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Demonstration of regional differences in equine ventricular myocardial velocity in normal 2-year-old Thoroughbreds with Doppler tissue imaging

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Summary

***Reasons for performing study*: Doppler tissue imaging (DTI) is a novel noninvasive method by which myocardial velocity can be assessed directly and it allows regional, rather than global, cardiac function to be evaluated.**

***Hypothesis*: That regional differences in myocardial velocities exist within the equine ventricle.**

***Objectives*: To develop a repeatable examination technique for DTI in horses, describe DTI findings in various regions of the normal equine ventricle, compare colour (CDTI) and spectral (SDTI) techniques of DTI, and document regional differences in myocardial velocity.**

***Methods*: Five regions of the ventricles (right ventricular wall, interventricular septum and left, right and caudal regions of the left ventricle) were evaluated using SDTI and CDTI in 20 clinically normal Thoroughbreds age 2 years. Individual repeatability of the method was determined by examination of one 6-year-old Thoroughbred on 6 occasions.**

***Results*: Three major movements were observed in the ventricular walls in systole, early diastole and late diastole. The interventricular septum had a complex pattern of movement. The left region of the left ventricle and interventricular septum had the most rapid movement. The individual repeatability of CDTI was poor, while in systole and early diastole, but not late diastole, SDTI produced repeatable estimates of maximal myocardial velocity. The different velocity estimates obtained with SDTI and CDTI are not interchangeable. Regional differences in the peak mean and maximal myocardial velocities were found in systole and early diastole ($P<0.05$), but were not identified in late diastole.**

***Conclusions*: The SDTI modality appears to produce the most repeatable data. There are regional differences in myocardial velocity within the equine ventricles for systole and early diastole.**

***Potential relevance*: DTI shows potential as a tool for studying regional myocardial movement both in clinical cases suspected of having myocardial dysfunction and in a research setting. In particular, SDTI offers potential as a direct and noninvasive means to study early diastolic function of the equine ventricles.**

Introduction

Doppler tissue imaging (DTI) is one of the more recent technological advances in echocardiography. It is a method by which the velocity of individual areas of the myocardium may be determined (Garcia *et al.* 1996; Gorcsan *et al.* 1996; Derumeaux *et al.* 1999). This modality is unique in that it provides a direct measure of intramural velocities during left ventricular relaxation and contraction (Sutherland *et al.* 1996) and it has the potential to objectively quantify regional, rather than global, left ventricular function (Katz *et al.* 1997). It is becoming established as a useful tool for the assessment of myocardial systolic and diastolic function (Bach *et al.* 1996). In man, DTI has primarily been applied in the diagnosis of regional wall motion abnormalities in patients with coronary artery disease and cardiomyopathies (Miyatake *et al.* 1995; Uematsu *et al.* 1995; Bach *et al.* 1996; Gorcsan *et al.* 1996; Sutherland *et al.* 1996; Katz *et al.* 1997; Oki *et al.* 1997; Derumeaux *et al.* 1999).

In conventional Doppler imaging, flow of relatively high velocity, corresponding to the velocities of the blood pool, is interrogated and low-velocity data are filtered out of the display to remove signals from cardiac structures. In DTI, lower velocity Doppler data generated from the myocardium are processed and imaged. Two DTI functions are available, each measuring different velocities; spectral DTI (SDTI) provides an estimate of the maximal velocity within a given region, whereas colour DTI (CDTI) produces a trace of the mean velocity within the sampled region of myocardium (Katz *et al.* 1997).

Regional differences in ventricular velocity have been documented in man and it was hypothesised that, similarly, regional differences would exist within the equine ventricle. The aims of this study were to develop a repeatable examination technique for DTI in horses, describe DTI findings in various regions of the equine ventricles, compare spectral (SDTI) and colour (CDTI) Doppler findings in normal horses, and determine the existence of regional differences in myocardial velocity within the equine ventricle.

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TABLE 1: Peak mean and maximal (± s.d.) myocardial velocities (m/sec) recorded with colour Doppler tissue imaging and spectral Doppler tissue imaging, respectively, in various regions of the ventricles in 20 Thoroughbreds age 2 years

	Late diastole		Systole		Early diastole	
Location	Peak mean	Maximal	Peak mean	Maximal	Peak mean	Maximal
RV	0.041 ± 0.015	0.082 ± 0.033	-0.057 ± 0.011	-0.105 ± 0.047	0.095 ± 0.020	0.102 ± 0.029
IVS	0.048 ± 0.036	0.061 ± 0.032	-0.057 ± 0.036	-0.093 ± 0.024	0.060 ± 0.018	0.171 ± 0.040
LLV	0.052 ± 0.019	0.082 ± 0.029	-0.054 ± 0.035	-0.096 ± 0.027	0.150 ± 0.052	0.173 ± 0.119
CLV	0.053 ± 0.034	0.051 ± 0.043	0.125 ± 0.178	0.099 ± 0.028	-0.140 ± 0.050	-0.160 ± 0.090
RLV	0.036 ± 0.037	0.134 ± 0.205	0.086 ± 0.170	0.129 ± 0.070	-0.160 ± 0.097	-0.220 ± 0.078

RV = right ventricle; IVS = interventricular septum; LLV = left region of left ventricle; CLV = caudal region of left ventricle; RLV = right region of left ventricle.

Materials and methods

Animals

Twenty Thoroughbreds age 2 years (11 entire males and 9 females), housed at a commercial training yard, were examined by one experienced ultrasonographer (C.M.M.) during 3 consecutive days. These horses were considered by their trainer to be healthy with no known history of cardiovascular disease. Cardiac auscultation was performed and horses with quiet (≤*Grade III*/*VI*), soft, blowing, localised murmurs without a precordial thrill were included in the study (Marr and Reef 1995). A 6-year-old Thoroughbred gelding with no audible murmurs was used in the individual repeatability study. This horse was examined by one experienced ultrasonographer (C.M.M.) 6 consecutive times at 2 h intervals on the same day.

Echocardiographic examination technique

All horses were examined in a quiet stable. Ultrasound coupling gel was applied to the left and right cardiac windows on the thorax. Echocardiographic examinations were performed with an ultrasonographic unit equipped with a 3.3 MHz phased array sector transducer set at an imaging depth of 28 cm with a 120° imaging angle (VingMed Sound System V)[1]. The pulse repetition frequency, frame rate and sampling depth and width were selected to produce an aliasing speed of 0.24 m/sec in 2-dimensional colour images, and a sampling site of 10 mm^2 and velocity range of ± 0.2 m/sec were selected in pulsed wave spectral mode. These machine settings were constant between examinations. An integrated unit was used to record the electrocardiograph (ECG) throughout the examinations. Each examination was recorded in real time on SVHS videotape and digital images were stored for further analysis using an integrated imaging archiving and image analysis computer programme (EchoPAC)[1].

Three consecutives cardiac cycles of right and left parasternal short-axis echocardiograms at the chordal level were recorded with CDTI. Conventional 2-dimensional right and left parasternal short-axis echocardiograms at the chordal level were used to guide sample placement for SDTI, and spectra of at least 5 cardiac cycles were obtained by placing the sampling site in the following locations, taking care to avoid the papillary muscles:

- Portion of right ventricular wall at the centre of the image (Fig 1a).
- Central region of the interventricular septum (Fig 1b).
- Left region of left ventricular wall (Fig 2a).
- Caudal region of left ventricular wall (Fig 2b).
- Right region of left ventricular wall, taking care to avoid the interventricular septum (Fig 2c).

Echocardiographic image analysis

Image analysis was performed with an ultrasound image archiving and analysis system (EchoPAC)[1]. All image analyses were performed by one operator (M.F.S.). An integrated electronic calliper system was used to determine the maximal velocities from SDTI traces in systole, early diastole and late diastole. Three cardiac cycles were averaged (except where subsequent review of the images revealed that only 2 cycles were of sufficient quality). An integrated CDTI image analysis function was used to create traces of mean velocity throughout the cardiac cycle. Regions corresponding to those described for SDTI were interrogated and peak mean velocities in systole, early diastole and late diastole were recorded; values were obtained by averaging 2–3 cardiac cycles.

Statistical analysis

Mean ± s.d. peak mean and maximal velocities were calculated for each wave of contraction within each ventricular region. To assess individual repeatability, coefficients of variation were determined for each wave, region and technique. Limits of agreement, bias and 95% confidence intervals between the maximal and peak mean velocities in each region in systole and early and late diastole were determined using the approach described by Bland and Altman (1986). Regional differences in myocardial velocity were identified using ANOVA for repeated measures and Bonferroni *post hoc* tests to compare the maximal velocity of the systolic wave within the 5 ventricular regions. A similar approach was used to determine regional differences in the maximal velocities within the early and late diastolic waves and compare regional peak mean velocities within each of the 3 waves (SPSS version 11.5.1)[2]. The level of significance was set at $P<0.05$.

Results

Fifteen horses had left systolic flow murmurs of *grade I* or *II*/*VI*; 2 had right-sided murmurs consistent with tricuspid regurgitation, of which one was *grade II*/*VI* and the other *III*/*VI*. Four horses had *grade I* or *II*/*VI* early diastolic squeaks, of which 2 were left-sided and 2 right-sided. Six horses had more than one murmur.

Three major myocardial movements were identified with peaks in systole, early diastole and late diastole in the right ventricular wall and all 3 regions of the left ventricular wall, in all horses examined (Fig 3). In the right ventricle and left region of the left ventricle, systolic movement was consistently opposite in

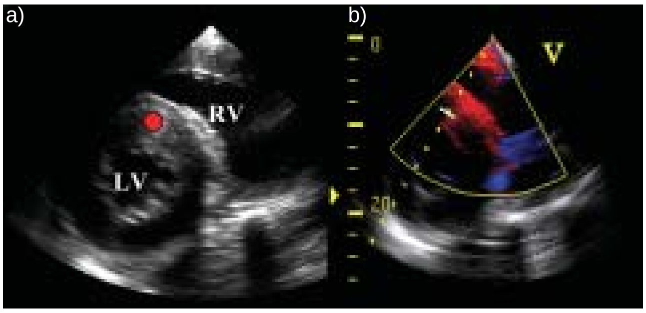

Fig 1: Two-dimensional and colour short-axis right parasternal images at the chordal level showing sampling sites for spectral (red dots) and colour (yellow bar) Doppler tissue imaging in a) the right ventricle (RV) and b) interventricular septum. LV = left ventricle.

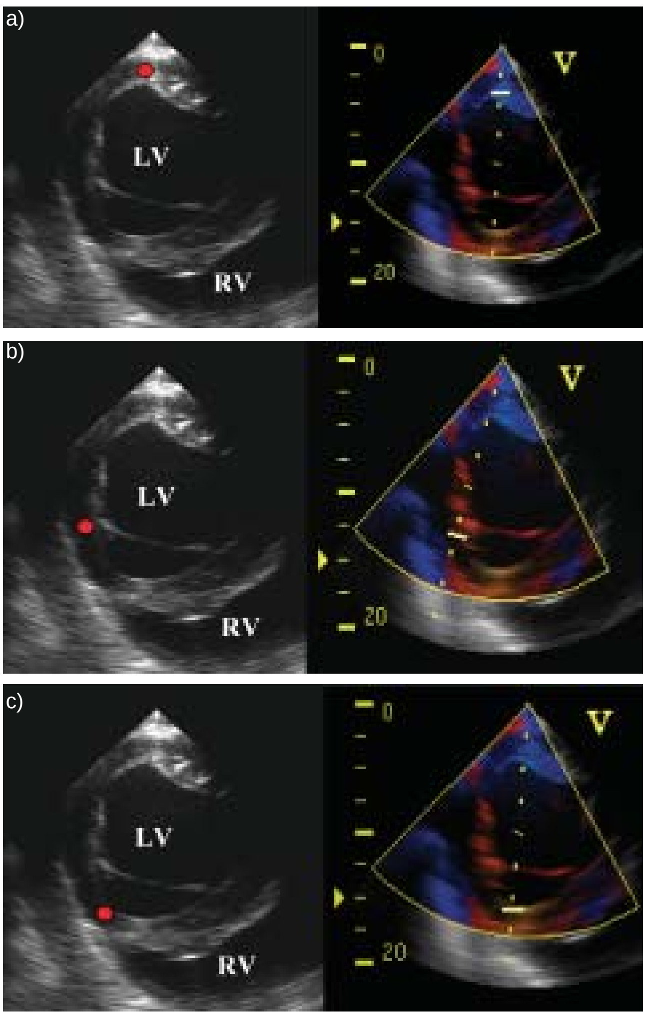

Fig 2: Two-dimensional and colour short-axis left parasternal images at the chordal level showing sampling sites for spectral (red dots) and colour (yellow bar) Doppler tissue imaging in a) left ventricular wall, b) caudal ventricular wall and c) right ventricular wall. LV = left ventricle; RV = right ventricle.

direction to diastolic movement. However, in the caudal and right regions of the left ventricle, early diastolic movement was opposite in direction to systolic movement, while the direction of movement in late diastole was not consistent (Table 1). The movement of the interventricular septum was more complex; movement associated with contraction occurred mainly in early

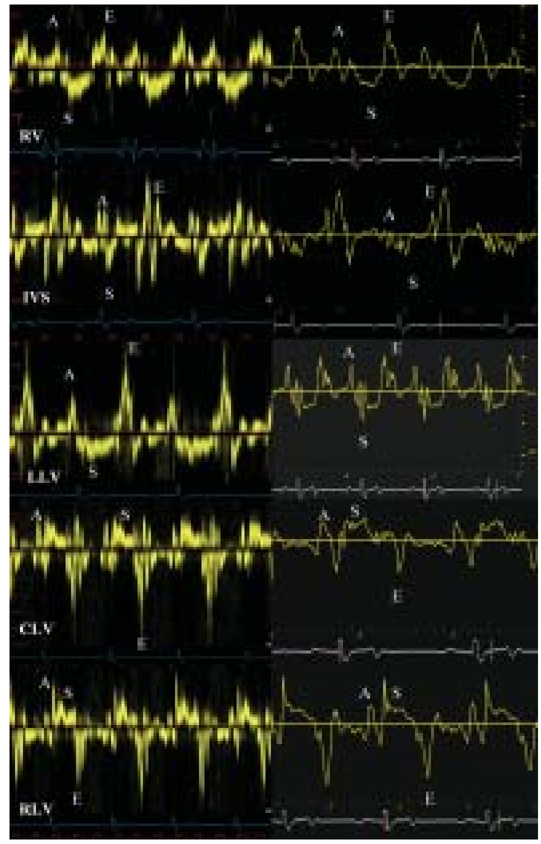

Fig 3: Spectral (left) and colour (right) Doppler tissue imaging traces of myocardial movement in the right ventricle (RV), interventricular septum (IVS) and the left (LLV), caudal (CLV) and right (RLV) regions of the left ventricle. The systolic (S), early (E) and late (A) diastolic waves are indicated. An ECG is superimposed for timing.

systole, in early diastole there were deflections both towards and away from the transducer, and in late diastole the direction of movement was not consistent, occurring in either direction in individual animals (Fig 3).

During the echocardiographic examination, mean ± s.d. heart rate was 37.5 ± 3 beats/min; median 36.9 beats/min; minimum 33 beats/min; and maximum 44 beats/min. The maximal and peak mean velocities in systole, early diastole and late diastole for each region are listed in Table 1. Generally, the highest maximal and peak mean velocities were recorded in early diastole and the lowest in late diastole, except in the right portion of the left ventricle, where the highest and lowest maximal velocities occurred in systole (Fig 3; Table 1).

The individual repeatability of the CDTI technique was poor, with coefficients of variation varying widely (Table 2). The individual repeatability of the SDTI technique was adequate, with coefficients of variation in the range of 10–15% in systole and early diastole in most regions, except in the case of maximal myocardial velocity in the caudal left ventricle in early diastole and peak mean velocity in the right region of the left ventricle in systole (Table 2).

There was no significant bias between the peak mean (CDTI) and maximal (SDTI) velocity measurements in the right ventricle, interventricular septum and left and caudal regions of the left ventricle during early diastole, in the caudal and right regions of the left ventricle in late diastole, and in the caudal region of the left

TABLE 2: Coefficient of variation for colour Doppler tissue imaging (CDTI) and spectral Doppler tissue imaging (SDTI) estimates of myocardial velocity in 5 regions of the ventricles during late diastole, systole and early diastole from one horse examined on 6 occasions

		Coefficient of variation		
Technique	Location	Late diastole	Systole	Early diastole
SDTI	RV	21.99	10.27	14.98
	IVS	27.57	12.73	12.21
	LLV	24.19	11.85	13.18
	CLV	32.67	21.15	11.95
	RLV	26.83	10.22	20.07
CDTI	RV	47.32	16.40	20.21
	IVS	59.14	12.43	20.31
	LLV	33.71	16.48	26.88
	CLV	195.77	34.68	24.60
	RLV	33.50	16.48	17.71

RV = right ventricle; IVS = interventricular septum; LLV = left region of left ventricle; CLV = caudal region of left ventricle; RLV = right region of left ventricle.

TABLE 3: Limits of agreement between spectral and colour Doppler tissue imaging estimates of myocardial velocity in 5 regions of the ventricles

	Late diastole			Systole			Early diastole		
	AB	LB	UB	AB	LB§	UB§	AB	LB	UB
RV	-0.040†	-0.103	0.023	0.049†	-0.042	0.140	-0.008	-0.073	0.057
IVS	-0.013	-0.101	0.074	0.037†	-0.043	0.117	-0.012	-0.200	0.176
LLV	-0.031†	-0.081	0.019	0.042†	-0.053	0.137	-0.024	-0.263	0.216
CLV	0.001	-0.118	0.121	0.026	-0.336	0.388	0.029	-0.166	0.224
RLV	-0.092	-0.522	0.338	-0.042*	-0.170	0.085	0.057*	-0.155	0.269

AB = Average bias; LB = lower bound; UB = upper bound; §mean ± 1.96 s.d.; †bias is significant at P≤0.001; *bias is significant at P≤0.05; RV = right ventricle; IVS = interventricular septum; LLV = left region of left ventricle; CLV = caudal region of left ventricle; RLV = right region of left ventricle.

ventricle in systole (Table 3). Significant biological bias was present at several locations (Table 3).

Regional differences in both maximal and peak mean myocardial velocities were detected between the right ventricle and the caudal and right regions of the left ventricle in systole and early diastole (P<0.001) and between the interventricular septum and all, except the left, regions of the left ventricle in systole and early diastole (P<0.001). Additionally, the maximal velocity in the caudal and right regions of the left ventricle were different in systole (P = 0.03). No regional differences in myocardial velocity were detected in late diastole.

Discussion

Myocardial movement occurs in 3 planes; longitudinal, rotational and circumferential shortening (Katz *et al.* 1997). In early diastole, left ventricular relaxation has 2 phases, elastic recoil after systole (passive relaxation) and active relaxation, mediated by calcium ion entry into the sarcoplasmic reticulum of myocytes in early diastole (Ohte *et al.* 1998). During late diastole, the atria contract to actively fill the ventricles. The longitudinal and lateral movements of the left ventricular wall in man have been studied with DTI using sampling sites based on examination of the ventricle in an apical to basilar plane (Donovan *et al.* 1995; Uematsu *et al.* 1995; Hada *et al.* 1996; Derumeaux *et al.* 1997; Galiuto *et al.* 1998). In the horse, it is anatomically impossible to obtain apical images, and the parasternal approach that was applied here is most likely to reflect lateral rather than longitudinal movement.

In this study, the imaging planes and 5 ventricular regions were chosen because they were easy to identify and, because they were located at the chordal level, they correspond to the planes used in 2-dimensional and M-mode echocardiography. Therefore, the 5 selected sample sites may not adequately represent either their local region or the ventricle as a whole. This study was concentrated on the evaluation of 3 major waves of movement. However, in both the SDTI and CDTI tracings, other waves of myocardial movement were present but not measured. In man, these other waves of movement can be explained by myocardial elasticity (Galiuto *et al.* 1998). Further work needs to be completed to characterise these smaller myocardial deflections, develop additional imaging planes and characterise the lateral, longitudinal and translational components of cardiac movement in the horse.

Each myocardial segment had a characteristic trace that was similar in both SDTI and CDTI traces and that could be easily recognised. The poor individual repeatability of the CDTI technique could be a major limitation in applying this technique in clinical practice and research studies, whereas the coefficients of variation of the SDTI technique in systole and early diastole were slightly higher than those achieved in some studies of 2-dimensional and M-mode echocardiography (Patteson *et al.* 1995; Kriz and Rose 2002), comparable with that of pulsed Doppler echocardiographic indices in horses (Young and Scott 1998). The caudal sampling site had poorest individual repeatability with SDTI, possibly because the angle of interrogation of this area was not parallel with the direction of movement. The individual repeatability of the SDTI measurements in late diastole was poor, suggesting that these imaging planes may not be appropriate for evaluation of movement during this phase of the cardiac cycle. Many additional factors influence the estimated velocity, including target velocity, target material, system receive gain and pulse train size (Weber *et al.* 1993). The mean velocity traces derived from CDTI images are not generated in real time; therefore, the quality of the traces could not be corrected at the time of the examination. Considerable computer storage capacity is required for CDTI. In this study, memory constraints created difficulties in recording more than 3 consecutive cardiac cycles. Where subsequent review demonstrated poor quality, averages were performed with only 2 measurements, these factors may have contributed to the disappointing individual repeatability observed with this technique. In this study, only one horse was used to provide data on individual repeatability. The purpose was to provide a guide to the minimal level of variation that might be expected with these DTI methods. However, more extensive studies on interobserver agreement, method repeatability and biological variability of this and other DTI techniques are needed.

There was considerable bias and inconsistent agreement between the CDTI and SDTI variables. This confirms that the techniques cannot be used interchangeably, as they provide estimates of different velocities. A spectral display of myocardial velocity against time is produced with SDTI, so that the maximal velocities within the sample site can be measured. Alternatively, with CDTI, mean velocities within multiple samples of myocardium are colour-coded and this information is superimposed on the conventional 2-dimensional images to provide a colour velocity map (Katz *et al.* 1997). The operator subsequently interrogates individual sites within the 2-dimensional images to produce a trace of mean myocardial velocity against time from which peak mean velocities within the specified sample site can be derived (Gorcsan *et al.* 1996).

In this study, regional differences in systolic and early diastolic myocardial velocities have been demonstrated in normal horses, as has previously been established in man (Donovan *et al.* 1995). In man, the posterior wall (which anatomically corresponds to the right and caudal regions of the left ventricular wall in the horse) moves most rapidly, while the interventricular septum moves most slowly (Donovan *et al.* 1995; Uematsu *et al.* 1995; Derumeaux *et al.* 1997). In the horse, the right and caudal regions of the left ventricle also moved faster than the other areas in all phases of the cardiac cycle. The systolic maximal and peak mean and the early diastolic peak mean velocities were lowest in the interventricular septum. Unlike findings in human studies (Galiuto *et al.* 1998), the equine interventricular septum seems to move at the same velocity as the other segments in late diastole. No regional differences were identified in the late diastolic movement; however, this phase represents active ventricular filling due to atria contraction and it is therefore likely that all the ventricular walls will deflect at a similar rate. The technical difficulties reflected in the poor individual repeatability of the late diastolic measurements may also have contributed to this result.

The current study confirms that DTI can be applied in equine subjects and confirms that there are regional differences between some ventricular myocardial velocities. It appears that SDTI can be used more easily and in a more repeatable manner than CDTI. The data may serve as a useful starting point for further development of the technique and its clinical application in horses. Possible applications in clinical cases include the identification of cardiomyopathy and as an adjunct to post exercise echocardiographic tests. Further studies examining the effects of cardiac disease and specific forms of valvular regurgitation are needed. The technique offers particular potential in a research setting, as a direct and noninvasive means of studying early diastolic function of the equine ventricles, since alternative means to study early diastolic function are currently limited.

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Manufacturers' addresses

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[2]Aspire Software International, Leesburg,Virginia, USA.

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