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## Left Ventricular Function After Prolonged Exercise in Equine Endurance Athletes

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**Background:** Prolonged exercise in human athletes is associated with transient impairment of left ventricular (LV) function, known as cardiac fatigue. Cardiac effects of prolonged exercise in horses remain unknown.

**Objectives:** To investigate the effects of prolonged exercise on LV systolic and diastolic function in horses.

**Animals:** Twenty-six horses competing in 120–160 km endurance rides.

**Methods:** Cross-sectional field study. Echocardiography was performed before and after rides, and the following morning, and included two-dimensional echocardiography, anatomical M-mode, pulsed-wave tissue Doppler imaging, and two-dimensional speckle tracking. Correlation between echocardiographic variables and cardiac troponin I was evaluated.

**Results:** Early diastolic myocardial velocities decreased significantly in longitudinal (baseline:  $-17.4 \pm 2.4$  cm/s; end of ride:  $-15.8 \pm 3.2$  cm/s ( $P = .013$ ); morning after:  $-15.4 \pm 3.0$  cm/s ( $P = .0033$ )) and radial directions ( $-32.8 \pm 3.4$  cm/s;  $-28.1 \pm 5.8$  cm/s ( $P < .001$ );  $-26.4 \pm 5.5$  cm/s ( $P < .001$ )). Early diastolic strain rates decreased significantly in longitudinal ( $1.58 \pm 0.27$  s $^{-1}$ ;  $1.45 \pm 0.26$  s $^{-1}$  ( $P = .036$ );  $1.41 \pm 0.25$  s $^{-1}$  ( $P = .013$ )) and circumferential directions ( $2.43 \pm 0.29$  s $^{-1}$ ;  $1.96 \pm 0.46$  s $^{-1}$  ( $P < .001$ );  $2.11 \pm 0.32$  s $^{-1}$  ( $P < .001$ )). Systolic variables showed ambiguous results. No correlations with serum cardiac troponin I concentrations were evident.

**Conclusions and Clinical Importance:** Prolonged exercise in horses is associated with impaired LV diastolic function. Reduced ventricular filling persisted for 7–21 hours despite normalization of biochemical indicators of hydration status, indicating that the observed changes were not entirely related to altered preload conditions. The clinical relevance of cardiac fatigue in horses remains uncertain.

**Key words:** Diastolic function; Echocardiography; Speckle tracking; Tissue Doppler imaging.

Studies have examined the cardiac effects of prolonged exercise in human athletes, and have shown evidence of a transient impairment of cardiac function after endurance exercise.<sup>1–4</sup> The echocardiographic findings are characterized by decreases in LV systolic and diastolic function, which have been interpreted as a sign of “cardiac fatigue”.<sup>5</sup> The majority of human studies agree on the presence of a diastolic dysfunction, whereas the results regarding LV systolic function after prolonged exercise are more inconsistent.<sup>6,7</sup> This is presumably because of methodological differences related to training status and exercise duration in the reported studies, as runners with lesser training mileage have greater changes in LV diastolic function after a marathon than runners

### Abbreviations:

2DE	two-dimensional echocardiography
2DST	two-dimensional speckle tracking
AMM	anatomical M-mode
cTnI	cardiac troponin I
$E_m/A_m$	ratio between the early diastolic and late diastolic radial myocardial velocity
LA	left atrium or left atrial
LAX-LA	long-axis 4-chamber view tilted for optimized view of the left atrium
LAX-LV	long-axis 4-chamber view of the left ventricle
LVFW	left ventricular free wall
LV	left ventricle or left ventricular
PW-TDI	pulsed-wave tissue Doppler imaging
ROI	region of interest
SAX-LV	short-axis view of the left ventricle

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with more training.<sup>8</sup> Furthermore, the results of a meta-analysis imply that systolic dysfunction will only occur after more than 6 hours of exercise, whereas diastolic dysfunction occurs at an earlier stage.<sup>9</sup> Endurance horses perform strenuous exercise for long periods of time (typically 8–12 hours in a 160 km ride) and could therefore be expected to sustain impaired cardiac function. The average completion rate in international endurance rides is 50%, and approximately 10% of the starting horses are eliminated for metabolic reasons.<sup>10</sup> Despite the immense physical strain of this discipline, the cardiac effects of endurance riding are largely unknown, with the exception of one study by Amory et al<sup>11</sup> indicating the presence of exercise-induced LV systolic dysfunction with significant decreases in stroke volume, ejection fraction, and fractional shortening.

Prolonged exercise is commonly associated with decreases in plasma volume and peripheral

redistribution of blood, leading to tachycardia and reduced venous return.<sup>12</sup> The interpretation of echocardiographic changes after prolonged exercise is therefore complicated by the confounding effects of altered heart rate and loading conditions. Two-dimensional speckle tracking (2DST) is a recently developed echocardiographic technique that allows quantitative assessment of myocardial deformation (strain) during contraction. While peak systolic strain is related to the traditional ejection phase indices (such as fractional shortening and ejection fraction) and is therefore largely load dependent, systolic strain rate has been shown to be a relatively load-independent measure of myocardial contractility.<sup>13,14</sup> This technique consequently provides an advantageous method for the assessment of myocardial performance after prolonged exercise and is frequently applied in studies of human endurance athletes.<sup>2,3,6,7</sup> The use of strain rate imaging has been validated in horses<sup>15-19</sup> and applied in equine stress echocardiography.<sup>20</sup> However, the technique has not yet been employed to assess the effects of prolonged exercise on equine cardiac function. The objective of this study was to investigate the effects of prolonged exercise on LV myocardial function in horses with conventional echocardiography, tissue Doppler imaging, and 2DST.

## Materials and Methods

### *Horses and Competitions*

Twenty-six Arabian horses (2 stallions, 11 mares, and 13 geldings, age  $11.1 \pm 2.5$  years, body weight  $423 \pm 31$  kg) were included in the study. Horses were recruited by convenience sampling among participants in three different international endurance competitions (Concours de Raid d'Endurance International) with comparable weather conditions and terrain between April and June 2012:

- Glimåkra (Sweden) CEI\*\*\* 160 km (n = 9)
- Gartow (Germany) CEI\*\*\* 160 km (n = 5) and CEI\*\*/CEIYRJ\*\* 120 km (n = 4)
- Nörten-Hardenberg (Germany) CEI\*\*\* 160 km (n = 1) and CEI\*\* 120 km (n = 7)

The number of horses recruited at each ride was limited by time constraints inherent to the study protocol. All horses were considered healthy after a veterinary inspection on the day before the ride. Data on timekeeping, speed, reasons for elimination, and distance completed before elimination were obtained from official records. A written informed consent was obtained from the owners. Permission to conduct the study was attained from national authorities where required (license number M 115-12/Dnr 31-3234-12). In addition, the examination protocol included electrocardiography and blood sampling as part of another study.<sup>21</sup> Measurements of cardiac troponin I (cTnI) concentration, hematocrit, and serum protein concentration have been published elsewhere.<sup>21</sup>

### *Echocardiography*

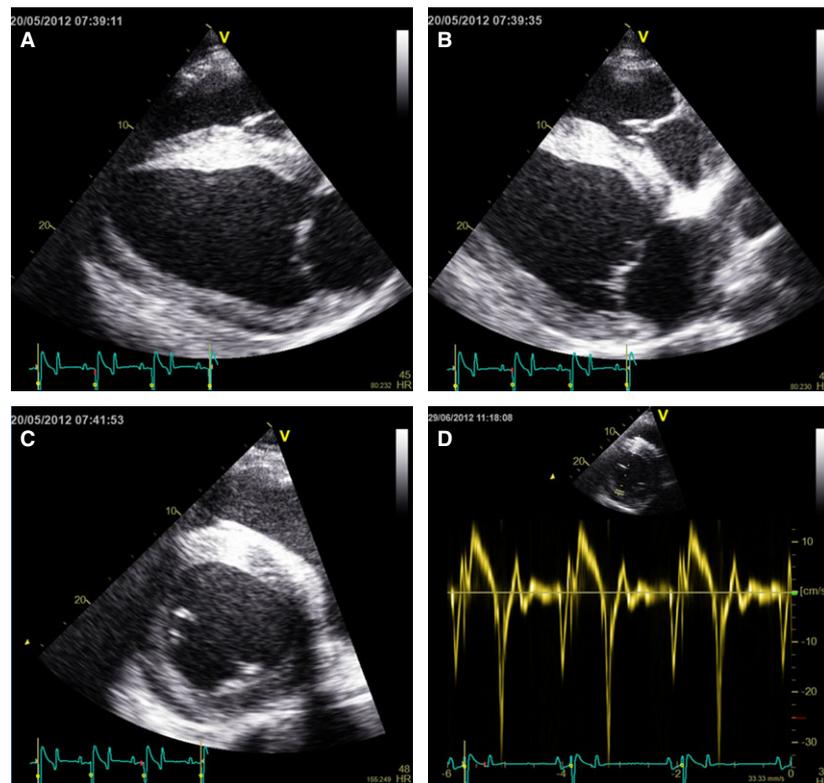
Echocardiography was performed by means of a portable ultrasound unit<sup>a</sup> with a phased array transducer<sup>b</sup> and simultaneous ECG recording. Horses were examined on three occasions: the day before the ride (baseline), as soon as possible after finishing the ride or after elimination, and the morning after the ride. The baseline

examination included cardiac auscultation, two-dimensional echocardiography (2DE), anatomical M-mode (AMM), color flow Doppler, pulsed-wave tissue Doppler imaging (PW-TDI), and 2DST. Subsequent examinations included only 2DE, AMM, PW-TDI, and 2DST. Cardiac murmurs of an intensity higher than 2 (on a scale of 1–6)<sup>22</sup> and regurgitating jets covering >30% of the luminal area in the receiving chamber (by the authors' standard presets for color gain, depth, and frequency) were considered to be exclusion criteria. The following right parasternal views were obtained in each examination (Fig 1): (1) long-axis 4-chamber view of the left ventricle (LAX-LV) with imaging depth of 26 cm and the sector angle reduced one step from its maximum width; (2) a similar view, slightly tilted for an optimized view of the left atrium (LAX-LA); (3) short-axis view of the left ventricle at chordal level (SAX-LV), where the sector angle was further reduced by one step and tilted on the screen to include as much of the myocardium as possible; and (4) a similar view, with PW-TDI applied to the left ventricular free wall (LVFW) to obtain the myocardial velocity profile (velocity scale  $\pm 49.5$  cm/s). Three cine loops of three consecutive cardiac cycles were obtained in each view and stored digitally for subsequent offline analysis. The frame rate was 52.2 frames/s in the LAX-LV view and 59.0 frames/s in the SAX-LV view.

### *Echocardiographic Measurements*

Measurements were performed offline, randomized, and blinded with a commercially available software package,<sup>c</sup> and values were reported as the average of three nonconsecutive cardiac cycles (one cycle from each cine loop). The RR interval of each cardiac cycle was measured and the instantaneous heart rate calculated as  $60,000/RR$ . A detailed description of measurement techniques and calculations is given in the Supporting information. Briefly, LA size and function were assessed in the LAX-LA view (Table S1).<sup>23</sup> One-dimensional indices of LV size and function were obtained by AMM applied to the SAX-LV view (Table S2).<sup>24</sup> Left ventricular internal volumes and systolic function were estimated in the LAX-LV view by single-plane modified Simpson's method (Table S3).<sup>20</sup> Three-dimensional (longitudinal, radial, and circumferential) LV myocardial wall motion was assessed by PW-TDI and 2DST. More specifically, radial wall motion velocity was obtained by PW-TDI in the basal LVFW (Table S4).<sup>19</sup> Estimates of longitudinal velocity, strain, and strain rate were obtained by 2DST in the LAX-LV view as described by DeCloedt et al<sup>17</sup> (Table S5), whereas radial and circumferential strain and strain rate were obtained in the SAX-LV view as described by Schwarzwald et al<sup>18</sup> (Table S6). This study utilized a newer version of the software<sup>c</sup> for 2DST analysis than previous studies. This software automatically reported longitudinal and circumferential strain as separate "layered" values of the endocardium, mid-myocardium, and epicardium, respectively. The strain values pertaining to the mid-myocardium is an average of the myocardial strain across the entire myocardial wall. In contrast, the strain values of the epi- and endocardium describe only the strain in the outermost and innermost layers of the myocardium, respectively.

The 2DST analysis assesses the myocardial function within an observer-defined region of interest (ROI). The software automatically divides the ROI into six segments and provides global values where the ROI is treated as one entity, as well as segmental values, which relate to the regional function. To reduce the number of reported values, only the global strain and strain rate values were reported, in addition to the average segmental value. However, as longitudinal velocity involves a base-apex gradient with basal segments moving faster than apical segments, it was considered irrational to report average segmental values of this variable. The longitudinal velocity was therefore only reported for the basal lateral segment, as this segment corresponded to the position of the



**Fig 1.** Right parasternal views. (A) Long-axis 4-chamber view of the left ventricle (LAX-LV); (B) Long-axis 4-chamber view tilted to optimize the view of left atrium (LAX-LA); (C) Short-axis view of the left ventricle at the level of the chordae tendineae (SAX-LV); (D) Radial myocardial velocity profile obtained by PW-TDI applied to the SAX-LV view. The imaging depth was 26 cm in all views.

ROI used for the PW-TDI measurements of the radial velocities in the LVFW.

### Blood Samples

Blood samples and echocardiographic examinations were obtained simultaneously. These results have previously been published, and revealed significant changes after exercise.<sup>21</sup> Cardiac troponin I concentration was determined by a high-sensitivity immunoassay<sup>d</sup> and included in this study as an indicator of myocardial damage. Hematocrit<sup>e</sup> and serum protein concentration<sup>f</sup> were included as indicators of the hydration status.

### Statistical Analyses

Pilot analyses by Student's *t*-test were used to draw comparisons between the results of horses in the 120 and 160 km rides, and between completing and eliminated horses. There were no systematic differences between the groups and the horses were therefore pooled in the subsequent statistical model. The effect of the ride on the echocardiographic variables was tested with a repeated measures mixed model ANOVA using Dunnett's test for post hoc comparisons with baseline values. Repeated measures were accounted for by including the examination time (Day) with an autoregressive covariance type 1 structure. The individual horse was included as random effect. Model control was performed by evaluating whether residuals were independent and normally distributed ( $0, \sigma^2$ ). Outliers were identified in the residual plots and excluded, providing this did not markedly change the results. The association between echocardiographic variables and cTnI was evaluated by linear regression. The level of significance was  $P < .05$ .

### Results

Seven horses completed a 120 km ride with an average speed of  $13.4 \pm 2.2$  km/h, and 10 horses completed 160 km with an average speed of  $14.7 \pm 2.1$  km/h. A total of 9 horses were eliminated during the rides, after completing an average of  $78 \pm 24$  km (median 90 km, range 30–100 km). When eliminated during the early stages of the competition, horses were permitted to leave the venue on the same day—otherwise they were required to stay until the next morning to allow the horses time to rest before transportation. One of the horses received fluid therapy when it was eliminated after 98 km as a result of metabolic disturbances, whereas none of the other horses received any treatment. The examinations after the ride were performed within 43 minutes (median, range 7–104 minutes) of entering the final veterinary inspection, and the next morning, 12.7 hours (median, range 7.0–21.2 hours) after the ride. All 26 horses were examined on the day before the ride (baseline) and upon completion of the ride, whereas only 20 horses were examined the next morning (including 7 eliminated horses).

The echocardiographic results along with the number of horses successfully examined for each variable are presented in Tables 1 and 2. No horses were excluded from the study based on valvular insufficiencies. Some data were missing because of inadequate image quality. This posed difficulties with the strain rate data in

**Table 1.** Measures of left atrial (LA) and left ventricular (LV) size and function assessed by two-dimensional echocardiography (2DE) and anatomical M-mode (AMM).

	Units	Before Ride	End of Ride	<i>P</i> Value	Morning After	<i>P</i> Value
Left atrium						
2DE		n = 21	n = 15	–	n = 17	–
Measured HR	min <sup>-1</sup>	38 ± 5	49 ± 7	<.001	39 ± 4	.028
LAD <sub>max</sub>	cm	11.3 ± 0.8	10.4 ± 0.6	<.001	10.7 ± 0.5	.0049
LAA <sub>max</sub>	cm <sup>2</sup>	82.3 ± 11.1	69.3 ± 9.2	<.001	73.8 ± 7.3	.0041
LAA <sub>a</sub>	cm <sup>2</sup>	62.1 ± 7.8	53.6 ± 5.7	<.001	55.1 ± 5.9	<.001
LAA <sub>min</sub>	cm <sup>2</sup>	51.3 ± 7.3	42.8 ± 5.7	<.001	43.7 ± 3.9	<.001
LA-FAC <sub>total</sub>	%	37.4 ± 5.4	37.7 ± 8.2	.92	40.5 ± 5.7	.26
LA-FAC <sub>passive</sub>	%	24.2 ± 5.7	22.1 ± 6.2	.59	25.1 ± 6.9	.66
LA-FAC <sub>active</sub>	%	17.2 ± 6.1	20.0 ± 6.8	.19	20.4 ± 5.2	.18
Left ventricle						
AMM		n = 24	n = 25	–	n = 19	–
Measured HR	min <sup>-1</sup>	39 ± 6	49 ± 7	<.001	40 ± 5	.88
IVSd	cm	3.1 ± 0.3	3.3 ± 0.3	.0028	3.3 ± 0.3	.0044
IVSs	cm	4.2 ± 0.6	4.1 ± 0.4	.93	4.2 ± 0.4	.99
LVIDd	cm	10.7 ± 0.9	9.8 ± 0.9	<.001	10.1 ± 0.7	<.001
LVIDs	cm	6.8 ± 0.7	6.3 ± 0.8	<.001	6.6 ± 0.7	.29
LFWd	cm	2.4 ± 0.3	2.4 ± 0.2	.45	2.4 ± 0.2	.10
LFWs	cm	3.7 ± 0.4	3.8 ± 0.3	.66	3.7 ± 0.3	.93
MWTd	cm	2.7 ± 0.2	2.8 ± 0.3	.0082	2.9 ± 0.2	<.001
RWTd	cm	0.5 ± 0.1	0.6 ± 0.1	<.001	0.6 ± 0.1	<.001
FS	%	36.8 ± 4.9	36.0 ± 5.4	.80	34.8 ± 4.5	.17
2DE		n = 26	n = 25	–	n = 20	–
Measured HR	min <sup>-1</sup>	38 ± 4	49 ± 7	<.001	40 ± 4	.10
LVIVd	mL	1108.3 ± 133.9	938.4 ± 193.9	<.001	965.1 ± 114.1	<.001
LVIVs	mL	389.2 ± 80.0	325.1 ± 79.3	<.001	325.1 ± 60.1	<.001
EF	%	65.0 ± 4.9	65.3 ± 4.2	.75	66.4 ± 4.0	.32
SV	mL	719.1 ± 93.4	613.3 ± 134.3	<.001	639.9 ± 79.6	.010
CO	L/min	27.2 ± 4.0	29.4 ± 4.8	.077	25.2 ± 3.4	.070

Mean ± SD. HR, heart rate; LAD, LA diameter; LAA, LA area; LA-FAC, LA fractional area change; IVS, interventricular septal thickness; d, end-diastolic measure; s, peak systolic measure; LVID, LV internal diameter; LFW, LV free wall; MWT, mean wall thickness; RWT, relative wall thickness; FS, fractional shortening; LVIV, LV internal volume; EF, ejection fraction; SV, stroke volume; CO, cardiac output. See Tables S1–S3 for details of subscripts and calculations.

*P* values indicate comparisons with baseline values, and bold *P* values indicate statistical significance.

particular, as a result of the inherent problems of 2DST analysis. The 2DST analysis was easier in the SAX-LV view than the LAX-LV view, and strain profiles were less prone to noise than velocity and strain rate curves. In general, the most robust examination methods were AMM, LV volume estimations, and PW-TDI, whereas it was more difficult to obtain LA area traces of sufficient quality, especially in the examinations performed immediately after the ride. Even though AMM and 2DST analysis (radial and circumferential variables) were performed in the same SAX-LV view, there was sometimes a difference in the number of successful measurements. The quality of the AMM was assessed manually by the observer. Typical reasons for excluding AMM images were poor visibility of the LV free wall in the depth of the image or rib shadows during systole that precluded reliable measurements. In the 2DST process, the tracking quality was automatically assessed by the software. This is conceivably the reason for the discrepancy in the number of successful measurements between the two techniques. A detailed description of missing data is given in the Supporting Information (Table S7).

Heart rate was significantly increased during the examination directly after the ride (Tables 1 and 2).

The LA diameter and area, as well as LV diameter, volume and stroke volume showed a significant decrease directly after the ride and the next morning (7–21 hours after exercise) (Table 1). No significant changes were found in LA fractional area changes or LV fractional shortening, ejection fraction, or cardiac output. Regarding the LV myocardial wall motion specifically, the early diastolic velocities and strain rates as well as some of the strain values were significantly decreased after exercise (Table 2). In addition, both longitudinal and circumferential strain showed clear transmural gradients with decreasing values from the endocardium to the epicardium (Fig 2). The blood analyses are summarized in Table 3. No linear correlations between cTnI and the echocardiographic variables were evident in the regression analyses.

## Discussion

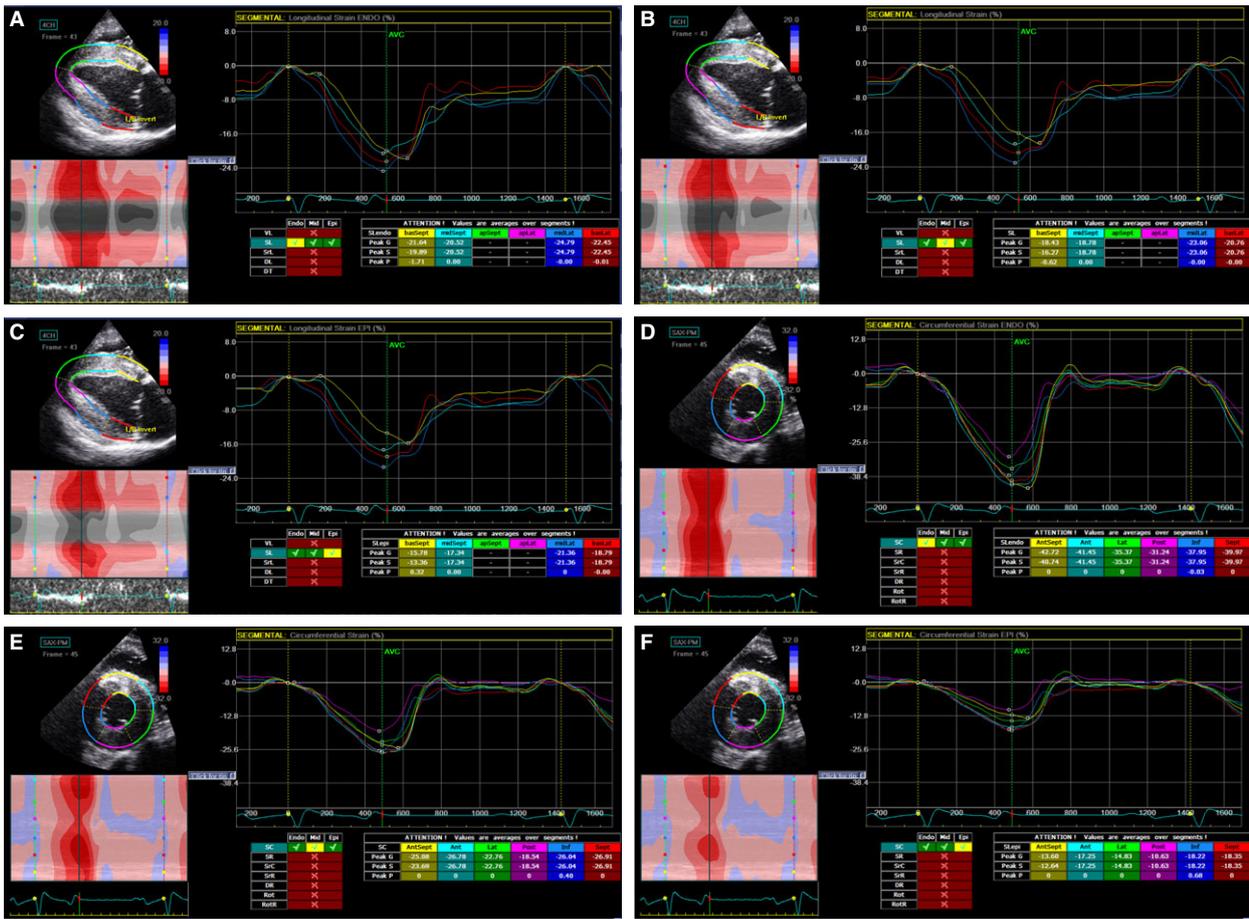
This echocardiographic investigation of equine cardiac function after prolonged exercise includes strain imaging for the evaluation of both systolic and diastolic LV function. Multiple echocardiographic variables are significantly changed in horses after an endurance ride,

**Table 2.** Indices of left ventricular (LV) myocardial wall motion assessed by two-dimensional speckle tracking (2DST) and pulsed-wave tissue Doppler imaging (PW-TDI).

	Units	Before Ride	End of Ride	<i>P</i> Value	Morning After	<i>P</i> Value
<b>2DST LAX</b>						
Measured HR	min <sup>-1</sup>	38 ± 4	49 ± 7	< .001	40 ± 4	.18
Longitudinal velocity		n = 25	n = 22	–	n = 20	–
Segmental values <sup>a</sup>						
VL <sub>S</sub>	cm/s	12.4 ± 1.6	12.3 ± 2.3	.91	10.8 ± 1.9	<b>.018</b>
VL <sub>E</sub>	cm/s	-17.4 ± 2.4	-15.8 ± 3.2	<b>.013</b>	-15.4 ± 3.0	<b>.0033</b>
VL <sub>A</sub>	cm/s	-10.9 ± 2.4	-10.4 ± 2.9	.79	-10.9 ± 2.5	.95
Longitudinal strain		n = 24	n = 23	–	n = 20	–
Global values						
SL <sub>endo</sub>	%	-25.2 ± 2.0	-25.5 ± 2.2	.56	-25.5 ± 1.8	.70
SL <sub>mid</sub>	%	-21.0 ± 1.8	-20.8 ± 2.1	.86	20.8 ± 1.7	.81
SL <sub>epi</sub>	%	-17.4 ± 1.7	-16.9 ± 2.1	.22	-16.9 ± 1.8	.22
Average segmental values						
SL <sub>endo</sub>	%	-23.0 ± 2.9	-21.6 ± 2.7	<b>.0034</b>	-22.2 ± 2.5	.36
SL <sub>mid</sub>	%	-21.0 ± 2.6	-19.4 ± 2.5	< .001	-19.8 ± 2.3	<b>.035</b>
SL <sub>epi</sub>	%	-19.2 ± 2.3	-17.3 ± 2.4	< .001	-17.7 ± 2.4	<b>.0065</b>
Longitudinal strain rate		n = 23	n = 20	–	n = 20	–
Global values						
SrL <sub>S</sub>	s <sup>-1</sup>	-0.86 ± 0.08	-0.94 ± 0.10	<b>.0075</b>	-0.81 ± 0.09	.073
SrL <sub>E</sub>	s <sup>-1</sup>	1.12 ± 0.19	1.09 ± 0.19	.69	1.06 ± 0.19	.31
SrL <sub>A</sub>	s <sup>-1</sup>	0.60 ± 0.17	0.60 ± 0.17	1.00	0.64 ± 0.13	.57
Average segmental values						
SrL <sub>S</sub>	s <sup>-1</sup>	-1.07 ± 0.10	-1.06 ± 0.16	.90	-0.94 ± 0.14	<b>.0027</b>
SrL <sub>E</sub>	s <sup>-1</sup>	1.58 ± 0.27	1.45 ± 0.26	<b>.036</b>	1.41 ± 0.25	<b>.013</b>
SrL <sub>A</sub>	s <sup>-1</sup>	0.65 ± 0.19	0.64 ± 0.18	.97	0.63 ± 0.20	.83
<b>PW-TDI</b>						
Measured HR	min <sup>-1</sup>	38 ± 4	50 ± 7	< .001	39 ± 4	.16
Radial velocity		n = 26	n = 26	–	n = 20	–
<i>S<sub>m</sub></i>	cm/s	11.1 ± 1.2	11.8 ± 1.7	<b>.043</b>	10.7 ± 1.2	.55
<i>E<sub>m</sub></i>	cm/s	-32.8 ± 3.4	-28.1 ± 5.8	< .001	-26.4 ± 5.5	< .001
<i>A<sub>m</sub></i>	cm/s	-9.2 ± 2.6	-11.5 ± 4.7	<b>.0026</b>	-10.8 ± 2.6	<b>.0034</b>
<i>E<sub>m</sub>/A<sub>m</sub></i>	cm/s	3.9 ± 1.1	2.8 ± 1.3	< .001	2.6 ± 0.8	< .001
<b>2DST SAX</b>						
Measured HR	min <sup>-1</sup>	39 ± 6	49 ± 7	< .001	40 ± 5	.83
Radial strain + strain rate		n = 24	n = 26	–	n = 19	–
Average segmental values						
SR	%	54.9 ± 14.7	51.5 ± 17.6	.45	57.4 ± 12.8	.85
SrR <sub>S</sub>	s <sup>-1</sup>	1.82 ± 0.37	1.71 ± 0.40	.39	1.78 ± 0.30	.99
SrR <sub>E</sub>	s <sup>-1</sup>	-2.30 ± 0.62	-2.36 ± 0.61	.85	-2.36 ± 0.48	.76
SrR <sub>A</sub>	s <sup>-1</sup>	-1.60 ± 0.36	-1.76 ± 0.61	.23	-1.76 ± 0.27	<b>.036</b>
Circumferential strain		n = 24	n = 26	–	n = 19	–
Global values						
SC <sub>endo</sub>	%	-33.0 ± 3.1	-32.6 ± 4.1	.79	-33.7 ± 2.2	.61
SC <sub>mid</sub>	%	-22.9 ± 2.2	-21.6 ± 2.9	<b>.023</b>	-22.8 ± 1.8	.94
SC <sub>epi</sub>	%	-16.0 ± 1.9	-14.4 ± 2.4	<b>.0028</b>	-15.5 ± 1.7	.55
Average segmental values						
SC <sub>endo</sub>	%	-33.4 ± 3.0	-32.8 ± 4.2	.68	-34.1 ± 2.1	.64
SC <sub>mid</sub>	%	-23.2 ± 2.1	-21.8 ± 2.9	<b>.011</b>	-23.0 ± 1.8	.85
SC <sub>epi</sub>	%	-16.3 ± 1.8	-14.6 ± 2.4	<b>.0024</b>	-15.7 ± 1.6	.47
Circumferential strain rate		n = 23	n = 26	–	n = 19	–
Global values						
SrC <sub>S</sub>	s <sup>-1</sup>	-0.79 ± 0.07	-0.83 ± 0.10	.17	-0.74 ± 0.06	.16
SrC <sub>E</sub>	s <sup>-1</sup>	2.10 ± 0.27	1.77 ± 0.43	< .001	1.83 ± 0.27	< .001
SrC <sub>A</sub>	s <sup>-1</sup>	0.44 ± 0.16	0.52 ± 0.22	.083	0.45 ± 0.12	.96
Average segmental values						
SrC <sub>S</sub>	s <sup>-1</sup>	-1.08 ± 0.11	-1.06 ± 0.11	.52	-0.99 ± 0.08	<b>.0018</b>
SrC <sub>E</sub>	s <sup>-1</sup>	2.43 ± 0.29	1.96 ± 0.46	< .001	2.11 ± 0.32	< .001
SrC <sub>A</sub>	s <sup>-1</sup>	0.58 ± 0.18	0.68 ± 0.23	<b>.032</b>	0.61 ± 0.14	.69

Mean ± SD. HR, heart rate; LAX, long-axis view; VL, longitudinal velocity; SL, longitudinal strain; SrL, longitudinal strain rate; *S<sub>m</sub>*, radial velocity in systole; *E<sub>m</sub>*, radial velocity in early diastole; *A<sub>m</sub>*, radial velocity in late diastole; SAX, short-axis view; SR, radial strain; SrR, radial strain rate; SC, circumferential strain; SrC, circumferential strain rate. Subscripts endo, mid, epi, endocardium, mid-myocardium, and epicardium, respectively. Subscripts S, E, A, systolic, early diastolic, and late diastolic, respectively. See Tables S4–S6 for details.

<sup>a</sup>Obtained in the basal segment of the left ventricular free wall. *P* values indicate comparisons with baseline values, and bold *P* values indicate statistical significance.



**Fig 2.** Transmurial strain gradients. Longitudinal strain of the: (A) endocardium, (B) mid-myocardium, and (C) epicardium. Circumferential strain of the: (D) endocardium, (E) mid-myocardium, and (F) epicardium.

**Table 3.** Summary of the blood analyses presented as median and range.

	Units	Before Ride (n = 26)	End of Ride (n = 26)	P Value	Morning After (n = 19)	P Value
cTnI <sup>a</sup>	ng/mL	0.003 (0.003–0.038)	0.007 (0.003–0.083)	<b>&lt; .001</b>	0.003 (0.003–0.026)	.21
Hematocrit	%	40.4 (31.6–47.7)	43.3 (36.8–55.7)	<b>&lt; .001</b>	39.8 (35.5–47.8)	.86
Serum protein	g/L	71.1 (60.5–78.1)	71.6 (59.0–84.2)	.089	68.5 (60.1–76.4)	<b>&lt; .001</b>

cTnI, cardiac troponin I.

<sup>a</sup>Non-normally distributed and log-transformed before analysis. P values indicate comparisons with baseline values, and bold P values indicate statistical significance.

and for the majority of these variables, the changes persist after a number of hours of recovery. The echocardiographic findings were generally comparable to those in human athletes, with indications of diastolic dysfunction and ambiguous results on variables of systolic function.<sup>1–4</sup>

The observed decrease in early diastolic myocardial velocities and strain rates (Table 2) is consistent with studies in human endurance athletes which show a significant decrease in early diastolic mitral flow and LV myocardial velocities, E/A ratio, and diastolic strain rates after endurance exercise.<sup>1,3,4,25–32</sup> However, the effects of altered loading conditions after exercise

caused by decreases in blood volume and/or peripheral redistribution of blood are difficult to assess. The significant decrease in LA area as well as LV diameter and volume during diastole indicated a reduction in preload after the ride, and the observed increase in heart rate might conceivably be caused by the baroreceptor reflex to compensate for a reduced stroke volume and maintain the cardiac output (Table 1). A reduction in central blood volume leading to a diminished venous return to the heart could contribute to the observed reduction in myocardial velocities and strain rates during the passive ventricular filling in early diastole. However, studies in human athletes have succeeded in demonstrating a

reduced diastolic function even under retained loading conditions, suggesting that echocardiographic changes after exercise are not entirely caused by altered loading conditions.<sup>1</sup> In this study, hematocrit and serum protein were used to assess the degree of volume depletion; however, these are only crude biochemical markers of the horses' hydration status. The significant increase in hematocrit directly after the ride could be attributable to release of the splenic erythrocyte reserve, and the lack of a significant increase in serum protein directly after the ride could suggest that the horses experienced only a minor degree of volume depletion. However, an interesting observation was the significant decrease in serum protein and nonsignificant decrease in hematocrit the morning after the ride compared to baseline values. This might indicate that the hydration status of the horses was already suboptimal on the day before the ride. Most horses had been transported for many hours and as water intake is commonly reduced during transportation<sup>33,34</sup> this could explain the relative decrease in serum protein and hematocrit in the after ride sampling period. A clinical evaluation of the hydration status including skin tent duration was also performed; however, as such clinical variables have been shown to be poorly correlated with plasma osmolality it was chosen not to include them in the statistical analyses.<sup>35</sup> Hypohydration has been shown to affect multiple echocardiographic variables pertaining to LV dimensions in horses hypohydrated to 4–7% of their bodyweight.<sup>36</sup> The decreases in LA diameter, LV systolic and diastolic diameter and diastolic volume were generally comparable or somewhat bigger than those observed in this study. It is not possible to determine the effects of altered loading conditions on the echocardiographic variables in this study, but it is worth noting that the significant decrease in diastolic variables persisted the next morning (7–21 hours after exercise), when the heart rate had returned to baseline. It therefore appears that the LV filling was still impaired the morning after the ride (7–21 hours after exercise), in face of the normalization of hematocrit and serum protein. This suggests that the decreases in diastolic variables were not caused exclusively by reduced preload. In cases of long-standing diastolic LV dysfunction (eg, caused by cardiomyopathy affecting the LV), the LA dimensions could be expected to enlarge, possibly even with an exaggerated active mechanical contraction to compensate for increased LV filling pressures (booster pump). However, the LA dimensions in this study decreased simultaneously with the LV dimensions. The reason for this cannot be ascertained, but it is possible that the acute functional changes leading to LV diastolic dysfunction possibly also affected the LA so that LA filling was also impaired.

The early diastolic strain rate denotes the speed of myocardial relaxation in early diastole, and although significantly decreased in both the longitudinal and circumferential dimensions after prolonged exercise in human athletes, the circumferential strain rate showed the largest decrease.<sup>32</sup> Ventricular filling in early diastole is driven by the relaxation of circumferentially

oriented fibers in the mid-myocardium and elastic recoil of the LV, resulting in a “suction effect”. It has consequently been suggested that diastolic dysfunction with reduced transmitral filling after exercise is caused by impaired myocardial relaxation and delayed ventricular untwisting.<sup>6</sup> Ventricular torsion was not assessed in this study, but might be an interesting perspective for future studies. In general, the results suggest the presence of a diastolic dysfunction in the equine heart after prolonged exercise that is only partly explained by altered loading conditions, although the causal mechanisms and clinical significance cannot be ascertained from this study. The exact causal mechanisms of cardiac fatigue in humans remain unknown, but proposed theories include modulations of the autonomic nervous system with vagal reactivation and desensitization of beta-receptors after prolonged exposure to catecholamines during exercise.<sup>12</sup> The clinical significance of cardiac fatigue is likewise unknown. The decrease in cardiac function after exercise is relatively small and resolves within 24–48 hours, but the long-term effects of such short-term reversible changes remain unclear.<sup>37</sup>

The results pertaining to the LV systolic function in this study were less clear. The overall systolic function appeared to be unchanged after the ride as there were no changes in ejection fraction or fractional shortening. Although the decreases in stroke volume and peak systolic strain indicated an impaired systolic function after exercise (Table 2), these indices are load dependent, so such changes are likely to reflect the reduction in preload noted in the present cases. One study<sup>11</sup> has previously investigated the effects of endurance riding on LV systolic function in horses and found significant decreases in multiple systolic variables (including ejection fraction, fractional shortening, stroke volume, and ejection time) after exercise. These changes were not correlated with heart rate and diastolic LV diameter, which led the authors to suggest that the reduced systolic function was not entirely caused by altered preload conditions. The 11 horses in the study competed in rides of 106–132 km with slightly higher average speed ( $16.3 \pm 1.3$  km/h). Thus, the distances were generally comparable or shorter than the distances in this study, and it would therefore appear that distance, and hence exercise duration, cannot explain the differing results between the two studies. On the other hand, the exercise intensity might have been higher because of the higher velocity, and this might have contributed to the observed systolic dysfunction. Another possible explanation is that the rides were conducted in more hilly terrain or during warmer weather conditions compared to this study which would also have challenged the horses further. The breed, age, weight, and sex distribution of the horses were comparable in both studies, but the training status of the horses remained unknown, providing another possible explanation for the differing results.

Studies in human endurance athletes have also shown conflicting systolic function results, significant decreases in multiple systolic variables after prolonged exercise. Left ventricular ejection fraction has been shown to be

decreased,<sup>1,4,25,26</sup> unchanged,<sup>4,27,28,30</sup> and even increased<sup>38,39</sup> after prolonged exercise. Similarly, systolic myocardial velocity (obtained by tissue Doppler imaging) has been shown to be both unchanged<sup>29</sup> and increased<sup>30</sup> after exercise. It has been suggested that these equivocal reports regarding systolic dysfunction after prolonged exercise are caused by differences in exercise duration.<sup>6</sup> The typical duration of a 120 km or 160 km endurance ride exceeds the 6-hour limit identified as the threshold for systolic dysfunction in trained athletes.<sup>9</sup> However, the intermediate compulsory rest periods of approximately 40 minutes for every 30–40 km during an endurance ride could act as a preventative factor for systolic dysfunction in endurance horses. Another possible explanation is that the impressive cardiac reserve and physical superiority of the horse compared to humans<sup>40</sup> imply that this species can endure physical exertion for longer periods of time before systolic dysfunction becomes evident.

Two-dimensional speckle tracking analyses in human athletes have shown more consistent results, with significant decreases in longitudinal, radial and circumferential systolic strain and strain rates.<sup>3,31</sup> However, the equivocal systolic strain rate results in this study preclude any conclusions regarding the effect of prolonged exercise on myocardial contractility in horses. The strain rate curves were often quite noisy, and between 10 and 25% of the measurements were excluded as a result of inadequate data quality (Table S7). It is possible that the systolic strain rate was indeed decreased after exercise, as observed in human studies, but that the data presented in this study were unable to track the changes. Both longitudinal and circumferential strain showed a transmural gradient with decreasing values from the endocardium to the epicardium (Fig 2). To our knowledge this is the first study to include layered strain in horses and a transmural strain gradient has therefore not previously been demonstrated in the equine heart. Unfortunately, we do not have any previous results to compare with and the variability of layered strain in horses is consequently unknown. Similar transmural strain gradients exist in humans, where circumferential strain has been shown to be 36% higher in the endocardium than the epicardium.<sup>41</sup> The LV endocardium is more vulnerable to ischemia in relation to myocardial infarction, and it has been suggested that “layer-specific” analysis of myocardial strain might be advantageous in the clinical assessment of cardiac disease in humans.<sup>41,42</sup> Interestingly, the strain values in this study showed larger decreases in the outer myocardial layers compared to the endocardium, and it appears that the equine mid-myocardium and epicardium might be more susceptible to the effects of prolonged exercise. However, because of the relatively small study population in this study, this hypothesis requires further investigation. Radial strain values were comparable to those previously reported in horses,<sup>15,18,20</sup> however, this variable was not significantly changed after exercise.

Exercise-induced myocardial damage has been proposed as a causal mechanism of cardiac fatigue, but a causal relationship between the release of troponin and

changes in echocardiographic variables after exercise has yet to be established.<sup>6</sup> No correlation was found between cTnI and echocardiographic measures in this study. Increases in cTnI have previously been shown in horses after prolonged exercise.<sup>43</sup> There are a number of different troponin assays available; however, there is a lack of knowledge concerning the release mechanisms of cardiac troponin in horses,<sup>44</sup> and the clinical relevance of troponin release after prolonged exercise in horses therefore remains unclear.

The primary limitation of this study was the relatively small study population, which precluded the comparison of completing and eliminated horses. Likewise, the effect of ride distance was not assessed. The echocardiographic variables were subject to a number of missing values caused by inadequate image quality which complicated the offline analyses. For instance, the most dorsal part of the LA endocardial border could not always be traced throughout the cardiac cycle and especially the maximum LA area could therefore not be measured. When this happened it was chosen to exclude the other LA measures pertaining to that cardiac cycle as well. This was done to ensure data quality at the expense of additional missing data. A limitation in relation to the 2DST analysis is that the algorithms are proprietary. The tracking quality and obtained values were therefore consistently manually checked by the observer to ensure the validity of the results. The number of available variables in the echocardiographic analyses is abundant and it was therefore chosen to limit our variables to traditional ejection phase indices (fractional shortening, ejection fraction, stroke volume, and cardiac output) and myocardial velocity, strain, and strain rate. Hence, no timing measurements were performed. The 2DST analysis does provide measures on time-to-peak strain etc., but in our experience these are quite variable measures in horses, and it was therefore decided not to include them in the study. Multiple echocardiographic variables had not returned to baseline on the morning after the ride (7–21 hours after exercise), and it would have been interesting to evaluate the time course of the effects. However, a follow-up examination was not possible after the horses had left the venue. Another important limitation that needs to be addressed is the variation in time between the echocardiographic reexaminations. This is probably most important in relation to the examination performed directly after the ride as the most pronounced physiologic changes could be expected to occur in the immediate phase of recovery. Of the 26 horses, 15 were examined within 30–60 minutes of exercise cessation, and of the 20 horses examined on the morning after the ride, 15 were examined within 10–16 hours after the ride. With regards to the examination directly after the ride it was attempted to examine the horses as soon as possible after the final veterinary inspection. The examination on the morning after the ride was performed at approximately 6:00–7:00 AM and the time period from cessation of exercise was therefore determined by the time the horse finished the ride.

In conclusion, a diastolic dysfunction characterized by small but significant decreases in early diastolic myocardial velocity and strain rate was observed in horses after

the endurance rides. Ventricular filling was still reduced the next morning despite normalization of biochemical indicators of hydration status, indicating that the observed changes were not caused exclusively by reduced preload. It is speculated that the diastolic dysfunction might be related to impaired myocardial relaxation and compromised ventricular recoil after exercise. The results regarding the presence of a systolic dysfunction in horses after prolonged exercise were equivocal. Overall, the effects of prolonged exercise were small and the clinical relevance of these findings remains to be clarified.

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## Footnotes

- <sup>a</sup> Vivid I, GE Healthcare, Horten, Norway  
<sup>b</sup> 3S Phased Array transducer, GE Healthcare  
<sup>c</sup> EchoPAC version BT13, GE Healthcare  
<sup>d</sup> ADVIA Centaur CP TnI-ultra, Siemens Healthcare Diagnostics Inc., Tarrytown, NY  
<sup>e</sup> ADVIA 2120 Hematology System, Siemens Healthcare Diagnostics Inc.  
<sup>f</sup> ADVIA 1800 Chemistry System, Siemens Healthcare Diagnostics Inc.
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*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** Measurement of left atrial dimensions.

**Figure S2** Anatomical M-mode.

**Figure S3** Measurement of left ventricular volume.

**Figure S4** Velocity profile of the left ventricular radial motion.

**Figure S5** Two-dimensional speckle tracking of the longitudinal left ventricular motion.

**Figure S6** Two-dimensional speckle tracking of the radial and circumferential left ventricular motion.

**Table S1** Two-dimensional echocardiographic variables for the assessment of left atrial size and function.

**Table S2** One-dimensional indices of left ventricular size and function.

**Table S3** Two-dimensional indices of left ventricular size and function.

**Table S4** Tissue Doppler imaging variables for the assessment of left ventricular radial velocity.

**Table S5** Two-dimensional speckle tracking variables for the assessment of left ventricular longitudinal motion.

**Table S6** Two-dimensional speckle tracking variables for the assessment of left ventricular circumferential and radial motion.

**Table S7** Missing data.