud et al., 2007). Echocardiography is a useful non-invasive method for the *in vivo* evaluation of ventricular dimensions and performance in experimental and clinical settings.

Doppler echocardiography provides useful additional information on cardiac conditions in both humans and small animals. Parameters obtained with tissue Doppler

imaging (TDI) have been shown to be more independent of pre- and after-load than classic haemodynamic Doppler measurements and can be used to quantify regional myocardial function accurately and more objectively (Sohn et al., 1997; Firstenberg et al., 2001; Nagueh et al., 2001). Pulsed TDI of the mitral annulus and myocardial wall has been suggested as a means to assess systolic and diastolic left ventricular (LV) function in human and veterinary medicine (Oki et al., 1999; Chetboul et al., 2005; Teshima et al., 2005; Chetboul et al., 2006; O'Sullivan et al., 2007). The Tei index, a new parameter to assess myocardial performance, has been proposed for the assessment of global cardiac performance (systolic and diastolic function) in a wide variety of congenital and acquired cardiac abnormalities (Dujardin et al., 1998; Bruch et al., 2002; Haque et al., 2002; Harjai et al., 2002; Gaibazzi et al., 2005; Dyer et al., 2006).

<sup>☆</sup> Presented in part at the 17th European College of Veterinary Internal Medicine Companion Animal Congress, Budapest, September, 2007.

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Reference values for various M-mode, flow Doppler and tissue Doppler echocardiographic parameters have been reported in rabbits in the conscious state as well as during different anaesthetic combinations (Fontes-Sousa et al., 2006; Stypmann et al., 2007). Nevertheless, examination of the awake rabbit is more difficult, more time-consuming, and needs special training, especially with research animals or animals less accustomed to handling. Anaesthesia can be used but might affect cardiac function, and the extent will depend on the type of anaesthesia (Schaefer et al., 2005). It is therefore important to know the effect of standardised sedation protocols on echocardiographic parameters. Recent studies have used ketamine- $\alpha_2$  agonist combinations to perform echocardiography in rabbits (Fontes-Sousa et al., 2006; Stypmann et al., 2007) but the major obstacle with this combination is its potential for cardiac and respiratory depression (Sanford and Colby, 1980). As an alternative, ketamine in combination with midazolam, a short-acting benzodiazepine, has been described for chemical restraint in rabbits associated with minimal cardiorespiratory depression (Dupras et al., 2001).

The purpose of the present study was to determine reference values for echocardiographic M-mode, Doppler, and pulsed TDI measurements in clinically healthy New Zealand white rabbits sedated with ketamine and midazolam.

## Materials and methods

The study was performed according to the Portuguese Law for Animal Welfare. The investigation conformed to the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Twenty-six young adult healthy male New Zealand white rabbits (16–20 weeks old and weighing  $2.3 \pm 0.4$  kg) were used. Rabbits were healthy and free from any sign of cardiovascular or respiratory tract disease on the basis of a physical examination that included careful thoracic auscultation and normal echocardiogram. The animals were housed in stainless steel cages in a controlled environment, at temperatures of 20–25 °C, with a 12:12 h light dark cycle, and were fed with a standard pellet diet and water ad libitum. The weight of each rabbit was recorded prior to anaesthesia.

A combination of ketamine–hydrochloride (20 mg/kg; Imalgene 1000, Merial) and midazolam (2 mg/kg; Midazolam APS, Farma-APS) was administered SC to each rabbit to minimise defensive movements and facilitate complete echocardiographic examination. Typically, the rabbits were completely immobilised within 5–10 min.

Echocardiography was carried out under light anaesthesia and spontaneous respiration, using a GE Vivid 7 system (GE VingMed) equipped with tissue Doppler technology. The standard phased-array, variable-frequency (3.5–6.9 MHz) transducer was used for two-dimensional, Doppler, and TDI recordings. Recordings were made under continuous ECG monitoring (lead II) by fixing the electrodes on the limbs at a sweep speed of 100 and 200 mm/s for off-line analysis. All echocardiographic acquisitions were made in sinus rhythm.

Rabbits were placed in right or left lateral recumbency to obtain right and left parasternal views, respectively, over a gap in the tabletop through which the ultrasound probe was brought from below and placed on a shaved area on the anterior aspect of the lower portion of the thoracic wall. Echocardiographic measurements were obtained from standard views (Thomas et al., 1993).

From the right parasternal short-axis view, two-dimensional guided M-mode tracings were made just below the mitral valve at the level of the papillary muscles for measurements of the interventricular septum (IVS), left ventricular internal diameter (LVID), and left ventricular free wall (LVFW) in diastole and systole. The right parasternal long-axis view with two-dimensional guided M-mode was used for the measurements of the E-point-to-septal separation interval in the plane of mitral valves. In this same view, the aortic and left atrial diameters were evaluated at the level of the aortic valve. These measurements were obtained applying the leading-edge method of the American Society of Echocardiography (Sahn et al., 1978). Left ventricular ejection fraction was calculated by use of the cube method according to the formula:  $\text{ejection fraction} = [(LVIDd^3 - LVIDs^3)/LVIDd^3] \times 100$ .

Doppler examinations were performed according to protocols established for dogs and cats (Gaber, 1991). Heart rate was calculated directly from the pulsed Doppler tracings. Variables recorded for each rabbit included maximal pulmonary artery and aortic outflow velocity, namely the maximal pulmonary outflow velocity ( $PA_{\max}$ ) and maximal aortic outflow velocity ( $AO_{\max}$ ), maximal E- and A-wave velocities, E:A ratio, isovolumetric relaxation time (IVRT), isovolumetric contraction time (IVCT), left ventricle ejection time (LVET) and Tei index.

The velocities were recorded as the maximal value on the outer edge of the peak velocity spectrum. Pulmonary artery flow velocity was determined by use of pulsed Doppler from the right parasternal short-axis view. The velocities of aortic flow and mitral flow-peak early diastolic wave (E) and peak atrial contraction wave (A) were recorded via pulsed Doppler from the

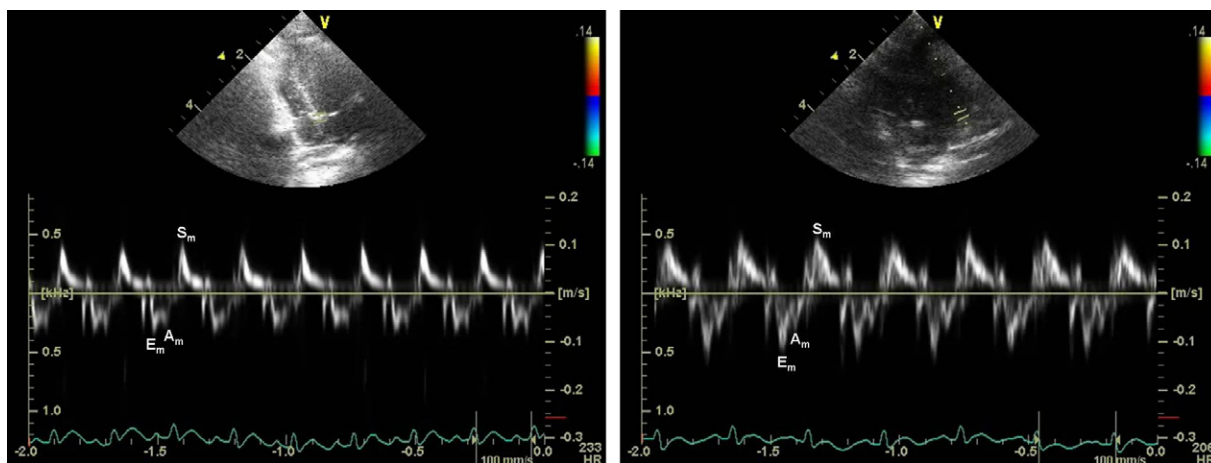


Fig. 1. The velocity profiles ( $E_m$ ,  $A_m$ ,  $S_m$ ) obtained from pulsed tissue Doppler imaging of septal (left) and lateral (right) mitral annulus in rabbits. The values of  $E_m$  peak velocities are significantly different between septal (lower values) and lateral side (higher values) of the mitral annulus.  $E_m$ : the peak early diastolic velocity.  $A_m$ : the peak atrial diastolic velocity.  $S_m$ : the peak systolic velocity.

left parasternal apical 5- and 4-chamber views. Mitral inflow velocity pattern was recorded with the sample volume between the tips of the leaflets. In the great vessels, the sample volume was positioned in the centre of the vessel, just beyond the valve leaflets, and colour Doppler was used to help align the cursor parallel to blood flow. Alignment was maximised in the two-dimensional view and no angle of correction was used.

IVRT was measured as the time interval between end of aortic outflow and onset of the mitral inflow by pulsed Doppler. IVCT was measured as the time interval between the end of mitral inflow and onset of aortic outflow by pulsed Doppler. The Tei index was calculated as described,  $Tei = (IVCT + IVRT)/LVET$  (Tei et al., 1995).

TDI was performed from the left parasternal apical 4-chamber view as previously described (Nagueh et al., 2001; Gan et al., 2004). In brief, the mitral annular motion was measured from the septal and lateral (free wall) side with pulsed TDI. Colour TDI was used to aid in sample volume placement, and the cursor was aligned as parallel as possible to the longitudinal axis of LV wall motion. Gain and filter settings were adjusted to eliminate background noise and to allow the recording of clear tissue signals. Measurements included peak early diastolic ( $E_m$ ), late diastolic ( $A_m$ ) and systolic ( $S_m$ ) mitral annular velocities (Fig. 1), with calculation of  $E_m:A_m$  and  $E:S_m$  ratios.

All data were collected by use of a trackball-driven cursor and ultrasound system software. The measured beats were selected on the basis of quality of the recording and presence of a regular cardiac rhythm. For each parameter the mean of three representative cardiac cycles was recorded. From these means, the overall mean, standard deviation (SD), and range for all variables measured in all rabbits were calculated. All images were stored digitally on optical discs and analysed retrospectively. The measurements were performed offline using dedicated software (EchoPAC 7).

### Statistical analysis

The statistical analysis was performed using the software SPSS for Windows, 15.0. Mean values, SD, maximum and minimum values (range) and percentiles for the echocardiographic parameters were computed. Pearson correlation coefficients ( $r$ ) were used to study the association between rabbit bodyweights and heart rates and their respective mean M-mode and Doppler echocardiographic measurements. The  $P$ -level for statistical significance was set at 0.05.

### Results

All 32 echocardiographic measurements (M-mode, 2D, Doppler echocardiography and TDI) were easily recorded in all rabbits in order to obtain reference values for the

breed when sedated with ketamine–midazolam. No animal died during or after the examination. Mean bodyweight was 2.2 kg (SD = 0.4, range 1.9–3.5 kg). Mean ( $\pm$ SD) heart rate was  $262 \pm 37$  bpm and remained stable throughout the examination. Recording was typically completed approximately 20 min after administration of ketamine and midazolam.  $A$ -wave was usually superimposed to  $E$ -wave due to the elevated heart rate, but it was possible to distinguish between the peak velocity of  $E$ - and  $A$ -wave with normal or higher frame rates (Fig. 2).

Tables 1 and 2 summarise the results of the two-dimensional and M-mode measurements, Doppler echocardiography including conventional Doppler and TDI. We found that heart rate correlated with few echocardiographic parameters. A weak significant negative correlation was found between heart rate and LVIDs ( $r = 0.50$ ,  $P = 0.01$ ). Weak positive correlations were found between heart

Table 1

Values for two-dimensional and M-mode echocardiographic variables in 26 male New Zealand white rabbits sedated with a combination of ketamine and midazolam

Parameter	Mean $\pm$ SD	5th percentile	95th percentile
IVS <sub>d</sub> (mm)	2.65 $\pm$ 0.31	2.23	3.20
IVS <sub>s</sub> (mm)	3.63 $\pm$ 0.34	2.97	4.13
LVID <sub>d</sub> (mm)	13.51 $\pm$ 1.05	11.97	15.23
LVID <sub>s</sub> (mm)	8.64 $\pm$ 0.82	7.37	10.00
LVFW <sub>d</sub> (mm)	2.25 $\pm$ 0.29	1.90	2.77
LVFW <sub>s</sub> (mm)	3.15 $\pm$ 0.38	2.60	3.93
FS (%)	36.01 $\pm$ 4.31	31.18	42.83
EF (%)	69.58 $\pm$ 5.33	62.99	77.73
Ao (mm)	6.57 $\pm$ 0.46	5.87	7.43
LA (mm)	7.49 $\pm$ 1.14	5.90	9.50
LA:Ao	1.15 $\pm$ 0.19	0.82	1.43
EPSS (mm)	1.41 $\pm$ 0.25	1.13	1.83

SD, standard deviation; IVS<sub>d</sub> and IVS<sub>s</sub>, thickness of the interventricular septum in diastole and systole, respectively; LVID<sub>d</sub> and LVID<sub>s</sub>, left ventricular internal diameter in diastole and systole, respectively; LVFW<sub>d</sub> and LVFW<sub>s</sub>, thickness of the left ventricular free wall in diastole and systole; FS, fractional shortening; EF, ejection fraction; Ao, aorta diameter; LA, left atrial diameter; and EPSS,  $E$ -point to septal separation.

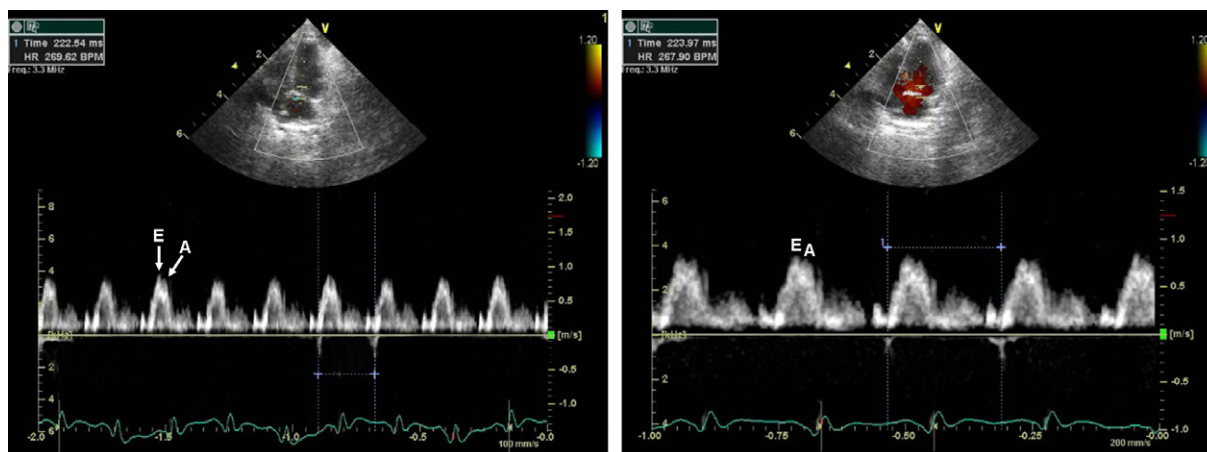


Fig. 2. Pulsed Doppler recordings of left ventricle inflow depicting the peak velocity of  $E$ - and  $A$ -waves, obtained at normal (left) and higher (right) sweep speeds from a rabbit with a heart rate above 250 bpm.

Table 2

Doppler echocardiographic measurements including tissue Doppler imaging (TDI) and calculated indices in 26 male New Zealand white rabbits sedated with a combination of ketamine and midazolam

Parameter	Mean $\pm$ SD	5th percentile	95th percentile
Doppler HR (bpm)	262.77 $\pm$ 37.17	213.01	329.03
Ao <sub>max</sub> (m/s)	0.86 $\pm$ 0.12	0.67	1.08
PA <sub>max</sub> (m/s)	0.78 $\pm$ 0.12	0.61	0.98
Mitral <i>E</i> (m/s)	0.78 $\pm$ 0.15	0.60	1.05
Mitral <i>A</i> (m/s)	0.55 $\pm$ 0.11	0.42	0.76
Mitral <i>E:A</i>	1.44 $\pm$ 0.16	1.26	1.65
IVRT (ms)	31.42 $\pm$ 6.19	23.77	39.94
IVCT (ms)	25.00 $\pm$ 3.68	19.02	30.43
LVET (ms)	95.72 $\pm$ 10.21	79.89	112.22
Tei index	0.60 $\pm$ 0.10	0.48	0.76
S <sub>m</sub> LW (m/s)	0.11 $\pm$ 0.02	0.08	0.14
E <sub>m</sub> LW (m/s)	0.16 $\pm$ 0.05	0.09	0.25
A <sub>m</sub> LW (m/s)	0.09 $\pm$ 0.03	0.06	0.13
E <sub>m</sub> :A <sub>m</sub> LW	1.83 $\pm$ 0.43	1.34	2.61
E:E <sub>m</sub> LW	5.24 $\pm$ 1.55	3.43	8.03
S <sub>m</sub> septal (m/s)	0.10 $\pm$ 0.02	0.07	0.12
E <sub>m</sub> septal (m/s)	0.11 $\pm$ 0.04	0.07	0.19
A <sub>m</sub> septal (m/s)	0.08 $\pm$ 0.02	0.04	0.12
E <sub>m</sub> :A <sub>m</sub> septal	1.55 $\pm$ 0.44	0.65	2.22
E:E <sub>m</sub> septal	7.75 $\pm$ 2.69	4.17	12.24

SD, standard deviation; HR, heart rate; Ao<sub>max</sub>, maximal aortic outflow velocity; PA<sub>max</sub>, maximal pulmonary outflow velocity; Mitral *E*, maximal mitral *E*-wave velocity; Mitral *A*, maximal mitral *A*-wave velocity; IVRT, isovolumetric relaxation time; IVCT, isovolumetric contraction time; LVET, left ventricle ejection time; S<sub>m</sub> LW, peak systolic mitral annular velocity from left wall; E<sub>m</sub> LW, peak early diastolic mitral annular velocity from left wall; A<sub>m</sub> LW, late early diastolic mitral annular velocity from left wall; S<sub>m</sub> septal, peak systolic velocity of septal mitral annulus; E<sub>m</sub> septal, peak early diastolic velocity of septal mitral annulus; and A<sub>m</sub> septal, late early diastolic velocity of septal mitral annulus.

rate and E<sub>m</sub> LW ( $r = 0.42$ ,  $P = 0.03$ ) and E<sub>m</sub>:A<sub>m</sub> LW ( $r = 0.50$ ,  $P = 0.01$ ). The bodyweight correlated weakly positive with Ao ( $r = 0.52$ ,  $P < 0.01$ ) and weakly negative with E<sub>m</sub>:A<sub>m</sub> septal ( $r = 0.44$ ,  $P = 0.03$ ).

## Discussion

Rabbits are an important model for cardiovascular research, mainly as they are small and relatively inexpensive but large enough to allow physiological experiments (Muders and Elsner, 2000). There are also various similarities between human and rabbit myocardium including a predominance of the  $\beta$ -myosin heavy-chain isoform, a positive force–frequency relationship and excitation–contraction coupling processes (Kavinsky et al., 1984; Ezzaher et al., 1992; Hasenfuss, 1998).

A complete Doppler echocardiographic examination including TDI evaluation was undertaken in rabbits anaesthetised with ketamine and midazolam. This anaesthetic combination yielded good immobilisation and allowed the ultrasonographer to obtain adequate 2D, M-mode, flow Doppler and TDI images for quantitative measurements. The combination is relatively common in research as well as in veterinary practice. In one study using this anaesthesia, some physiological and blood parameters were

reduced (mean arterial pressure, CO<sub>2</sub> arterial pressure) or not affected (O<sub>2</sub> arterial pressure) relative to ketamine–midazolam–xylazine and tiletamine–zolazepam–xylazine anaesthesia (Dupras et al., 2001).

The mean heart rate observed in the present study was slightly higher than the range of mean heart rates previously reported for conscious rabbits (180–250 bpm) (Mariano et al., 1996; Gil et al., 2004), and considerably higher when compared with rabbits anaesthetised with ketamine– $\alpha_2$  agonists (medetomidine or xylazine) (Fontes-Sousa et al., 2006; Stypmann et al., 2007). Our results are in accordance with a previous study using rabbits, which found that ketamine–midazolam produced the highest heart rate compared with other anaesthetic combinations (Dupras et al., 2001). In fact, although these agents cause minimal cardiorespiratory depression, it has also been reported that they may lead to increased heart rate in humans (Marlow et al., 1991).

Some echocardiographic parameters are particularly sensitive to high heart rates, e.g., mitral *E*- and *A*-wave that fuse and may not be distinguishable. Nevertheless, the use of high-speed tracing (100–200 m/s) allowed the assessment of *E*- and *A*-wave peak flow velocities, even at elevated heart rates, but deceleration time of the *E*-wave could not be measured.

In recent years, TDI has emerged as a new modality that is less affected by loading conditions and so provides a strong complementary role in the assessment of diastolic function (Leite-Moreira, 2006). In the present study, mitral annulus velocity obtained from the septal and lateral (free wall) side with pulsed TDI was markedly higher than the values observed in awake rabbits or those anaesthetised with ketamine–xylazine (Stypmann et al., 2007). This higher mitral annulus velocity could be explained by sympathetic stimulation induced by ketamine (positive chronotropic and inotropic effects) and the minimal cardiovascular effects associated with midazolam anaesthesia (Dupras et al., 2001).

The ratio obtained between transmitral *E* velocity and annular E<sub>m</sub>, E:E<sub>m</sub> ratio, has been reported to be an accurate index of the level of filling pressure of the assessed ventricular chamber. In previous studies in humans this ratio had a strong correlation with pulmonary capillary wedge pressure (PCWP) and LV diastolic pressure (LVDP) (Nagueh et al., 1999; Ommen et al., 2000). In small animal medicine, it has been reported that an E:E<sub>m</sub> value  $>9.1$  indicated a mean left atrial pressure  $>20$  mmHg in dogs with experimentally induced acute mitral regurgitation (Oyama et al., 2004).

Some potential limitations of the current study deserve attention, since we only used healthy anaesthetised rabbits. Firstly, the work only partially allowed us to assess the influence of the specific sedation used on the various echocardiographic parameters, since the same rabbits had not been examined in the conscious state. Nevertheless, some of the systolic and diastolic parameters were similar to those reported previously in the conscious state (fractional

shortening, Tei index, and maximal mitral *E*- and *A*-wave velocities) (Stypmann et al., 2007). Secondly, the work did not allow an assessment of the efficacy of the newer echocardiographic parameters, such as Tei index and TDI, or possible superiority above conventional parameters for detecting myocardial disease. This will have to be demonstrated in future studies in rabbits with induced or spontaneous cardiomyopathies.

Finally, we did not evaluate intra-operator variability. Poor repeatability has been reported in the acquisition of the velocities from the long-axis posterior wall and inter-ventricular septum using pulsed TDI analysis (Simpson et al., 2007). Another study showed that the intra-examination variability was better under anaesthetised conditions (Chetboul et al., 2004), which was attributed to perfect immobility of the animal that improved repeatability of TDI measurements.

## Conclusions

Echocardiographic reference values for New Zealand white rabbits anaesthetised with ketamine–midazolam are presented providing reference values for future studies. Emphasis was given to more recent indices that simultaneously reflect systolic and diastolic cardiac function, such as the Tei index derived from pulsed Doppler echocardiography and the pulsed TDI of the mitral annulus. Most of the results were comparable to those found in non-anaesthetised rabbits, and thus ketamine–midazolam anaesthesia may offer a good alternative when sedation is necessary.

## Conflict of interest statement

There are no financial or other relations that could lead to a conflict of interest.

## Acknowledgments

This work was supported by the Portuguese Foundation for Science and Technology (No. POCI/SAU-FCT/60803/2004) through Cardiovascular R&D Unit (FCT nr. 51/94).

Ana Patrícia Fontes-Sousa is supported by a Grant from the Portuguese Foundation for Science and Technology (No. SFRH/BD/22590/2005). The authors would like to thank Pedro Leitão for technical support during echocardiographic examination.

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