

# Echocardiographic comparison of left ventricular dimensions and function after standardized treadmill exercise in trained and untrained healthy warmblood horses

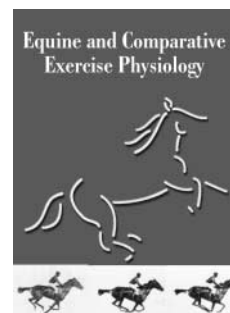
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Submitted 4 January 2005; Accepted 1 December 2005

Research Paper

## Abstract

The purpose of the present study was to determine the influence of fitness on cardiac function, particularly on left ventricular function parameters. Fifteen healthy 'three-day event' warmblood horses were examined at rest and immediately after high-speed treadmill exercise (3% incline, 3 min 1.8 m s<sup>-1</sup>, 3 min 4 m s<sup>-1</sup>, 3 min 5 m s<sup>-1</sup>, 3 min 6 and 3 min 7 m s<sup>-1</sup>, 1.5 min 8 m s<sup>-1</sup>). Horses were divided into two groups. Group 1 consisted of nine conditioned horses and group 2 included six unconditioned horses. Left ventricular dimensions and function were acquired using standardized echocardiographic indices. To assess the level of fitness, heart rate and blood lactate concentration were determined at rest and immediately after exercise. The group of conditioned horses showed a significantly lower blood lactate concentration (mean value 2.39 mmol l<sup>-1</sup>) after high-speed treadmill exercise than did the group of unconditioned horses (mean value 3.81 mmol l<sup>-1</sup>), which clearly revealed the difference in fitness between the two groups. During exercise the heart rate was not significantly different between both groups. Only in the recovery phase did the trained horses show a significant faster decrease in heart rate than did the untrained horses. Mean heart rate during echocardiography immediately after exercise (within the first 2 min) was 105 bpm in the group of trained horses and 113 bpm in the group of untrained horses. Within each group of horses, several echocardiographic parameters differed significantly between resting values and values after treadmill exercise. Particularly, in the group of trained horses, 17 out of 30 echocardiographic parameters (most diastolic) differed significantly between rest and exercise. In the group of untrained horses, only six out of 30 parameters were significantly different. At rest, left ventricular diameter at the *apex cordis*, left ventricular free wall at papillary muscle level, left ventricular volume and stroke volume, as well as fractional shortening (at the *apex cordis* and at papillary muscle level) were significantly different between both groups. After treadmill exercise comparison of echocardiographic parameters of the conditioned to those of the unconditioned animals showed no significant differences. In the present study, data have been provided for stress echocardiography in conditioned and unconditioned warmblood horses without any disorders of the cardiovascular system.

**Keywords:** warmblood horses; heart rate; stress echocardiography; fitness level; treadmill exercise, three-day event

## Introduction

Echocardiographic examination at rest tells us very little about cardiac function under stress load. Consequently, and in analogy to human cardiology, stress

echocardiography shortly after exercise could have an important additional diagnostic value in the horse<sup>1–4</sup>. In human medicine, cardiac stress is commonly induced by treadmill or bicycle exercise<sup>5</sup>. It results in an increase

in heart rate, blood pressure and inotropy of the heart<sup>5</sup>. Stress echocardiography has been used as an additional examination in horses<sup>1-4,6</sup>. In people and in horses, training leads to alterations in chamber dimensions and cardiac function, which can be detected echocardiographically<sup>7</sup>. Specific changes in single echocardiographic parameters, however, are controversially discussed in human science as well as in equine medicine<sup>6,8</sup>. Data are published about echocardiography at rest indicating changes in left ventricular myocardial function in trained Thoroughbred racehorses and in trotters in comparison to untrained horses<sup>9-11</sup>. In warmblood horses, the influence of dressage and showjumping training on echocardiographic parameters was investigated, and an effect of training on some parameters was described<sup>12</sup>. Stress echocardiography has also been carried out in healthy Standardbreds before and 8 weeks after training, and a positive effect of training on measured cardiac sizes and function immediately after an incremental exercise test was observed<sup>13</sup>.

The purpose of the present study was to determine whether there are any significant differences in echocardiographic left ventricular function parameters between healthy conditioned and unconditioned warmblood horses at rest and after treadmill exercise.

## Materials and methods

Nine conditioned and six unconditioned healthy warmblood horses used for 'three-day event' competition and owned by the Federal Agricultural Research Centre (FAL) and the Institute for Animal Breeding Mariensee (Braunschweig, Germany) were included in the present study. The conditioned horses had been regularly trained and used for competition at German class L (comparable to a CCI\*) before they were integrated into this study. The unconditioned horses were all retired 'three-day event' horses which had been maintained at pasture and which had not been exercised for the last two years. Additionally to competition history, the fitness level was assessed by blood lactate concentration after treadmill exercise<sup>17,18</sup>.

A physical and a specific examination of the respiratory and cardiovascular systems, including electrocardiography and echocardiography, were performed on all horses. None of the horses had any respiratory, cardiovascular or musculoskeletal disorders. Basic data on these horses (breed, sex, age, body weight, height and state of fitness) are listed in Table 1.

### Echocardiographic examination

Echocardiography at rest and after treadmill exercise included M-mode and B-mode techniques ('Ving Med 600E'; VINGMED 600E, General Electrics, Garching, Germany) with a 2.5 MHz-phased-array transducer. A base-apex ECG was recorded simultaneously for timing

of the cardiac cycles. For subsequent analysis, the echocardiographic sequences were documented on the S-VHS video recorder (AG 7350, Panasonic, Osaka, Japan) Echocardiography was carried out at rest and directly after exercise. To minimize inter- and intra-observer variability, all echocardiographic examinations and measurements were performed by the same person and repeated at five consecutive heart cycles.

### B-mode examination

For the B-mode echocardiography, standardized long-axis real time echocardiograms were used<sup>14</sup>. The heart was visualized in a right parasternal long-axis (four-chamber view).

To gain detailed information about the left ventricle, the B-mode measurements were repeated at different locations. Peak-systolic and peak-diastolic (at the onset of the QRS complex) septal thickness, the thickness of the left ventricular free wall and the left ventricular diameter at the level of the apex (A) at papillary muscle level (PPM) and close to the heart base (below the mitral valve, uMV) were determined in the four-chamber view (Fig. 1, measurements 1-9). Additionally, the longitudinal diameter of the left ventricle (measurement from the *apex cordis* to the level of the mitral valves) at peak-systole and peak-diastole was measured (Fig. 1, measurement 10).

All measurements were performed at rest and immediately after exercise.

### Regional left ventricular function (M-mode examination)

Assessment of the left ventricular function was performed using M-mode techniques. For assessment of the regional left ventricular function, the fractional shortening (FS%) was measured in the right parasternal long-axis view of the left ventricle at three different locations (Fig. 1, I-III): at the level of the apex (A), at papillary muscle level (PPM) and below the mitral valve (uMV) using the following formula:

$$FS(\%) = 100 \times \frac{LVDD - LVDS}{LVDD} \quad (1)$$

LVDD, left ventricular diastolic diameter; LVDS, left ventricular systolic diameter.

### Global left ventricular function

For assessment of the global left ventricular function, the left ventricular area was determined planimetrically (rounding the endocardial line) and the left ventricular volume was calculated with the Simpson's disc summation method, which was done automatically by Ving Med software. The ejection fraction was determined and the left ventricular stroke volume was calculated from the systolic (LVSV) and the diastolic

**Table 1** Breed, sex, age, weight, height and level of fitness of all 15 horses

| Horse | Breed | Sex | Age (years) | Weight (kg) | Height (cm) | Level of fitness |
|-------|-------|-----|-------------|-------------|-------------|------------------|
| 1     | H     | G   | 9           | 630         | 168         | Unconditioned    |
| 2     | H     | G   | 9           | 728         | 172         | Unconditioned    |
| 3     | H     | G   | 9           | 686         | 169         | Unconditioned    |
| 4     | H     | G   | 9           | 662         | 172         | Unconditioned    |
| 5     | H     | G   | 7           | 772         | 170         | Unconditioned    |
| 6     | H     | G   | 7           | 568         | 163         | Unconditioned    |
| Mean  |       |     | 8.3         | 674         | 169         |                  |
| 7     | H     | G   | 7           | 586         | 163         | Conditioned      |
| 8     | H     | G   | 7           | 560         | 163         | Conditioned      |
| 9     | H     | G   | 7           | 572         | 170         | Conditioned      |
| 10    | B     | G   | 7           | 550         | 164         | Conditioned      |
| 11    | B     | G   | 7           | 580         | 166         | Conditioned      |
| 12    | SA    | G   | 7           | 604         | 167         | Conditioned      |
| 13    | SA    | G   | 7           | 524         | 163         | Conditioned      |
| 14    | O     | G   | 7           | 536         | 166         | Conditioned      |
| 15    | H     | G   | 7           | 574         | 168         | Conditioned      |
| Mean  |       |     | 7           | 565         | 166         |                  |

B, Brandenburger; H, Hannoveraner; O, Oldenburger; SA, Sachsen-Anhalt; G, Gelding.

(LVDV) volume of the left ventricle using the following formulas:

$$SV = LVDV - LVS$$
 (2)

$$EF(\%) = 100 \times \frac{LVDV - LVS}{LVDV}$$
 (3)

### Treadmill exercise

Exercise was performed on a high-speed treadmill (Mustang 2200, Kagra AG Fahrwangen, Switzerland). During treadmill exercise and during echocardiographic examination, the heart rate was continuously recorded by a telemetry system (Elmed ETM, Polar Horse Trainer® SE 610, Augsburg, Germany). The horses were exercised on the treadmill with 3% incline, using the following exercise protocol: 3 min of walk ( $1.8 \text{ m s}^{-1}$ ;  $t_1$ - $t_3$ ), 3 min of trotting ( $4 \text{ m s}^{-1}$ ;  $t_4$ - $t_6$ ), 3 min of fast trotting ( $5 \text{ m s}^{-1}$ ;  $t_7$ - $t_9$ ), 6 min of cantering ( $6 \text{ m s}^{-1}$ ;  $t_{10}$ - $t_{12}$  and  $7 \text{ m s}^{-1}$ ;  $t_{13}$ - $t_{15}$ ) and 1.5 min of hard cantering ( $8 \text{ m s}^{-1}$ ;  $t_{16}$ ,  $t_{17}$ )<sup>15</sup>. Immediately after cessation of the exercise protocol, the treadmill was stopped, the horses were taken off the treadmill and echocardiography was performed within the next 2 min ( $t_{18}$ - $t_{19}$ ). Subsequently, the horses were walked for c. 15 min, until normal heart rate and respiratory rate were re-established. Prior to and directly after exercise, blood samples (lithium-heparin) were taken from the jugular vein to determine the blood lactate concentration<sup>16,17</sup>.

### Statistical analysis

The objective of the study was to identify differences in echocardiographic parameters between trained and untrained healthy horses at rest and after treadmill exercise, respectively.

Both groups show statistical significant differences in body weight and height. Therefore, weight was included

as a covariate in the statistical model, while considering height as a covariate in addition to weight would not alter the results.

Echocardiographic parameters at rest, after treadmill exercise and the differences between both groups were calculated by one-way analysis of covariance model, which incorporates the fixed factor training as well as the individual weight of the horses. Least square means were calculated for both training groups. These are within-group means appropriately adjusted for the covariate (body weight) in the model<sup>18</sup>.

All data were included in a descriptive analysis. Normal distribution of the echocardiographic variables was confirmed by visual assessment of normal probability plots.

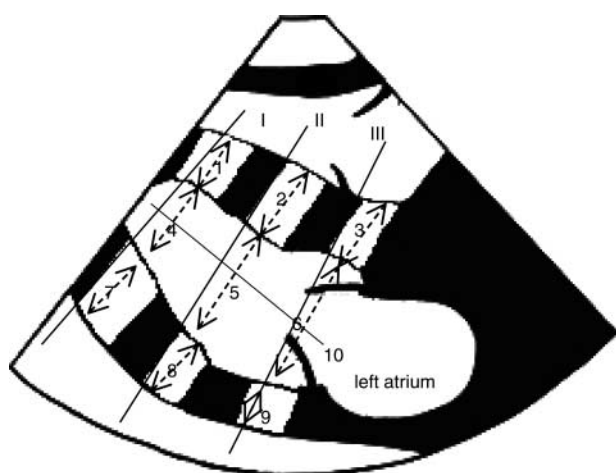
Least square means of the various echocardiographic parameters and the corresponding standard errors are tabulated in the figures and tables. For derived variables for which normal distribution could not be assumed (e.g. EF%, ejection fraction in %), logarithmic transformation was performed prior to analysis; hence the results were tabulated on the original scale after exponential retransformation.

Analyses were carried out with the statistical software SAS, version 9.1 (SAS Institute, Cary, NC, USA). For the analysis of the linear model, the GLM procedure was used. Significance was set at  $P < 0.05$ .

## Results

### Horses

Both groups showed statistical significant differences in body weight and height ( $t$ -test,  $P < 0.05$ ). There was a considerable difference in body weight between both groups. For unconditioned horses the mean weight was 674 kg, and for the conditioned horses it was 565 kg. An influence on echocardiographic parameters



**Fig. 1** Localization of the B-mode measurements (long axis, 'four-chamber view') of the left ventricle during systole and diastole: the interventricular septum: at the level of the apex (1), at papillary muscle level (2), below the mitral valve (3), the inner diameter of the left ventricle: close to the apex (4), at papillary muscle level (5), below the mitral valve (6), the left ventricular free wall: at the level of the apex (7), at papillary muscle level (8), below the mitral valve (9), left ventricular longitudinal axis (10), and location of the M-mode cursor (1–3) to determine left ventricular fractional shortening (FS%) at I) the level of the apex, FS A; II) at papillary muscle level, FS PPM and III) below the mitral valve, FS uMV

cannot be excluded and, therefore, this variable was included as a covariate in the statistical model.

The mean height differed by 3.4 cm between both groups and seemed not to be biologically meaningful. Considering this variable a covariate in addition to the weight would not alter the results. It was, therefore, not incorporated into the statistical model.

### Heart rate

During treadmill exercise, the mean maximum heart rate of the untrained horses was 163 bpm and of the

trained horses 162 bpm, and significant differences between both groups were not observed.

Within the first 2 min after exercise, while post-exercise echocardiography was performed, the mean heart rate of the trained horses was 105 bpm and of the untrained horses 113 bpm. During echocardiography and in the recovery phase, the trained horses showed a significantly faster decrease in heart rate than did the untrained horses (Table 2).

### Blood lactate concentration before and after exercise

Blood lactate concentrations obtained from all 15 horses before and after treadmill exercise are listed in Fig. 2. Mean value of blood lactate concentration at rest was  $1.2 \text{ mmol l}^{-1}$  for both conditioned and unconditioned horses. The group of trained horses had a significantly ( $P = 0.035$ ) lower blood lactate concentration ( $2.39 \text{ mmol l}^{-1}$ ) after exercise than the group of untrained horses ( $3.81 \text{ mmol l}^{-1}$ ).

### Echocardiography

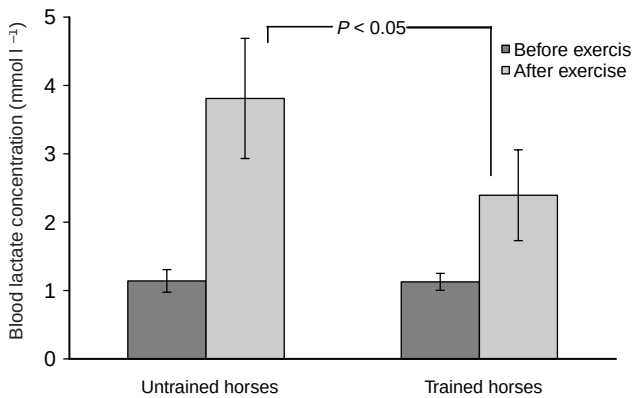
#### Differences of echocardiographic parameters between rest and after exercise in conditioned and in unconditioned horses

Within the group of trained horses, most of the echocardiographic parameters showed a significant difference between values obtained at rest and after exercise (66%). In the group of untrained horses, only 24% of all echocardiographic parameters were significantly different between rest and after exercise. Most of the significant differences were found in the diastolic measurements (Table 3).

**Table 2** Mean heart rate (HR; least square means adjusted to weight) of conditioned and of unconditioned horses during and after treadmill exercise

| Exercise              | Time     | Untrained horses' heart rate, LSmeans $\pm$ SE (bpm) | Trained horses' heart rate, LSmeans $\pm$ SE (bpm) | P-value |
|-----------------------|----------|------------------------------------------------------|----------------------------------------------------|---------|
| $1.8 \text{ ms}^{-1}$ | $t_1$    | $48.4 \pm 3.6$                                       | $43.0 \pm 2.7$                                     | n.s.    |
| $1.8 \text{ ms}^{-1}$ | $t_2$    | $68.4 \pm 4.9$                                       | $61.4 \pm 3.7$                                     | n.s.    |
| $1.8 \text{ ms}^{-1}$ | $t_3$    | $62.6 \pm 3.7$                                       | $67.5 \pm 2.7$                                     | n.s.    |
| $4 \text{ ms}^{-1}$   | $t_4$    | $69.1 \pm 3.8$                                       | $67.6 \pm 2.8$                                     | n.s.    |
| $4 \text{ ms}^{-1}$   | $t_5$    | $119.4 \pm 10.0$                                     | $113.6 \pm 7.5$                                    | n.s.    |
| $4 \text{ ms}^{-1}$   | $t_6$    | $113.1 \pm 8.3$                                      | $113.4 \pm 6.3$                                    | n.s.    |
| $5 \text{ ms}^{-1}$   | $t_7$    | $113.5 \pm 8.0$                                      | $113.1 \pm 6.0$                                    | n.s.    |
| $5 \text{ ms}^{-1}$   | $t_8$    | $134.2 \pm 6.6$                                      | $123.1 \pm 5.0$                                    | n.s.    |
| $5 \text{ ms}^{-1}$   | $t_9$    | $127.1 \pm 5.8$                                      | $132.1 \pm 4.3$                                    | n.s.    |
| $6 \text{ ms}^{-1}$   | $t_{10}$ | $126.2 \pm 5.9$                                      | $131.3 \pm 4.4$                                    | n.s.    |
| $6 \text{ ms}^{-1}$   | $t_{11}$ | $131.4 \pm 6.4$                                      | $136.8 \pm 4.8$                                    | n.s.    |
| $6 \text{ ms}^{-1}$   | $t_{12}$ | $128.4 \pm 8.3$                                      | $139.6 \pm 6.2$                                    | n.s.    |
| $7 \text{ ms}^{-1}$   | $t_{13}$ | $129.2 \pm 8.0$                                      | $147.4 \pm 6.0$                                    | n.s.    |
| $7 \text{ ms}^{-1}$   | $t_{14}$ | $144.9 \pm 13.0$                                     | $142.8 \pm 10.0$                                   | n.s.    |
| $7 \text{ ms}^{-1}$   | $t_{15}$ | $146.3 \pm 11.0$                                     | $150.9 \pm 8.0$                                    | n.s.    |
| $8 \text{ ms}^{-1}$   | $t_{16}$ | $143.9 \pm 12.0$                                     | $159.5 \pm 9.0$                                    | n.s.    |
| $8 \text{ ms}^{-1}$   | $t_{17}$ | $163.2 \pm 9.0$                                      | $162.4 \pm 7.0$                                    | n.s.    |
| Stop                  | $t_{18}$ | $144.8 \pm 3.0$                                      | $137.1 \pm 2.0$                                    | n.s.    |
| Examination           | $t_{19}$ | $117.8 \pm 3.0$                                      | $108.6 \pm 2.0$                                    | 0.032   |
| Examination           | $t_{20}$ | $109.1 \pm 1.4$                                      | $101.7 \pm 1.0$                                    | 0.0025  |
| Walk                  | $t_{21}$ | $84.6 \pm 2.5$                                       | $71.9 \pm 1.8$                                     | 0.0043  |

## Left ventricular dimensions and function



**Fig. 2** Least square means of blood lactate concentration (adjusted to weight) with 95% confidence limits before and after treadmill exercise in conditioned and unconditioned horses

*Comparison of echocardiographic parameters at rest and after exercise between conditioned and unconditioned horses*

At rest and after exercise, the systolic echocardiographic parameters showed no significant differences between the trained and untrained horses. Only a few diastolic measurements (i.e. left ventricular diameter at the *apex cordis*, left ventricular free wall

at papillary muscle level and the left ventricular volume) showed significant differences between both groups at rest.

The results of the echocardiographic examination at rest and after treadmill exercise are listed in Table 4. The parameters of the regional left ventricular function (M-mode) were also not significantly different between both groups (Table 4).

## Discussion

It is a known fact that physical exercise improves cardiac function in humans<sup>11</sup> and that the level of fitness leads to a change in the parameters of cardiac function not only during exercise, but also at rest<sup>8</sup>.

In the present study, mean maximum heart rates of 163 bpm were recorded. In comparison to the documented peak heart rates of 190 bpm during showjumping and of 154 during dressage competition in warmbloods<sup>12</sup>, treadmill exercise was considered to be an appropriate cardiac stress test in both trained and untrained warmblood horses. No significant differences between heart rates of the unconditioned and

**Table 3** Differences in echocardiographic parameters (least square means and standard error adjusted to weight) within the trained and untrained horses between rest and treadmill exercise (*P*, statistic deviation; n.s., not significant)

| Parameter                  | Hp | Untrained LSmeans ± SE | <i>P</i> -value | Trained LSmeans ± SE | <i>P</i> -value |
|----------------------------|----|------------------------|-----------------|----------------------|-----------------|
| IVS A (mm)                 | D  | -4.0 ± 2.4             | n.s.            | -2.6 ± 1.8           | n.s.            |
|                            | S  | -0.4 ± 2.0             | n.s.            | -7.3 ± 1.5           | 0.0004          |
| IVS PPM (mm)               | D  | -3.8 ± 1.5             | 0.025           | -3.7 ± 1.1           | 0.006           |
|                            | S  | -4.2 ± 1.9             | 0.05            | -5.9 ± 1.5           | 0.0016          |
| IVS uMV (mm)               | D  | -0.9 ± 1.3             | n.s.            | -0.3 ± 1.0           | n.s.            |
|                            | S  | 1.5 ± 3.0              | n.s.            | -4.0 ± 2.3           | n.s.            |
| LV A (mm)                  | D  | 4.8 ± 5.9              | n.s.            | 18.3 ± 4.4           | 0.0014          |
|                            | S  | 9.1 ± 4.3              | n.s.            | 5.9 ± 3.2            | n.s.            |
| LV PPM (mm)                | D  | 8.5 ± 5.3              | n.s.            | 20.0 ± 4.0           | 0.0003          |
|                            | S  | 11.5 ± 5.4             | n.s.            | 7.36 ± 4.1           | n.s.            |
| LV uMV (mm)                | D  | 11.3 ± 5.2             | 0.0499          | 15.0 ± 4.0           | 0.0024          |
|                            | S  | 8.1 ± 3.2              | 0.025           | 5.0 ± 2.4            | n.s.            |
| LVW A (mm)                 | D  | 1.1 ± 2.6              | n.s.            | -2.4 ± 1.9           | n.s.            |
|                            | S  | -3.9 ± 2.1             | n.s.            | -7.6 ± 1.6           | 0.0005          |
| LVW PPM (mm)               | D  | 0.2 ± 2.9              | n.s.            | -6.7 ± 2.2           | 0.010           |
|                            | S  | -5.7 ± 3.1             | n.s.            | -8.9 ± 2.4           | 0.0025          |
| LVW uMV (mm)               | D  | 0.3 ± 1.6              | n.s.            | -3.4 ± 1.2           | 0.0158          |
|                            | S  | -2.8 ± 4.4             | n.s.            | -4.8 ± 3.3           | n.s.            |
| LD LV (mm)                 | D  | 14.1 ± 5.5             | 0.025           | 22.3 ± 4.1           | 0.0002          |
|                            | S  | 9.6 ± 6.5              | n.s.            | 21.3 ± 4.9           | 0.0009          |
| Area LV (cm <sup>2</sup> ) | D  | 14.6 ± 5.1             | 0.015           | 20.6 ± 3.8           | 0.0002          |
|                            | S  | 75.0 ± 6.3             | n.s.            | 61.0 ± 4.2           | n.s.            |
| Volume LV (ml)             | D  | 110.0 ± 64.0           | n.s.            | 142.0 ± 48.0         | 0.013           |
|                            | S  | 420.0 ± 64.0           | n.s.            | 309.0 ± 25.0         | n.s.            |
| FS A (%) <sup>a</sup>      |    | -6.1 ± 1.1             | n.s.            | -17.1 ± 1.1          | 0.0043          |
| FS PPM (%) <sup>a</sup>    |    | -6.4 ± 1.1             | n.s.            | -11.3 ± 1.1          | 0.0009          |
| FS uMV (%) <sup>a</sup>    |    | -7.4 ± 1.1             | n.s.            | -5.3 ± 1.1           | n.s.            |
| EF (%) <sup>a</sup>        |    | -3.2 ± 1.1             | n.s.            | 3.6 ± 1.0            | n.s.            |
| SV (ml)                    |    | 108.0 ± 93.7           | n.s.            | 348.0 ± 70.5         | 0.0003          |

IVS, interventricular septum; LV, left ventricle; LVW, left ventricular free wall; LD LV, left ventricular longitudinal diameter; FS, fractional shortening; EF, ejection fraction; SV, stroke volume; uMV, below the mitral valve; PPM, at papillary muscle level; A, at the level of the apex of the heart; Hp, heart phase; D, end-diastolic; S, end-systolic.

<sup>a</sup>Model calculations were performed after logarithmic transformation; results were reported after taken exponentials.



**Table 4** Differences in echocardiographic parameters (least square means and standard error adjusted to weight) between trained and untrained horses before and after treadmill exercise (*P*, statistic deviation, n.s., not significant)

| Parameter                  | Hp | Data at rest           |                      |                 | Data after exercise    |                      |                 |
|----------------------------|----|------------------------|----------------------|-----------------|------------------------|----------------------|-----------------|
|                            |    | Untrained LSmeans ± SE | Trained LSmeans ± SE | <i>P</i> -value | Untrained LSmeans ± SE | Trained LSmeans ± SE | <i>P</i> -value |
| IVS A (mm)                 | D  | 24 ± 1.5               | 25 ± 1.4             | n.s.            | 28 ± 2.4               | 28 ± 1.8             | n.s.            |
|                            | S  | 48 ± 3.0               | 44 ± 2.0             | n.s.            | 48 ± 4.0               | 52 ± 3.0             | n.s.            |
| IVS PPM (mm)               | D  | 34 ± 1.8               | 31 ± 1.3             | n.s.            | 38 ± 1.9               | 35 ± 1.4             | n.s.            |
|                            | S  | 49 ± 2.0               | 45 ± 2.0             | n.s.            | 54 ± 3.0               | 50 ± 2.0             | n.s.            |
| IVS uMV (mm)               | D  | 23 ± 1.5               | 24 ± 1.1             | n.s.            | 24 ± 1.4               | 24 ± 1.0             | n.s.            |
|                            | S  | 31 ± 3.0               | 30 ± 1.8             | n.s.            | 29 ± 2.4               | 34 ± 1.8             | n.s.            |
| LV A (mm)                  | D  | 64 ± 3.6               | 82 ± 2.7             | 0.005           | 59 ± 5.0               | 64 ± 4.0             | n.s.            |
|                            | S  | 27 ± 5.2               | 36 ± 4.0             | n.s.            | 18 ± 4.4               | 30 ± 3.3             | n.s.            |
| LV PPM (mm)                | D  | 96 ± 3.4               | 105 ± 2.5            | n.s.            | 88 ± 5.0               | 85 ± 4.0             | n.s.            |
|                            | S  | 42 ± 5.8               | 53 ± 4.4             | n.s.            | 30 ± 6.6               | 45 ± 4.9             | n.s.            |
| LV uMV (mm)                | D  | 123 ± 2.2              | 129 ± 1.6            | n.s.            | 111 ± 5.5              | 114 ± 4.0            | n.s.            |
|                            | S  | 86 ± 5.0               | 88 ± 3.8             | n.s.            | 77 ± 4.0               | 83 ± 3.0             | n.s.            |
| LVW A (mm)                 | D  | 30 ± 2.0               | 25 ± 1.6             | n.s.            | 29 ± 3.0               | 27 ± 2.2             | n.s.            |
|                            | S  | 46 ± 4.3               | 39 ± 3.2             | n.s.            | 50 ± 3.3               | 47 ± 2.5             | n.s.            |
| LVW PPM (mm)               | D  | 33 ± 2.0               | 26 ± 1.5             | 0.044           | 33 ± 3.0               | 33 ± 2.0             | n.s.            |
|                            | S  | 51 ± 4.5               | 43 ± 3.4             | n.s.            | 56 ± 2.7               | 51 ± 2.0             | n.s.            |
| LVW uMV (mm)               | D  | 23 ± 1.0               | 22 ± 1.0             | n.s.            | 23 ± 1.0               | 25 ± 1.0             | n.s.            |
|                            | S  | 33 ± 3.5               | 33 ± 2.6             | n.s.            | 36 ± 2.4               | 38 ± 1.8             | n.s.            |
| LD LV (mm)                 | D  | 162 ± 4.0              | 158 ± 3.0            | n.s.            | 148 ± 5.0              | 136 ± 4.0            | n.s.            |
|                            | S  | 120 ± 6.7              | 131 ± 5.0            | n.s.            | 110 ± 6.4              | 110 ± 5.0            | n.s.            |
| Area LV (cm <sup>2</sup> ) | D  | 156 ± 5.5              | 171 ± 4.0            | n.s.            | 128 ± 6.0              | 127 ± 5.0            | n.s.            |
|                            | S  | 75 ± 6.3               | 85 ± 4.7             | n.s.            | 61 ± 4.2               | 64 ± 3.2             | n.s.            |
| Volume LV (ml)             | D  | 1155 ± 68              | 1390 ± 51            | 0.037           | 935 ± 87               | 900 ± 65             | n.s.            |
|                            | S  | 420 ± 64               | 458 ± 48             | n.s.            | 309 ± 25               | 316 ± 18             | n.s.            |
| FS A (%) <sup>a</sup>      |    | 71 ± 1.0               | 57 ± 1.0             | 0.046           | 77 ± 1.0               | 74 ± 1.0             | n.s.            |
| FS PPM (%) <sup>a</sup>    |    | 58 ± 1.0               | 47 ± 1.0             | 0.004           | 64 ± 1.1               | 58 ± 1.0             | n.s.            |
| FS uMV (%) <sup>a</sup>    |    | 32 ± 1.1               | 30 ± 1.1             | n.s.            | 39 ± 1.1               | 36 ± 1.1             | n.s.            |
| EF (%) <sup>a</sup>        |    | 64 ± 1.1               | 67 ± 1.0             | n.s.            | 67 ± 1.1               | 64 ± 1.0             | n.s.            |
| SV (ml)                    |    | 734 ± 36               | 932 ± 28             | 0.003           | 627 ± 80               | 583 ± 60             | n.s.            |

IVS, interventricular septum; LV, left ventricle; LVW, left ventricular free wall; LD LV, left ventricular longitudinal diameter; FS, fractional shortening (long axis); uMV, below the mitral valve; PPM, at papillary muscle level; A, at the level of the apex of the heart; EF, ejection fraction; SV, stroke volume; Hp, heart phase; D, end-diastolic; S, end-systolic.

<sup>a</sup> Model calculations were performed after logarithmic transformation; results were reported after taken exponentials.

the conditioned horses were detected during exercise in our study. Immediately after cessation of the exercise protocol, the heart rate dropped remarkably within minutes. This phenomenon has also been described by several other authors<sup>4,19,20</sup>. Furthermore, and in conformity with some published data<sup>4,21,22</sup>, the conditioned horses in the present study showed a significantly faster decrease in heart rate during recovery period than did the unconditioned ones. Other authors found contradictory results<sup>9,11,17</sup>. In their studies, a difference in heart rate decrease during recovery between trained and untrained horses was not observed. The reason for this opposing result could be differences in the state of condition and/or exercise levels, but it should also be taken into account that the individual variation between horses could also influence the differences which we have seen between trained and untrained horses.

In humans, training leads to a decrease in heart rate at rest, which is described as exercise-induced bradycardia (an increase in vagal-induced cardiac modulation leads to a decrease in heart rate at rest)<sup>23</sup>. Neither in the study described here nor in other published equine studies<sup>11,17,21,22</sup> was an exercise-induced bradycardia observed in trained horses at rest.

In order to minimize a possible influence of the heart rate on left ventricular dimensions, as is described in humans<sup>24</sup>, echocardiography was performed within 2 min after exercise and with the heart rate at about 100 bpm, as described by other authors. Such examination procedure requires an experienced examiner who is confident with echocardiographic techniques and who has examined the horse echocardiographically at rest before exercise was carried out.

In human athletes, training induces compensatory changes in cardiac dimensions and function. They are considered a physiological remodelling, resulting in a hypertrophy of the heart muscle cells<sup>25</sup>. Regarding the clinical relevance, it may be difficult to distinguish such a training-induced hypertrophy from a pathological cardiomyopathy and vice versa<sup>26</sup>.

The results of this study show that training affects echocardiographic parameters of left ventricular dimensions and left ventricular function in three-day event warmblood horses moderately at rest. Only some of the diastolic B-mode parameters and some of the M-mode parameters were significantly different between conditioned and unconditioned horses. Particularly, the diastolic left ventricular volume, the stroke volume and the left ventricular diameter at the *apex cordis* were significantly greater in the conditioned horses at rest. This could imply an exercise-induced cardiac remodelling with an increased filling of the left ventricle at rest, as described in humans. In human athletes, the stroke volume at rest is generally greater than in

untrained people<sup>27</sup>. In equine science, an increase in stroke volume in trained yearling Thoroughbred racehorses has also been described<sup>28</sup>, but this increase might have been attributed to growth rather than to training. In our study, we also found a significantly greater stroke volume at rest (27%) in the conditioned three-day event horses in comparison to the unconditioned ones. As the possible effect of growth has been excluded, this must be an effect of training, which results in an increased systolic and diastolic left ventricular volume.

It is controversially discussed whether the greater cardiac function of well-trained human athletes during exercise includes an improvement in systolic and/or diastolic parameters. Some authors consider an improved cardiac function under stress load solely a consequence of changes in systolic parameters, e.g. an increased ejection fraction<sup>26,27</sup>. Other authors, however, did not find any difference in ejection fraction between trained and untrained people at rest and during exercise<sup>28</sup>. They support the theory that there exists a correlation between changes in diastolic parameters and improvement in cardiac function in human athletes<sup>28-30</sup>. In the present study, no changes were determined in the ejection fraction either at rest or during exercise in the trained compared to the untrained horses.

Depending on the type of exercise and the subsequent extent and intensity of cardiac stress load, different changes in cardiac dimensions after exercise have been described in humans<sup>31</sup>. In top-performance athletes, a greater thickness of the left ventricular wall was documented. In human endurance athletes not only a slight increase in thickness of the left ventricular wall, but also a symmetric enlargement of both ventricles and both atria were described in trained individuals<sup>31</sup>. In horses, a similar phenomenon may exist.

In equine science, data published about echocardiographic changes immediately after exercise are controversial. On the one hand, a significant increase in diastolic left ventricular diameter, fractional shortening and ejection fraction was described after treadmill exercise in horses<sup>19</sup>.

On the other hand, no changes in left ventricular diameter were observed directly after treadmill exercise under standardized conditions in five horses<sup>32</sup>. In conformity to our study, Sampson *et al.* also observed neither changes in left ventricular diameter, in septal thickness nor in thickness of the left ventricular posterior wall in six Thoroughbred racehorses after treadmill exercise (heart rate > 100 bpm)<sup>1</sup>. However, in a study with healthy Standardbreds a positive effect of training was observed on echocardiographically measured cardiac sizes and function immediately after an incremental exercise after 8 weeks' training<sup>13</sup>.

In the present study, thickness of the left ventricular free wall (at papillary muscle level) and the FS (%) at the *apex cordis* and at papillary muscle level were significantly decreased at rest in the trained horses, but a significantly greater increase in fractional shortening at the *apex cordis* was observed after exercise in the trained horses (23%) compared to the untrained ones (8%). Our results at the *apex cordis* are in conformity with a study about bicycle exercise in humans in which an increase in FS (from 29 to 47%), and thus an increase in myocardial contractility, is described immediately after exercise<sup>26</sup>.

Examination of myocardial contractility (FS) at papillary muscle level and at the *apex cordis* has not been carried out before in horses, neither at rest nor after exercise. Thus, information and experiences of other examiners about this myocardial area are lacking. Nevertheless additional measurements of myocardial contractility at different locations of the left ventricle seem to be helpful in graduating general left ventricular function, especially after exercise. Standardization of measurement positions and establishment of norm values of this additional 'non-standard' M-mode measurement are necessary to evaluate its further usefulness.

After exercise, measurement of FS (% below the mitral valve) showed no differences between trained and untrained horses in the present study. This stands in contrast to a study in which an increase in mean FS (%) immediately after treadmill exercise was observed in Thoroughbreds<sup>33</sup>. One reason for this and a reason for the general lack of significant differences in echocardiographic parameters after exercise could be that the differences in training status between three-day event warmbloods and untrained warmbloods were probably not compared significantly enough to more athletic trained and untrained Thoroughbreds. Another reason for the lack of difference in echocardiographic parameters between three-day-event horses and the untrained ones, especially after exercise, could be due to the stress level, which is probably not strong enough to reveal significant differences. Additionally, the heart rate of the trained horses dropped more rapidly during examination than the heart rate of the untrained ones, and this could also have an influence on echocardiographic parameters. A correlation of echocardiographic parameters to heart rate could be helpful in avoiding this effect.

Also to be taken into account should be the combination of post-exercise stress echocardiography with other cardiac values, e.g. exercising right ventricular pressure dynamics<sup>34</sup>.

One limitation of our study is that the horses have not been used as their own control (e.g. untrained horses examined, trained, then re-examined) to ensure similar fitness levels and to decrease the effect of individual variation on measurements between trained and untrained

horses. Individual variations in echocardiographic measurements between trained and untrained horses have been examined and the effect on individual variation of echocardiographic parameters was 14.3% of the whole variation<sup>35</sup>.

In our study the left ventricular volume, stroke volume and the ejection fraction were performed automatically by the Ving Med ultrasound software. Although this software has been established for human cardiology, the methods seems to be suitable for horses in determining intra- and inter-individual variations between different training groups, as in our study. For measurement of quantitative real values this could be a limitation for use in equine cardiology.

Despite the need of further investigations, the present study demonstrates that the level of fitness in three-day event warmblood horses has only minimal effect on echocardiographic parameters after treadmill exercise.

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