Received: 2 July 2024 | Accepted: 5 November 2024

DOI: 10.1111/jvim.17253

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE

Equine Cardiology

A consort-guided randomized, blinded, controlled clinical trial on the effects of 6 weeks training on heart rate variability in thoroughbred horses

Emma Santosuosso[1] | Renaud Léguillette[1] | Sierra Shoemaker[2] | Ryan Baumwart[2] | Sierra Temple[2] | Kaneesha Hemmerling[2] | Tessa Kell[2] | Warwick Bayly[2]

[1]Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada

[2]Department of Veterinary Clinical Sciences, Washington State University, Pullman, Washington, USA

Correspondence
Renaud Léguillette, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.
Email: rleguill@ucalgary.ca

Funding information
Calgary Chair in Eqiune Sports Medicine, University of Calgary

Abstract

Background: Fitness assessment of horses remains challenging. Heart rate variability (HRV) can be used to monitor human athlete's training, but its value is unknown in horses.

Hypothesis: The linear domain HRV variables are affected by fitness.

Animals: Twelve healthy untrained thoroughbreds were randomly split into a training group (6 weeks of incremental racetrack training) and a control group (no training).

Methods: Linear domain HRV variables were analyzed (high frequency [HF], low frequency [LF], their normalized units [Hf_{nu}, Lf_{nu}], root mean square of successive differences between beats [RMSSD], Poincaré plot features [SD1 and SD2]) while resting overnight before (baseline) and after 2, 4 and 6 weeks of training. $\dot{V}O_{2max}$ and echocardiographic indexes were measured at baseline and after 6 weeks. Changes in HRV variables over time (ANOVA), and correlation with $\dot{V}O_{2max}$ (Pearson's chi-squared test) were tested ($P < .05$ significance).

Results: $\dot{V}O_{2max}$, LF/HF ratio, and LF_{nu} increased while HF_{nu} decreased in the training group (before and after training mean [SD] values: $\dot{V}O_{2max}$ 134 [12.8]-146 [16.5] mL/[kg min]; $P < .001$; LF/HF ratio 0.51 [0.2]-0.91 [0.3] [$P = .02$]; LF_{nu} 37.5 [10.1]-46.8 [7.8] [$P = .02$]; HF_{nu} 76.2 [7.9]-53.2 [7.7] [$P < .001$]). Training did not affect RMSSD, SD1, SD2, resting HR, or echocardiographic indexes. Strong correlations were found between $\dot{V}O_{2max}$ and HRV variables ($\dot{V}O_{2max}$ and LF_{nu} [$r = -0.59$, $P = .04$]; $\Delta\dot{V}O_{2max}$ and the corresponding ΔLF [$r = -0.88$, $P = .02$]).

Conclusions and Clinical Importance: Six weeks of training affected some frequency domain HRV variables. Further studies are necessary to validate the use of HRV for monitoring horses' responses to training.

Abbreviations: AAD, aortic annular dimension before systole; ANOVA, one-way analysis of variation; ANS, autonomic nervous system; BAV2, second-degree atri-ventricular block; CG, control group; ECG, echocardiograms; FS %, fractional shortening; HF, high frequency; HF_{nu}, normalized units high frequency; HRV, heart rate variability; IVSs, interventricular septum during systole; LADmax, left atrial dimensions maximum; LADmin, left atrial dimensions minimum; LF, low frequency; LF_{nu}, normalized units low frequency; LVFWd, left ventricular free wall systole; LVFWs, left ventricular free wall systole; LVIDd, left ventricular internal dimensions during diastole; LVIDs, left ventricular internal dimensions during systole; RMSSD, root mean squares of successive R-R intervals; SD1, SD perpendicular to the line of identity; SD2, SD of a Poincaré plot along the line of identity; SET, standardized exercise tests; TG, trained group; V200, velocity at which heart rate reaches 200 beats per min; VLA4, velocity at which lactate reaches 4 mmol/L.

KEYWORDS
echocardiography, fitness, racetrack conditioning, training, $\dot{V}O_{2max}$

1 | INTRODUCTION

Several methods have been proposed to assess equine athletes' fitness under field or laboratory conditions.[1-15] Fitness testing protocols commonly use incremental speed standardized exercise tests (SET) to estimate physiological variables like VLA_4 (velocity at which lactate reaches 4 mmol/L) and V_{200} (velocity at which heart rate reaches 200 beats per min).[2,5,6,12,16-19] However, the requirements of a standardized workload necessary for these tests are difficult to meet under field conditions.

Heart rate variability (HRV) reflects fluctuations in the interval between consecutive heartbeats, and various HRV variables are usually assessed noninvasively by analyzing R-R intervals on electrocardiograms. Variability in heart rate intervals is a normal phenomenon that occurs secondary to variations in the balance between the sympathetic and parasympathetic (vagal) contributions to the autonomic nervous system (ANS) control of the heart rate at rest.[20-23]

Algorithms have been developed to analyze HRV using linear (which includes time and frequency HRV indexes) and nonlinear domain measurements.[24] In time-domain analysis, indexes are derived from the analysis of consecutive R-R intervals. Such indexes include the SD of R-R intervals (SD perpendicular to the line of identity, SD1, and SD of a Poincaré plot along the line of identity, SD2) and the root mean squares of successive R-R intervals (RMSSD).[25] Frequency domain indexes have bands that are specific to each species and include the low frequency (LF; 0.01-0.07 Hz for horses) and the high frequency (HF; 0.07-0.6 Hz for horses) bands of HRV.[20] The LF/HF ratio and the relative power of LF and HF expressed in normalized units (LF_{nu}, HF_{nu}) are based on spectral analysis and represent the sympathovagal ANS balance at rest in humans.[25]

Some HRV variables are a practical way to evaluate athletic fitness in humans[25-30] with HRV changes in linear domain indexes observed after training and with improvements in physical performance in some disciplines.[29,31,32] Similarly, HRV changes occur in horses after training,[20,21,23,33] and greater LF/HF ratios were associated with better racing results.[34] However, although fitness was assumed to improve with training, the horses' fitness level was not objectively measured[20,23,33] preventing the analysis of potential correlations between aerobic capacity and HRV variables.

Therefore, the objective of the present study was to quantify, in a randomized controlled trial, the ANS's response to standardized training using linear analysis of HRV and to compare it to a traditional reference indicator of fitness ($\dot{V}O_{2max}$). We hypothesized that a training-induced increase in $\dot{V}O_{2max}$ (measured in a SET) would be correlated with changes in the HRV variables (measured at rest) in the frequency domain (with an increase in LF and a decrease in HF) but not in the time domain.

2 | MATERIALS AND METHODS

2.1 | Horses

Twelve thoroughbred racehorses retired from racing (8 geldings, 4 mares; mean age 8.5 ± 6.4 years) owned by the College of Veterinary Medicine, Washington State University were enrolled. Inclusion criteria were the absence of abnormal findings on clinical examination, echocardiographic examinations, and complete blood cell count. An additional criterion was not having been trained for at least a year. The sample size was based on previous training and $\dot{V}O_{2max}$ improvement data collected in the same environment ($P = .05$, power = 0.80). The horses were randomly divided into a trained group (TG, n = 6) and a control group (CG, n = 6) by one of the authors (WB) using the RAND function in Microsoft Excel. The persons who analyzed the measured outcomes (ES, RL, RB) were blinded to the grouping of the horses. All horses lived together in small paddocks for the duration of the study except for when the TG horses were training, or horses were scheduled for the recording of their ECG during the night.

2.2 | Experimental setting

This was a prospective, randomized, blinded, and controlled study using a parallel group design performed at the College of Veterinary Medicine, Washington State University. All horses had been housed in the same environment for several months before starting the study. The outcomes measured were done in each horse before and after training (or control) and included echocardiography, HRV analysis at rest, and a peak oxygen consumption ($\dot{V}O_{2max}$) assessment during a SET on a high-speed treadmill (see SET protocol below). Measurements obtained before training for both groups were defined as baseline values.

2.3 | Training protocol

After baseline measurements, horses in the TG underwent 6 weeks of training. The training program consisted of ridden exercise 6 days per week on an 800 m racetrack with the intensity of exercise being progressively increased over the 6 weeks period. Weeks 5 and 6 included breezes of 600 and 800 m, respectively, as fast as the horses were capable of running them (>15 m/s; Table 1).

2.4 | Echocardiography

Echocardiographic examination was performed by transthoracic echocardiography and included both 2-dimensional (2D), M-mode evaluation, and color Doppler.[35] Horses were restrained in standard stocks.

TABLE 1 Training program for horses in the training group.

	Warm-up	Fast trot	Canter	Gallop	Slow trot
Week 1	2 laps slow trot	2-3 laps	1-3 laps		0.5-2 laps
Week 2	3 laps slow trot	2 laps	2-3 laps		0.5-2 laps
Week 3	2 laps slow trot	2 laps	2.5-3 laps	1 lap at ≈15 s/200 m	0.5-1 lap
Week 4	2 laps slow trot	2 laps	2-3.5 laps	1.5-3.5 laps, ≈15 s/200 m	0.5-1 lap
Week 5	2 laps slow trot	0-1 lap	1-3 laps	1.5-3.5 laps, ≈15 s/200 m 600 m breeze	0.5-1 lap
Week 6	2 laps	1-2 laps	1-4 laps	1.5-3.5 laps, ≈15 s/200 m 800 m breeze	0.5-1 lap

Note: Protocols varied from day to day and from horse to horse. Overall, the training distance increased each week as did the speed at which the canter and the gallop were completed. Lap distance was 800 m.

Images were obtained from the left and right sides. Left ventricular internal dimensions during systole (LVIDs) and diastole (LVIDd), interventricular septum width during systole (IVSs) and diastole (IVSd), left ventricular free wall thickness in systole (LVFWs) and diastole (LVFWd), and fractional shortening (FS%) were determined from the M-mode study at the left ventricular papillary level. The minimum and maximum left atrial diameters (LADmin, LADmax, respectively), and aortic annular dimension before systole (AAD) were taken from the right parasternal long axis. The examinations were conducted without sedation. All echocardiograms were performed before baseline SET and at the end of the training period.

2.5 | Resting ECG analysis and HRV calculation

Horses were not exercised for 2 days before any ECG data acquisition. Resting ECGs were recorded for 2 hours overnight between 09:00 and 11:00 PM on the night before the SET using a telemetric ECG device (TELEVET 100. Engel Engineering Service GmbH. Software version 7.0.0) at baseline and after 2, 4, and 6 weeks of the training (in the TG) or control periods (in the CG). Nighttime was chosen to reduce environmental stress and barn activity (feeding and traffic) that could possibly have affected HRV data. A modified resting base/apex ECG electrode configuration was used with an elastic chest band covering the equipment.[36] The horse's identity was removed from the ECGs and they were first analyzed for arrhythmias (ES and RL) and the number of second-degree atrioventricular blocks (BAV2) was reported. The R-R intervals were detected using the ECG software function (TELEVET. Version 7.0.2 Build 002, Engel Engineering Services GmbH, Germany) and manually verified, and data were then exported and downloaded to an HRV analysis software (KUBIOS. Version 2.2. Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The frequency range of the LF component of HRV was set to be between 0.01 and 0.07 Hz and the HF analysis was set between 0.07 and 0.6 Hz as previously described.[20] Based on previously reported ECG analysis recommendations,[37] a "low" artifact correction filter (0.35 s) from the KUBIOS software was applied. Mean HR was obtained and RMSSD, SD1, SD2, LF and HF domain of HRV, LF/HF ratio and LF_{nu}, HF_{nu} were calculated.

2.6 | Standardized exercise test and $\dot{V}O_2$ measurement

On the morning following the recording of the baseline and end of training (6 weeks) ECGs, $\dot{V}O_{2max}$ was measured using a SET on a high-speed treadmill inclined at 10% using a previously validated ergospirometry mask.[38] The horses had all been acclimated to the treadmill and mask before the study. After a 4-min warm-up period at 4 m/s, the SET consisted of 1 minute incremental bouts of exercise at 7, 9, 10, 11, and 12 m/s or until the horse could not maintain the speed despite strong verbal encouragement. Calibration of the ergospirometry system (flowmeter and oxygen sensor) was conducted immediately before each SET, and oxygen consumption was measured continuously during the exercise. The $\dot{V}O_2$ values were calculated for the speed for which the full minute was completed by the horses to determine $\dot{V}O_{2max}$ as previously described.[10] Heart rate and ECG were continuously recorded during the SET. The speed at which $\dot{V}O_{2max}$ was reached was calculated using linear regression of the linear portion of the $\dot{V}O_2$-speed curve.

2.7 | Statistical analysis

Statistical analysis was conducted (GraphPad Prism 9.3.1 GraphPad Software LLC) after testing the normality of the data distribution with the Shapiro-Wilk test. Before and after the training period HR, HRV indicators, echocardiographic indices, and $\dot{V}O_{2max}$ data for TG and CG were compared using a paired *t* test, and the respective effect size was calculated using Cohen's d. A Wilcoxon signed rank test was performed to compare the number of BAV2 between the ECGs over time. A Pearson correlation was used to assess the correlation between the changes in $\dot{V}O_{2max}$ and each HRV variable. Changes in HRV variables over time were tested with a one-way analysis of variation (ANOVA). A *P*-value <.05 was

TABLE 2 Physiological data at baseline and after training (6 weeks) in the control group and the training group.

	Control group		Training group	
	Before training (n = 6)	After training (n = 5)	Before training (n = 6)	After training (n = 6)
Resting HR (bpm)	39.8 [3.6]	38.1 [4.4]	36.4 [2.3]	35.7 [2.5]
$\dot{V}O2_{max}$ (mL/[kg min])	134 [12.8]	146 [16.5]	131 [25.9]	151 [20.5][a]
Mean $\dot{V}O2_{max}$ speed (m/s)	11.6 [1.4]	10.9 [0.9]	11.3 [0.4]	11.9 [0.4][a]

Note: Mean [SD] reported.
[a]Indicates a significant difference between baseline and posttraining values.

considered statistically significant. Normally distributed data were reported as mean ± SD.

3 | RESULTS

One horse from the CG developed a lameness before the posttraining treadmill test and was therefore excluded from the study. No other negative effects were noted during the study. Data distribution was normal for all the variables studied.

3.1 | Physiological variables

Resting HR and $\dot{V}O_{2max}$ measured during the SETs are reported in Table 2. A significant increase in $\dot{V}O_{2max}$ was observed after training in the TG (from 131.3 ± 25.8 to 150.5 ± 22.7 mL/[kg min]; $P = .004$) while no change was detected in the CG. The speed at which horses reached $\dot{V}O_{2max}$ increased ($P = .03$) after 6 weeks in the TG (Table 2).

3.2 | Echocardiographic indices

There was no significant change between baseline and posttraining in any echocardiographic measurement in the TG or the CG groups. Cardiac dimension values are reported in Table 3.

3.3 | Resting ECG analysis and HRV calculation

Training was not associated with a change in resting HR in TG, and there was no difference in resting HR between the CG and TG horses after the 6 weeks period (CG: 37.7 bpm ± 2.6; TG: 37.5 bpm ± 4.7; Table 2). No arrhythmias other than the BAV2s were observed in the ECGs. There was no difference in the number of BAV2 between the ECG time points over the study period.

Significant changes in HRV were only observed in the TG, where 5 out of the 6 horses showed an increase in some of the HRV variables. All HRV variables analyzed are reported in Table 4. A significant increase in LF/HF ratio (+38.7%, $P = .04$), LF_{nu} (+32.0%, $P = .02$) and decrease in HF_{nu} (−17.5%, $P = .02$) were noted after 6 weeks training in the TG but not in the CG (Table 4). Effect size was 0.11 for LF/HF ratio, 0.42 for LF_{nu} and 0.42 for HF_{nu}. No other HRV variables (RMSSD, SD1, SD2) changed over the study time in either of the groups.

TABLE 3 Echocardiographic indices in the trained group before and after training (mean [SD] reported).

	Before training	After training	*P* value
LVIDs (mm)	70 [11.0]	75 [13.0]	.51
LVIDd (mm)	106 [11.0]	115 [12.0]	.14
IVSs (mm)	39 [6.1]	43 [3.9]	.56
IVSd (mm)	26 [3.0]	26 [2.9]	.37
LV freeWs (mm)	40 [4.1]	42 [3.4]	.70
LV freeWd (mm)	25 [3.1]	24 [3.7]	.20
LV shortening (%)	0.35 [0.070]	0.37 [0.03]	.74
LA diam max (mm)	113 [8.6]	116 [7.7]	.45
LA diam min (mm)	90 [8.1]	91 [11.0]	.66
AAD (mm)	60 [5.2]	62 [6.7]	.96

Abbreviations: AAD, ascending aorta diameter; IVSd, intraventricular septal thickness end—diastole; IVSs, intraventricular septal thickness at end—systole; LA diam max, maximal left atrial diameter; LA diam min, minimal left atrial diameter; LV freeWd, left ventricular free wall diastolic; LV freeWs, left ventricular free wall systolic; LV, left ventricular; LVIDd, left ventricular internal diametral systolic; LVIDs, left ventricular internal diametral systolic.

There was a strong correlation between $\dot{V}O_{2max}$ and LF_{nu} ($r = -0.59$, $P = .04$) as well as a strong correlation between the changes in $\dot{V}O_{2max}$ (after training − baseline values) and the corresponding changes in LF ($r = -0.88$, $P = .02$).

4 | DISCUSSION

This study collectively evaluates responses of aerobic capacity, echocardiographic indices, and HRV in horses to training. All horses in the TG showed an improvement in their aerobic capacity as reflected by an increase in $\dot{V}O_{2max}$ and the speed at which $\dot{V}O_{2max}$ was reached. The changes in the other cardiac physiological variables (resting HR and echocardiographic indexes) were not significant. This was not unexpected given the previous training history of the horses, the relatively short training period, and high vagal tone associated with resting HR in trained and untrained horses.

TABLE 4 Linear domain variables of HRV at baseline and posttraining (mean [SD] data reported) in the control group and training group.

	Control group				Training group			
	T0	T2weeks	T4weeks	T6weeks	T0	T2weeks	T4weeks	T6weeks
RMSSD	58.6 [13.3]	80.1 [31.1]	79.6 [22.7]	71.1 [36.9]	72.3 [22.2]	73.6 [21.6]	64.9 [17.4]	71.1 [19.5]
SD1	41.5 [9.4]	56.6 [21.9]	56.3 [16.1]	50.2 [26.1]	51.4 [15.7]	52.1 [15.2]	46.0 [12.3]	50.2 [13.8]
SD2	61.1 [13.7]	70.9 [7.9]	76.7 [8.9]	61.5 [11.6]	63.1 [14.7]	66.8 [10.9]	70.2 [15.7]	71.8 [17.3]
LF/HF	0.87 [0.3]	0.72 [0.5]	0.87 [0.6]	0.85 [0.7]	0.51 [0.2][a,b]	0.71 [0.2][a]	0.94 [0.4]	0.91 [0.3][b]
LF_{nu}	54.7 [12.4]	49.8 [26.4]	42.1 [17.4]	40.2 [20.1]	37.5 [10.1][a,b]	50.5 [12.2][a]	53.9 [25.6]	46.8 [7.8][b]
HF_{nu}	66.3 [12.9]	75.9 [12.1]	57.9 [17.5]	59.7 [20.1]	76.2 [7.9][a,b]	73 [5.5]	57.2 [6.1][a]	53.2 [7.7][b]

Note: T0 indicates baseline measurements, T2weeks, T4weeks, and T6weeks are 2, 4, and 6 weeks posttraining time points, respectively. Letters indicate a significant difference between the values indicated with the same letter.

The increases in LF/HF ratio and LF_{nu} that we observed were consistent with reported changes in response to field training in horses.[20,23] In horses, injection with the sympathetic β-blocker, propranolol, enhanced LF and LF/HF ratio,[20] whereas administration of atropine decreased LF and LF/HF ratio in a dose-dependent manner while HF decreased after a dose of atropine.[18,20] Therefore, HF seems to be less sensitive to ANS variation compared to LF. In this sense, the variation in HF domain in response to training was less marked when compared to LF variation in field conditions,[20] and similar results were reported in the present study. Again, the greater vagal tone in horses compared to other species might explain those findings.[39] Our results showing an increase in LF/HF ratio and LF_{nu} were in accordance with an increase in vagal tone and decreased β-receptor stimulation at rest posttraining.[40]

We trained the horses for 6 weeks expecting a measurable improvement in fitness since an increase in $\dot{V}O_{2max}$ typically occurs after 4 weeks of training.[23] We opted to avoid longer duration training to limit the possibility of a decrease in resting HR associated with response to training. Such a decrease in resting HR was observed in one previous HRV study after 7 months of training. As HRV is affected by HR,[24] a decrease due to training would have been likely to bias the HRV analysis in the present study. The fact that resting HR remained unchanged after the 6-week training protocol in the present study, while LF/HF ratio, LF_{nu}, and HF_{nu} decreased, suggests that HRV indices are more sensitive than resting HR as indicators of ANS changes following improvement of aerobic capacity. This might lead to some application of LF/HF ratio, LF_{nu} and HF_{nu} to the monitoring of horses' fitness during training. These variables are from the linear domain of HRV analysis and done on resting ECGs, but further research is necessary to explore the value of nonlinear HRV variables in horses.

Horses in the TG were the only individuals with improvement in $\dot{V}O_{2max}$ and 5 of the 6 trained horses displayed significant HRV changes in response to training. Furthermore, we found a significant correlation between $\dot{V}O_{2max}$ and LF_{nu} and a strong correlation between the changes in $\dot{V}O_{2max}$ associated with 6 weeks training and the corresponding changes in LF. The fact that such associations were found for LF but not for HF variables might be due to their different response to training. Low frequency is affected by both the orthosympathetic and parasympathetic systems' tone compared to the HF domain which reflects more specifically the parasympathetic tone in horses[18] Atropine administration to horses affects the LF domain first.[22] Altogether these results suggest the possibility that LF might change more than HF, or earlier than HF during training, possibly due to some additional effect of the orthosympathetic system on LF.

A challenge when evaluating HRV following interventions like training is the many sources of variation that could interfere with the analysis. For example, a horse's ANS is influenced by its age, breed, and sex.[22] After some months in training, ANS of young horses might be affected both by their growth and the improvement in their aerobic capacity. Therefore, growth might bias the results and including young horses in HRV studies over a long period should be carefully considered. Furthermore, field studies during a racing season should take into account stress and fatigue as potential confounding factors in the analysis. In humans, fatigue and stress factors[41] have a marked impact on frequency domain HRV variables (both LF and HF). It is also for this reason that in other species, including cows and horses, HRV has also been used to assess the animals' stress level.[42-44]

A lack of standardization represents the main limitation when comparing HRV data between studies in the veterinary field. In addition to a lack of standardization of the analysis settings, practical aspects such as the time of day and the duration of ECG recordings for HRV analysis have been controversial. Analysis of ECGs recorded overnight is known to be characterized by greater parasympathetic tone.[20] However, there is no consensus about the best time of day or night during which to record ECGs for subsequent HRV analysis. In the present study, ECG data acquisition was repeated under the same conditions with respect to location, time of day, and duration of recording before and after the training period. The recording duration of 2 hours during the late evening period was chosen to decrease HRV variability since the horses had been fed over 4 hours before the recording time and there was limited human activity.

The use of software to calculate HRV has also been questioned due to the lack of clear recommendations regarding the standardization of software settings.[45] As a result, HRV studies continue to report results using different or even undisclosed ranges of frequencies for the HF and LF domains[20,23] which is a source of variability between studies. In human medicine,[20] efforts have been made to standardize HRV methodology and interpretation. We felt that it was important to use and report settings that had been previously described in equine studies.[20,37,45] Thus, we elected to use the range of 0.07 to 0.6 Hz for HF and 0.01 to 0.07 Hz for LF although they are different to the ranges utilized in HRV studies involving people.

Arrhythmias displayed during the ECG recording are also likely to affect HRV analysis. Although resting arrhythmias might be secondary to changes in ANS tone, they might affect HRV calculations.[43,45] This is particularly true for arrhythmias affecting R-R intervals, like BAV2, as can be identified in the Poincaré plot.[45] One approach proposed to circumvent this problem was to perform HRV analysis on the P-P intervals,[45] but this is not commonly done. No pathological arrhythmia was observed in the ECGs recorded in the present study, but some horses displayed physiological BAV2. The prevalence of these BAV2 was the same before and after training. However, the clinical relevance of arrhythmias at rest, in recovery, and during exercise on HRV warrants additional documentation in future studies.

The 6 weeks training was not associated with changes in any of the measured echocardiographic variables in the thoroughbreds from the TG. Whether this reflected their previous extensive athletic histories, or the relative brevity of the training period is unclear. Reports on the effects of training on echocardiographic measurements of 2-year-old thoroughbred and standardbred racehorses have indicated that training over periods of 18 weeks to 18 months results in increases in measures of ventricular chamber dimensions and myocardial muscle mass.[46,47] However, we could find no reports on the effects of physical maturity or years of training and racing on echocardiographic measures, and it is possible that the hearts of horses in the TG had already hypertrophied to such an extent that the 6 weeks training period was unlikely to induce a statistically detectable increase. One study conducted echocardiographic assessments of 53 standardbreds when they were aged 2 to 5.5 years.[48] Forty of them had been in training and racing throughout this period while 13 others had raced until they were about 5 years old but had been out of full training for 1-6 months at the time of the last echocardiographic examination. Unfortunately, the results at 3.5 and 5.5 years were not specifically compared. The only difference between the horses still racing at 5.5 years of age and those out of training at that age was in LVIDd but it was not clear if that assessment took into account differences in sex and body weight. Given our sample size, and a power of 80% and $P = .05$, the findings in our study would have been significant had we observed an increase of 12.5 mm between baseline and post-training LVIDd, whereas we noted an increase of 9 mm. We cannot confirm if the training effect is truly 9 mm or if the number of animals was not sufficient to detect a statistically significant difference (type II error). Finally, echocardiogram measurements in our study were all 2D and M-mode linear measurements. Other echocardiographic measurements such as chamber area, chamber volume, strain, and strain rate might have been more sensitive in detecting responses to the 6 weeks of training.

5 | LIMITATIONS

The small number of horses used in the present study was a limitation, notably with respect to data for which we failed to show significant changes between time points, potentially due to lack of statistical power. However, enrolling athletic horses in a controlled training program study presents economical and logistical challenges that were a limiting factor for the number of animals included. This study combined field training of unfit racehorses with state-of-the-art laboratory physiological measurements of fitness. The HRV and physiological results obtained in the present study were in accordance with those of previous publications. Furthermore, to compensate for the low number of animals enrolled, data was compared using a paired design using each horse as its own reference. Additionally, blood pressure was not assessed in either group, and changes in blood pressure would likely affect both heart rate variables and echocardiographic variables.

6 | CONCLUSION

Horses with an improvement in aerobic capacity after racetrack training displayed significant changes in some frequency domain HRV variables recorded at rest for 2 hours in the late evening. Furthermore, this study showed a strong correlation between HRV LF_{nu} and LF variables with $\dot{V}O_{2max}$. Although these results show promising potential applications for HRV in the monitoring of equine athletes' fitness, our sample size was small, and further studies on larger groups with different breeds (eg, standardbred, warmbloods) and disciplines (eg, eventing, endurance) are necessary to validate the benefits of assessing resting HRV for the equine industry.

ACKNOWLEDGMENT

Funding provided by the Calgary Chair in Eqiune Sports Medicine, University of Calgary.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Washington State University IACUC (ASF# 6783).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Emma Santosuosso https://orcid.org/0009-0008-9777-6003
Renaud Léguillette https://orcid.org/0000-0003-0381-3640
Warwick Bayly https://orcid.org/0000-0002-1403-6245

REFERENCES

1. Bayly WM, Gabel AA, Barr SA. Cardiovascular effects of submaximal aerobic training on a treadmill in standardbred horses, using a standardized exercise test. *Am J Vet Res*. 1983;44:544-553.

2. Rose RJ, Hendrickson DK, Knight PK. Clinical exercise testing in the normal thoroughbred racehorse. *Aust Vet J*. 1990;67:345-348.
3. Seeherman HJ, Morris EA. Methodology and repeatability of a standardised treadmill exercise test for clinical evaluation of fitness in horses. *Equine Vet J Suppl*. 1990;20-25.
4. Courouce A. Field exercise testing for assessing fitness in French standardbred trotters. *Vet J*. 1999;157:112-122.
5. Courouce A, Geffroy O, Barrey E, et al. Comparison of exercise tests in French trotters under training track, racetrack and treadmill conditions. *Equine Vet J Suppl*. 1999;528-532.
6. Vermeulen AD, Evans DL. Measurements of fitness in thoroughbred racehorses using field studies of heart rate and velocity with a global positioning system. *Equine Vet J Suppl*. 2006;113-117.
7. Lindner A, Mosen H, Kissenbeck S, et al. Effect of blood lactate-guided conditioning of horses with exercises of differing durations and intensities on heart rate and biochemical blood variables. *J Anim Sci*. 2009;87:3211-3217.
8. Munsters CC, van Iwaarden A, van Weeren R, et al. Exercise testing in warmblood sport horses under field conditions. *Vet J*. 2014;202:11-19.
9. Hiraga A, Sugano S. Studies on exercise physiology of the racehorse performed in Japan during the period from the 1930s to the 1970s: respiration and heart rate during exercise and the effect of exercise on blood characteristics. *J Equine Sci*. 2016;27:37-48.
10. Bond SL, Greco-Otto P, Sides R, et al. Assessment of two methods to determine the relative contributions of the aerobic and anaerobic energy systems in racehorses. *J Appl Physiol*. 1985;2019(126):1390-1398.
11. Navas de Solis C. Cardiovascular response to exercise and training, exercise testing in horses. *Vet Clin North Am Equine Pract*. 2019;35:159-173.
12. Leguillette R, Bond SL, Lawlor K, et al. Comparison of physiological demands in warmblood show jumping horses over a standardized 1.10 m jumping course versus a standardized exercise test on a track. *BMC Vet Res*. 2020;16:182.
13. Kirsch K, Fercher C, Horstmann S, et al. Monitoring performance in show jumping horses: validity of non-specific and discipline-specific field exercise tests for a practicable assessment of aerobic performance. *Front Physiol*. 2021;12:818381.
14. Siegers E, van den Broek J, Sloet van Oldruitenborgh-Oosterbaan M, et al. Longitudinal training and workload assessment in Young Friesian stallions in relation to fitness, Part 2—an adapted training program. *Animals (Basel)*. 2023;13.
15. Titotto AC, Santos MM, Ramos GV, et al. Effect of lactate minimum speed-guided training on the fluid, electrolyte and acid-base status of horses. *Animals (Basel)*. 2023;13.
16. Gottlieb-Vedi M, Persson S, Erickson H, et al. Cardiovascular, respiratory and metabolic effects of interval training at VLA4. *Zentralbl Veterinarmed A*. 1995;42:165-175.
17. Persson SG. Heart rate and blood lactate responses to submaximal treadmill exercise in the normally performing standardbred trotter—age and sex variations and predictability from the total red blood cell volume. *Zentralbl Veterinarmed A*. 1997;44:125-132.
18. Ohmura H, Hiraga A, Aida H, et al. Effects of repeated atropine injection on heart rate variability in thoroughbred horses. *J Vet Med Sci*. 2001;63:1359-1360.
19. Fraipont A, Van Erck E, Ramery E, et al. Assessing fitness in endurance horses. *Can Vet J*. 2012;53:311-314.
20. Kuwahara M, Hashimoto S, Ishii K, et al. Assessment of autonomic nervous function by power spectral analysis of heart rate variability in the horse. *J Auton Nerv Syst*. 1996;60:43-48.
21. Physick-Sheard PW, Marlin DJ, Thornhill R, et al. Frequency domain analysis of heart rate variability in horses at rest and during exercise. *Equine Vet J*. 2000;32:253-262.
22. Ohmura H, Jones JH. Changes in heart rate and heart rate variability as a function of age in thoroughbred horses. *J Equine Sci*. 2017;28:99-103.
23. Nyerges-Bohak Z, Nagy K, Rozsa L, et al. Heart rate variability before and after 14 weeks of training in thoroughbred horses and standardbred trotters with different training experience. *PLoS One*. 2021;16:e0259933.
24. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public Health*. 2017;5:258.
25. Shin K, Minamitani H, Onishi S, et al. Assessment of training-induced autonomic adaptations in athletes with spectral analysis of cardiovascular variability signals. *Jpn J Physiol*. 1995;45:1053-1069.
26. Hedelin R, Wiklund U, Bjerle P, et al. Pre- and post-season heart rate variability in adolescent cross-country skiers. *Scand J Med Sci Sports*. 2000;10:298-303.
27. Baumert M, Brechtel L, Lock J, et al. Changes in heart rate variability of athletes during a training camp. *Biomed Tech (Berl)*. 2006;51:201-204.
28. Gamelin FX, Berthoin S, Sayah H, et al. Effect of training and detraining on heart rate variability in healthy young men. *Int J Sports Med*. 2007;28:564-570.
29. Plews DJ, Laursen PB, Stanley J, et al. Training adaptation and heart rate variability in elite endurance athletes: opening the door to effective monitoring. *Sports Med*. 2013;43:773-781.
30. Souza HCD, Philbois SV, Veiga AC, et al. Heart rate variability and cardiovascular fitness: what we know so far. *Vasc Health Risk Manag*. 2021;17:701-711.
31. Sacknoff DM, Gleim GW, Stachenfeld N, et al. Effect of athletic training on heart rate variability. *Am Heart J*. 1994;127:1275-1278.
32. Oliveira RS, Leicht AS, Bishop D, et al. Seasonal changes in physical performance and heart rate variability in high level futsal players. *Int J Sports Med*. 2013;34:424-430.
33. Kinnunen S, Laukkanen R, Haldi J, et al. Heart rate variability in trotters during different training periods. *Equine Vet J Suppl*. 2006;214-217.
34. Janczarek IKW, Stachurska A. Autonomic nervous system activity in purebred Arabian horses evaluated according to the low frequency and high frequency spectrum versus racing performance. *Acta Veterinaria Brno*. 2016;85:355-362.
35. Schwarzwald CC. Equine echocardiography. *Vet Clin North Am Equine Pract*. 2019;35:43-64.
36. Massie SL, Bezugley RJ, McDonald KJ, et al. Prevalence of cardiac arrhythmias and R-R interval variation in healthy thoroughbred horses during official Chuckwagon races and recovery. *Vet J*. 2021;267:105583.
37. Flethoj M, Kanters JK, Pedersen PJ, et al. Appropriate threshold levels of cardiac beat-to-beat variation in semi-automatic analysis of equine ECG recordings. *BMC Vet Res*. 2016;12:266.
38. Sides RH, Kirkpatrick R, Renner E, et al. Validation of masks for determination of $\dot{V}O2$ max in horses exercising at high intensity. *Equine Vet J*. 2018;50:91-97.
39. Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res*. 1986;59:178-193.
40. Kuwahara M, Hiraga Kai M, Tsubone H, Sugano S. Influence of training on autonomic nervous function in horses: evaluation by power spectral analysis of heart rate variability. *Equine Vet J Suppl*. 1999;30:178-180.
41. Tran YWN, Tarvainen M, Karjalainen P, Craig A. The relationship between spectral changes in heart rate variability and fatigue. *J Psychophysiol*. 2009;23:143-151.
42. Mohr E, Langbein J, Nurnberg G. Heart rate variability: a noninvasive approach to measure stress in calves and cows. *Physiol Behav*. 2002;75:251-259.

43. von Borell E, Langbein J, Despres G, et al. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. *Physiol Behav*. 2007;92:293-316.
44. Ohmura H, Hobo S, Hiraga A, et al. Changes in heart rate and heart rate variability during transportation of horses by road and air. *Am J Vet Res*. 2012;73:515-521.
45. Mitchell KJ, Schwarzwald CC. Heart rate variability analysis in horses for the diagnosis of arrhythmias. *Vet J*. 2021;268:105590.
46. Young LE. Cardiac responses to training in 2-year-old thoroughbreds: an echocardiographic study. *Equine Vet J Suppl*. 1999;195-198.
47. Buhl R, Ersboll AK, Eriksen L, et al. Changes over time in echocardiographic measurements in young standardbred racehorses undergoing training and racing and association with racing performance. *J Am Vet Med Assoc*. 2005;226:1881-1887.
48. Buhl R, Ersboll AK. Echocardiographic evaluation of changes in left ventricular size and valvular regurgitation associated with physical training during and after maturity in standardbred trotters. *J Am Vet Med Assoc*. 2012;240:205-212.

How to cite this article: Santosuosso E, Léguillette R, Shoemaker S, et al. A consort-guided randomized, blinded, controlled clinical trial on the effects of 6 weeks training on heart rate variability in thoroughbred horses. *J Vet Intern Med*. 2025;39(1):e17253. doi:10.1111/jvim.17253