**Cardiovascular training adaptations in Criollo breed horses: biochemical markers and morphofunctional parameters**

P. F. Ribeiro, G. L. B. L. Pizzi, P. M. Silva, G. A. de O. Cavalcanti, F. R. P. Bruhn, P. P. C. Costa, R. T. França, K. Holz, R F de Godoy and C. F. Martins

*Published in:* Comparative Exercise Physiology *Publication date:* 2023

*The re-use license for this item is:* CC BY-NC-ND

*This document version is the:* Accepted version

*The final published version is available direct from the publisher website at:* <https://doi.org/10.1163/17552559-20230018>

**Cardiovascular training adaptations in Criollo breed horses: biochemical markers and morphofunctional parameters**

P. F. Ribeiro 1, G. L. B. L. Pizzi 1, P. M. Silva 1, G. A. de O. Cavalcanti 1, F. R. P. Bruhn 1, P. P. C. Costa 1, R. T. França 1, K. Holz 1, R F de Godoy 2 and C. F. Martins 1

1 Federal University of Pelotas, Capão do Leão, Rio Grande do Sul, Brazil

2 Writtle University College, Chelmsford, United Kingdom.

*\*Corresponding author:* priscilafri@hotmail.com

**Abstract**

The aim of the present study was to investigate the cardiovascular morphofunctional and biochemical training adaptations of Criollo breed horses. Thirty-one animals were evaluated, arranged in two groups- untrained horses, used as a control group (n = 17); trained horses, in training for the *Freio de Ouro* competition (n = 14). The means and standard deviation of echocardiographic, electrocardiographic and biochemical variables were measured and, subsequently, compared between groups of trained and untrained animals using Student's t test, considering p<0.05. Trained horses had lower biochemical levels of CK, CKMB and cTnI I when compared to untrained animals. The echocardiographic variables Interventricular septum during diastole (IVSd), LVFWs Left ventricular free wall during systole(Left ventricular free wall during diastole, LVFWd, LVMass Left ventricular mass, Diameter of the aorta (Ao) and Diameter of the left atrium (LA) have shown higher values in trained horses compared to untrained. Duration of the T wave was the only electrocardiographic variable that was influenced by training, showing higher values for untrained animals. Therefore, there is influence of training on biochemical variables, in addition to on morphological and functional echocardiographic/electrocardiographic characters in trained Criollo breed horses, characterized by concentric cardiac hypertrophy, when compared to untrained individuals. In addition, a majority of normal sinus heart rhythm was observed in the animals evaluated.

Keywords: athletes; echocardiogram; electrocardiogram; equine

*Conflicts of interest statement:* The authors declare no conflicts of interest

1. **Introduction**

More often, new modalities of equestrian sports, with different training loads and requirements, are incorporated into the equine routine, requiring systematic cardiovascular monitoring for the design of training programs. The heart, as well as skeletal muscle, undergoes significant adjustments resulting from exercise and training (Hamer 2006; Pinho et al., 2010), this cardiac remodeling is observed in human athletes (Gerche et al, 2014; George et al., 1991; Weiner & Baggish 2012), and has been called “athlete's heart”, as well as in dogs (Benito & Boutigny, 2020; RIedhammer et al., 1976; Restan et al., 2022; Trachsel et al., 2018). The adaptations of metabolic activities, resulting in biochemical, electrical, morphological and mechanical adjustments of the heart muscle (Filho et al., 2020), together provide a mechanism for prevention and health promotion, in addition to a more efficient functionality of the cardiovascular system, causing the animal to reach its best performance during exercise due to its superior capacity to meet its needs (Bello et al., 2011; Gonçalves and Alchieri 2010; Li et al., 2020).

Cardiac adaptations that occur due to exercise are dependent on its frequency, intensity and duration, varying in different sports, in different training systems, according to individual responses, and also with race (Bello et al. 2011; Pluim et al, 1999; Ugo & Giannuzzi, 2006). These adaptations have already been considered in other studies involving other horse breeds (Al-Haidar et al., 2013; Gehlen et al., 2007), information that remains scarce in the Creole breed. The Criollo breed is the native horse of the region between Uruguay, Argentina, Paraguay, southern Brazil and Chile, referred to as the “Pampa region”. This breed is one of the most important [horse breeds](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/horse-breed) in Brazil, with around 570 thousand horses registered (*Associação Brasileira de Criadores de Cavalos Crioulos* - ABCCC). The main equestrian field test of the Criollo breed is called *Freio de Ouro*. This field test includes conformation evaluation as well as nine functional tests including a reining-like test and a cutting-like test, which serves to test the horse’s ability to handle cattle handing and to work in cattle farms. The Freio de Ouro is a competition of medium to high intensity of external work, demanding intense physical preparation of the competing animals. Among the athletic requirements, there is the need for both anaerobic training, in order to acquire better muscle adaptation for the exercises to be performed, and aerobic training, requiring the optimization of oxygen consumption and, consequently, a decrease in energy expenditure values (GARCIA et al, 2020). Nonetheless, little is known about the influence of training for this equestrian modality on cardiac muscle and cardiovascular electrical activity. Therefore, the identification of the adaptations of the cardiac muscle, function and myoelectric activity triggered by the moderate to high intensity exercise performed during the training for this competition, aimed to make it possible to monitor the external workload training and the effects of training on the cardiovascular system, differentiating them between physiological and pathological (Evans, 2000; NRC, 2007; Coenen, 2008). Within this context, the aim of the present study was to investigate the cardiac morphofunctional and biochemical adaptations Criollo breed horses trained for the *Freio de Ouro*.

1. **Materials and methods**

*Study Design*

All procedures performed were approved by the Ethics Committee on Animal Experimentation (CEEA) of the *Universidade Federal de Pelotas*, under approval number 51839-2019.

*Experimental design*

Thirty-one Criollo breed horses were evaluated, aged between 7±3.02 and 10±3.10 years, for trained and untrained animals respectively, with an average weight of 400.23 ± 81.89 kg, height of 1.41 ± 0.03 m and chest circumference of 1.74 ± 0.05 m, distributed in two groups, untrained horses (n = 17), used as a control group; trained horses (n = 14). The trained animals were in a training center, and the untrained ones were in *cabañas* and breeding centers located in the southern region of *Rio Grande do Sul* (RS) state, Brazil. The biotype of the sample was homogeneous and consistent with the phenotypic patterns of the breed, since the animals from the establishments, regardless of gender groups and levels of athletic activity, belonged to the Criollo breed and were healthy.

The group of non-trained animals was formed by individuals kept in the pasture and with water ad libitum, without any specific management, mainly related to physical training. On the other hand, the group of horses considered as trained in this study, during a minimum period of two years, were submitted to a weekly routine of aerobic and anaerobic training, participating in qualifying competitions for the *Freio de Ouro*. The training protocol for the horses consisted of alternating walks and trots (walk-trot-walk-trot) of 10 minutes duration each and canter exercise of the same duration (10 minutes), with cool-down walks to return the heart rate to physiological limits. This training program was performed regularly five days a week. In addition, technical maneuvers are trained two to three times a week to improve technique as it is used in competitions. These maneuvers include exercises with and without the presence of cattle as well as sliding stops and canter pirouettes.

*Biochemical analysis, echocardiographic evaluation, and electrocardiographic assessment*

Firstly, blood was collected from the fasting horses, at 6 AM, for biochemical analyses, later the trained animals received their diet, and the untrained ones were released again to feed. After 1 hour, an echocardiogram was performed, followed by an electrocardiogram.

*Biochemical analysis*

Blood samples were collected in a Vacutainer® tube without anticoagulant from the jugular vein of the horses. Aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase isoenzyme MB (CKMB), were analysed on an automatic biochemical analyser Cobas C111 Roche and Cardiac Troponin I (cTnI) DXI 800 Beckman Coulter (High Sensitivity Troponin I Kit).

*Echocardiographic evaluation*

An echocardiographic assessment was performed for each individual. The animals were evaluated only in one moment, which was at rest, in the morning, without having performed physical activity in the hours before the exam. The device used for the echocardiographic analysis was a *Sonosite® MicroMaxx* model, with a *Sonosite® P17* sectorial, electronic and multi-frequency transducer from 1 to 5 MHz, with a range of 35 cm in depth.

The echocardiographic examination was performed in the region between the 3rd and 5th intercostal spaces of the right cardiac window, with the application of ethyl alcohol and ultrasound contact gel according to the American Society of Echocardiography guidelines (Gottdiener et al., 2004).

To perform the two-dimensional mode (B), the recommendations of Boon (1998) were followed. In B-mode, the cardiac chambers, myocardial contractility and morphological aspect of the heart valves were evaluated. In the right transverse parasternal position, in the basal region of the heart, the following echocardiographic variables were measured: diameters of the left atrium (LA) and aorta (Ao), at the beginning of diastole, similar to that performed by Rishniw and Erb (2000), for the relationship between these parameters (LA/Ao) is calculated. The Ao was measured along the line of closure of the right coronary and non-coronary leaflets. The LA was measured from a line parallel to the closing line of the non-coronary and left coronary leaflets. The images in the two-dimensional mode served as a guideline for performing the Motion Mode (M-mode).

In the M-mode in the right parasternal section of the chordal plane region, the following parameters were evaluated: interventricular septum thickness (IVS), left ventricular free wall (LVSW) and left ventricular internal diameter (LVID) during the end diastole and peak systole, left ventricular end-systolic volume (LVIVs), left ventricular end-diastolic volume (LVIVd), interventricular septum thickening fraction (TF), left ventricular fractional shortening (FS), left ventricular mass (LVMass), left ventricular ejection fraction (EF) and stroke volume (SV).

Fractional shortening (FS) was calculated from the values obtained from LVID and LVIDs through the formula: FS=[(LVIDd-LVIDs)/LVDd] x 100, as well as the ejection fraction (EF) was obtained by the formula: EF%=[(LVIVD-LVIVS)/LVIVD] × 100 (Nyland and Mattoon, 2002). Left ventricular end-systolic volume (LVEDV) and left ventricular end-diastolic volume (LVESV) were determined by the modified Teicholz formulae: LVEDV= [7.0 (LVIDd)3]/[2.4 + LVIDd]; LVESV= [7.0 (LVIDs)3 ]/[2.4 + LVIDs] (Rovira et al., 2008; Teichholz et al., 1976). Finally, the stroke volume (SV) was obtained by the difference between LVEDV and LVESV, and cardiac output (CO) was obtained multiplying SV by HR (Nyland and Mattoon, 2002). The LV mass was calculated by the formulae: left ventricular mass =1,04 x 0,8 [(LVSW+LVID) – (LVID)] + 0,6 g (Devereux et al., 1986).

*Electrocardiographic assessment*

The electrocardiographic examination was performed with the *Incardio Duo®* device, using the Fré method, for 20 minutes. The duration of the exam took into account the stabilization of the animal's behavior and HR, in order to avoid possible external interferences in the electrocardiographic tracings. The electrodes were fixed to the skin of the horses using metallic conductors, alligator type and moistened with alcohol, positioned according to the methodology used by Costa (2017).

The animals were submitted only to an electrocardiographic evaluation, being evaluated at rest, in the morning, standing, without having been submitted to physical activity in the hours before the exam.

Data analysis was performed on the dedicated software inCardio duo® 2.7.4, in a semi-automatic manner. The following measurements were performed: determination of heart rhythm, morphology of electrocardiographic waves, heart rate (bpm), P peak (ms), P and T wave duration (ms), QRS complex (ms) and of the QT interval (ms), in addition to the amplitudes (mV) of the P, R and S wave, the QRS complex, the ST segment and the T wave in lead DII. In the middle electrical axis, the P-wave axis, which analyzes the depolarization of the atrium, and the QRS complex axis, which is based on the evaluation of the ventricle, were evaluated, being the second most used because the ventricular mass is greater than the atrial one. The values of the cardiac axis were obtained according to the calculation of Santilli and Perego (2009).

 *Statistical Analysis*

Matched pairing was performed by the variable sex, including the same proportion of males and females between the two groups. All variables were tested for normality distribution using the Shapiro-Wilk test, considering the means of parametric variables compared between groups trained v*s.* untrained using independent samples t-test and non-parametric variables using the Mann-Whitney U test. Statistical tests were performed using SPSS 20.0 software. For all tests, a significance level of P < 0.05 was considered.

In addition, the normality of the variables AST, CK, CKMB and cTnI was tested, and after verifying that CK, CKMB and cTnI were non-parametric, a logarithmic transformation was performed. To verify the difference in the distribution of means between groups trained *vs.* untrained, the dependent variables were tested using Student's t-tests, considering a minimum confidence level of 95% (P < 0.05).

# Results

Training has elicted morphofunctional and biochemical adaptive changes in the heart, with trained horses presenting lower levels of CK, CKMB and cTnI I and higher values of IVSd, LVFWs, LVFWd, LVMass, Ao and LA. Furthermore, trained horses presented lower T wave duration.

*Biochemical analysis*

Trained horses exhibited lower biochemical indices of CK, CKMB and cTnI when compared to untrained animals (P < 0.05). On the other hand, AST showed no difference between the groups (P > 0.05) (Table 1).

**Table 1.** Effect of training on laboratory variables (mean values and standard deviation) of healthy Criollo breed horses

|  |  |  |
| --- | --- | --- |
| **Laboratory variables** | **Untrained horses**  | **Trained horses**  |
| $\overbar{X}$**±σ** | $\overbar{X}$**±σ** |
| **AST (U/I)** | 330.0±49.31a | 283.70±61.72a |
| **CK (U/I)** | 665.2±266.91a | 205.40±85.05b |
| **CKMB (U/I)** | 708.7±232.26a | 226.60±106.16b |
| **cTnI (pg/mL)** | 3.51±2.12a | 1.6±1.86b |

Means followed by different letters on the same line differ statistically (p≤0.05).

*Echocardiographic analysis*

In the echocardiographic parameters of untrained and trained horses, variability of the measured echocardiographic characteristics was verified. The variables IVSd, LVSWd, IVSs, LVSWs, LVMass, Ao and LA varied between untrained and trained horses (P = 0.0001, 0.011, 0.043, 0.0001, 0.002, 0.028 and 0.006 respectively), with higher measurements for the trained group. The other variables were similar (P > 0.05, Table 2).

**Table 2.** Effects of training on echocardiographic variables (mean values and standard deviation) of Criollo breed horses

|  |  |  |
| --- | --- | --- |
| **Echocardiographic variables** | **Untrained horses**  | **Trained horses**  |
| $\overbar{X}$**±σ** | $\overbar{X}$**±σ** |
| **IVSd (cm)** | 2.72±0.43b\* | 3.44±0.82a\* |
| **LVIDd (cm)** | 11.90±4.06a\* | 12.73±2.77a\* |
| **LVSWd (cm)** | 2.73±0.73b | 3.48±0.80a |
| **IVSs (cm)** | 4.44±0.56b | 4.93±0.69a |
| **LVIDs (cm)** | 7.06±2.94a\* | 7.56±3.11a\* |
| **LVSWs (cm)** | 4.13±0.75b | 5.46±1.08a |
| **LVIVS (ml)** | 296.50±272.95a\* | 274.60±277.40a\* |
| **LVIVD (ml)** | 795.10±538.05a\* | 910.10±395.05a\* |
| **TF (%)** | 63.92±22.04a | 49.83±20.16a |
| **FS (%)** | 35.30±4.19a | 36.34±6.45a |
| **LVMass (g)** | 2771.00±1140.00b\* | 3969.00±1181.25a\* |
| **EF (%)** | 61.47±5.91a | 62.71±8.67a |
| **SV (ml)** | 529.75±165.17a | 520.19±112.85a |
| **Ao (cm)** | 6.04±0.43b | 6.40±0.41a |
| **LA (cm)** | 7.37±0.94b | 8.16±0.49a |
| **LA/Ao (cm)** | 1.23±0.15a |  1.27±0.09a |

\* Median followed by interquartile difference

Means/medians followed by different letters on the same line differ statistically (p≤0.05). IVSd: Interventricular septum durin in diastole; LVIDd: Left ventricular internal diameter in diastole; LVSWd: Left ventricular free wall in diastole; LVIVS: Left ventricular end-systelus volume; LVIVD: Left ventricular en-diastolic volume; TF: Interventricular septum thickening fraction; FS: Left ventricular fraction shortening; LVMass: Left ventricular mass; EF: Left ventricular ejection fraction; SV: Stroke volume; AO: Diameter of the aorta; LA: Diameter of the left atrium; LA/AO: Ratio between left atrium and aorta.

*Electrocardiographic analysis*

The heart rhythm most observed in Criollo breed horses was normal sinus. The mean morphology of the bifid P wave in the Criollo breed was 58.82% in untrained horses and 71% in trained horses (mean: 64.91%). There was a percentage presence of the QRS complex in 41.17% in untrained horses, and 64.28% in trained horses (mean: 52.72%). The morphological pattern of the T wave was biphasic 70.58% in untrained horses and 57.14% in trained horses (mean: 63.86%).

The only electrocardiographic variable that showed a difference between untrained and trained animals was the T wave duration (P = 0.025), with a higher value for the group of untrained animals. All other electrocardiographic variables showed similar measurements between groups (Table 3).

 **Table 3.** Effect of training on electrocardiographic variables (mean values and standard deviation) of healthy Criollo breed horses

|  |  |  |
| --- | --- | --- |
| **Electrocardiographic variables** | **Untrained horses** | **Trained horses** |
| **𝑿̅±σ** | **𝑿̅±σ** |
| **P-axis (º)** | 47.70±8.79a | 43.98±10.57a |
| **QRS-axis (º)** | -49.32±25.88a | -44.39±21.73a |
| **T-wave (mV)** | -0.12±0.52a\* | 0.15±0.50a\* |
| **QT Interval (ms)** | 514.49±122.50a\* | 536.50±35.00a\* |
| **S-wave (mV)** | -0.14±0.32a\* | -0.16±0.31a\* |
| **T Duration (ms)** | 136.41±21.76a | 118.00±18.41b |
| **P-wave (mV)** | 0.14±0.02a | 0.13±0.005a |
| **QRS Interval (ms)** | 113.47±14.54a | 107.14±12.37a |
| **R-wave (mV)** | 0.08±0.15a\* | 0.15±0.17a\* |
| **P duration (ms)** | 97.82±14.76a | 90.00±11.26a |
| **HR** | 44.58±14.24a | 38.50±5.17a |

\* Median followed by interquartile difference

Means followed by different lowercase and uppercase letters in the same row differ statistically (p≤0.05). P-axis: P-wave axis; QRS-axis: axis of the QRS complex; Amplitude T: T-wave amplitude; Q-T: Interval between Q and T waves; S amplitude: S-wave amplitude; T duration: T-wave duration; P amplitude: P-wave amplitude; QRS Duration: Duration of the QRS complex; Amplitude R: R-wave amplitude; P duration: P-wave duration; HR: Mean heart rate

1. **Discussion**

*Biochemical analysis*

Cardiac markers are indicators of biological, physiological, pathogenic or pharmacological intervention processes, and may vary depending on heart disease, lesion extension and prognosis (Oyama and Sisson, 2004), in addition to monitoring athletes and their performance (Brown et al., 1997), cardiac troponin I (cTnI) and the isoenzyme creatine kinase MB (CKMB) are examples. For greater reliability and specificity, the association of AST and total CK enzymes was considered, which when elevated together indicate alterations in the skeletal muscles (Valberg, 1996), as did not occur in the present study. Mean CK concentration varied between groups of untrained and trained horses (P < 0.001), results close to the rates found by Michima et al. (2010) for Arabian and crossbred horses and lower than those determined by Fernandes (1994), in Arabian Horses and Toledo et al. (2001) in the Thoroughbreds. The higher concentration of the total CK variable in untrained horses can be influenced by factors that do not represent muscle damage, such as sarcomere shear (Soares et al., 2012) but by the increase of its MB isoenzyme, due to a positive correlation (R = 0.96) of total CK with CKMB, according to previous records (Jaffe et al., 1984). In the evaluation of the CKMB enzyme, the group of untrained animals was superior to the rates observed in trained horses (P < 0.001). In human athletes, serum CK values are lower when compared to sedentary individuals undergoing the same physical activity (Goldfeder, 2010; Lisboa, 2010), similar results to those verified in the present study, with lower CK and CKMB indices in trained horses, even if the animals have not been subjected to physical exercise.

Cardiac Troponin I concentration did not exceed serum values for clinically healthy horses (below 0.3 ng/ml) reported by Cornelisse et al. (2000), referring that the animals monitored in the present study did not present pathological cardiac alterations, despite the variability of the cTnI concentrations verified between the groups (P = 0.018), a fact that, due to the adaptations of the cardiac muscle to the consecutive athletic challenges of the groups of horses in training has provided an increase in their rates.

Although the biochemical variables CKMB and cardiac troponin have clinical significance in heart diseases, the differences between groups of trained and untrained horses for these variables remained within the values already identified in other studies (Cornelisse et al., 2000; Oliveira et al., 2011) and, therefore, not being considered as pathological cardiac alterations, but physiological ones resulting from the adaptability of the cardiac muscle to the constant stimuli promoted by the daily exercises practiced.

*Echocardiogram*

The echocardiographic indices considered in the present study have clinical predictive value to investigate cardiac alterations in Criollo breed horses. Differences in echocardiographic indices were evidenced between untrained and trained individuals, guiding the interpretation of these cardiological variables in animals with different aptitudes. These differences have already been considered in other studies involving other horse breeds (Al-Haidar et al., 2013; Gehlen et al., 2007) and, therefore, the results obtained are unprecedented for the Criollo breed. This scenario constitutes valuable auxiliary elements that provide objective information for the interpretation of these cardiac indices.

# *Echocardiographic morphological indices*

The present study showed that horses undergoing training showed increased myocardial thickness (IVSd, IVSs, LVSWd, LVSWs) and left ventricular mass (LVMass), corroborating other authors; Young (1999) evaluated horses before and after 18 months of training and found an increase in LVMass and LVSWd. In contrast, Gehlen et al. (2006) and Bello et al. (2012) evaluated horses shortly after Polo training and in a complete riding competition (CCE) and found reduced rates of IVSd, IVSs, LVSWd, LVSWs; this identifies that isolated physical activity does not influence, but daily training does.

The echocardiographic variables IVSd, IVSs, LVSWd, LVSWs and LVMass in the present study differed between groups with different levels of physical activity, a result explained by Young (1999) and Hodgson et al. (2013) due to cardiac hypertrophy secondary to physical training.

There was no difference in left ventricular internal diameter (LVID) in systole and diastole between groups (P > 0.05). Morganroth et al. (1975) identified that human athletes who performed hypertrophic training, despite not presenting alterations in the diastolic diameter, manifested thickening in the posterior wall of the left ventricle (LVSW) and interventricular septum (IVS), results similar to those found in the present study. This fact can be explained by the strength training performed by the horses, imposing pressure overload on the heart, promoting an increase in the myocardial walls with little change in the internal diameter of the left ventricle (Miranda et al., 2012). In horses, researchers have found a significant decrease in LVIDd and LVIDs after endurance exercise, and these echocardiographic findings are associated with metabolic disturbances and/or dehydration (Michima, 2007; Bello et al., 2011). For Di Bello et al. (1996), different alterations in the cardiac dimension can occur after the exercise in humans and they vary with the type of exercise, duration and intensity of the cardiac load. In the present study, due to the enlargement of the left ventricular wall without alterations in the diameter of the cardiac chamber, it can be affirmed that the Criollo breed horses in training presented concentric cardiac hypertrophy, possibly due to the adaptation of the cardiovascular system to the exercise, data as verified by Fernandes et al. (2015).

Another adaptation to exercise occurs when the greater blood flow in the athlete's aorta implies an increase in its diameter. In other terms, physical activity tends to promote an increase in this index (Evans, 1994), but the anatomical characteristic prevails and, thus, the animals from different breeds may present variability in their morphological indices, as observed in the present study, due to the variability of the aortic diameter between trained and untrained animals (P < 0.05). The left atrium diameter also changed, with higher measurements in trained horses (P < 0.001). These findings had already been identified in other studies, both in humans (Ghorayeb et al., 2005) and in Thoroughbred horses (Paterick et al., 2014). According to Paterick et al. (2014) the morphological amplification of the LA index is proportional to the increase in the ventricles, therefore, it is also affected by the type of physical training.

The variability of these echocardiographic morphological indices between studies can be explained by the biometric differences between different genetic groups of horses, levels and/or stages of training assigned and requirements for the different modalities of equestrian sports to which the horses are submitted.

*Echocardiographic functional indices*

In the present study, no difference in shortening fraction was observed between trained and untrained animals (P > 0.05), corroborating the findings described by Ghorayeb et al. (2005), showing the variable within the reference standards for the species, despite the sharp increase in the cavity and ventricular thickness (Pelliccia et al., 1991).

The left ventricular diameter shortening fraction (SF) represents the ventricular systolic performance, with this characteristic being similar for trained and untrained Criollo breed horses (P > 0.05), validating Miranda et al. (2012) who did not observe a significant difference between athletes and sedentary humans, identifying that the groups had similar functional aspects of the heart.

*Electrocardiogram*

The predominant rhythm in Criollo breed horses was the normal sinus rhythm, considered physiological in the equine species (Patteson, 1996), a result similar to that verified in the breed by Schade et al., (2014) and in Arabian Horses (Dumont et al., 2010), in addition to distinct in the Andalusian breed (Ayala et al., 1995), in which the most frequent rhythm was sinus arrhythmia. Compared to other domestic species, healthy horses have a high incidence of cardiac arrhythmias (Melchert et al., 2012), findings considered physiological in most cases, but which may be associated with poor athletic performance (Mitten, 1996). Therefore, no rhythm or electrical conduction disturbances were observed, which is justified by Schade et al. (2014) due to the low incidence of arrhythmias detected in Criollo breed horses. However, future long-term electrocardiographic studies (Holter system) may help in the investigation of possible cardiac arrhythmias in horses from this genetic group (Bakos and Lohne, 2009).

The P wave is a parameter that changes its morphology and, generally, in the horse, this wave appears in a bifid form (Yonezawa et al., 2009). In the Criollo breed, it was identified a prevalence of bifid P wave in 64.91% of the horses studied, similar results to those found in other studies (Schade et al., 2014; Pascon et al., 2015). On the other hand, Gama (2018), studying miniature horse racing, identified the prevalence of biphasic P waves in their tracings, this phenomenon being justified by the different points of atrial activation and confirmed by means of vectorcardiography (Hamlin et al., 1970).

The QRS complex was complete in most tracings in the Criollo breed (52.72%), a result similar to those observed by Dumont et al. (2010) in Arabian Thoroughbred horses. On the other hand, Pascon et al. (2015) found a prevalence of rS in Criollo breed horses and for Diniz et al. (2008), the Q wave was observed in only 3.3%; R in 73.3% of tracings and S in 100% in *Mangalarga Marchador*, using a different electrocardiographic lead. These variations identified in the presentation of the QRS complex in the Criollo breed are considered physiological electrocardiological parameters for the species and dependent on the derivation used in the electrocardiographic examination.

The T wave seems to be the most variable parameter in the equine electrocardiogram (Piccione et al., 2003). The morphological pattern of this wave in the Criollo breed was biphasic in 63.86% of the tracings, similar results verified by Diniz et al. (2008), but different from those obtained by Schade et al. (2014) for the breed (91%). Animals that were not in training had a longer T wave duration compared to horses in training (P < 0.05). T wave changes are common in normally performing trained horses (Patteson, 1996), and are considered nonspecific and of little use in diagnosing heart disease or poor performance (Evans, 1991).

Although the QT interval represents ventricular repolarization and is inversely proportional to heart rate, it was expected that HR would decrease significantly with training and that the QT interval would increase. This fact occurred in the present study, but there was no difference between the groups (P > 0.05), and this can be explained by the handling of the animals during the exams, which directly influences their HR. Thus, it becomes necessary to use other assessment methods together, such as heart rate variability, which allows the assessment of the autonomic nervous system, resulting in more accurate HR analysis.

The amplitude of the P wave was similar between trained and untrained animals; however, this variable has clinical relevance, due to the alterations that occur in the cardiac positioning of the animals that undergo training in relation to the horses that do not practice physical activities, due to the size body and hypertrophies. The P wave values observed in the present study are physiological, since the acceptable values for the species are less than 0.17s (Diniz et al., 2011). When animals are subjected to stress or physical exhaustion, it is possible to observe inversion of polarity of the P wave and increase in amplitude that can surpass the QRS complex (Piccione et al., 2003).

The cardiac axis reveals the orientation and direction of ventricular depolarization, providing pertinent information about hypertrophy and/or dilation of cardiac chambers, in addition to identifying or suggesting intraventricular conduction defects (Pastore et al., 2016). In the present study, this index was more negative in trained horses, that is, with deviation to the left, although there was no difference between groups (P > 0.05). Result similar to that exposed by Dumont et al. (2010) and different from that found by Ayala et al. (1998) and Fernandes et al. (2004), who observed a predominance of positivity. Axis deviation to the left suggests left ventricular hypertrophy, possibly due to adaptations to training, a result that, added to the echocardiographic examination obtained in the present study, confirms the occurrence of concentric cardiac hypertrophy. Serial assessment of LV mechanical function would have been useful in clarify if this cardiac hypertrophy was physiological or pathological. However, as ‘physiological’ cardiac hypertrophy can be provoked by exercise training and is characterized by normal cardiac morphology with a normal and/or enhanced cardiac function (Kavazis, 2015) and the biomarkers assessed gives us a good indication of physiological adaptations, further assessments could have been useful to definitely rule out pathological changes.

As for the heart rate index (HR), the untrained Criollo breed horses had an average HR of 44.58 bpm and 38.50 bpm for the trained ones (P > 0.05), values that were concentrated within the physiological parameters for the species (26 to 50 bpm) (Fregin, 1992). Other studies had already identified the absence of significant variability for HR (Albernaz et al., 2011) before and after training, but there was a decreasing trend, as in the present study, corroborating the statement that trained and well-conditioned horses present a lower HR than unconditioned horses (Littlejohn, 1987).

The variability of the indices and electrocardiographic leads discussed in the present study highlights the need for future dynamic investigations of Criollo breed horses in training, in order to understand what these oscillations represent. The echo and electrocardiographic variables were within the standards described for the species, reflecting the conditions of animal welfare. The absence of follow-up and veterinary medical examinations before, during and after the practice of exercises, makes cases of sudden death and loss of performance in horses much more frequent than in previously examined animals. In addition, due to the existence of few studies related to cardiac biomarkers in horses and their possible low specificity, it is identified the importance of associating the analysis of cardiac biomarkers in addition to complementary exams of the system and enzymes related to musculoskeletal alterations, to rule out possible injuries. muscles and tendons, as proposed in the present study.

Altogether, the study brings as important points that the association of the analysis of cardiac biomarkers with complementary exams of the heart helps in the identification and differentiation of physiological adaptations from pathological ones. In addition, training provides a reduction in CKMB and cTnI in horses, although these levels are within the reference values for the species. Criollo horses in training showed physiological cardiac hypertrophy identified by the increase in IVSd, IVSs, LVSWd, LVSWs, LAMass, Ao and LA, in addition to the electrical axis with deviation to the left. Hypertrophy was characterized as concentric due to increased thickness of the left ventricle, without changes in cavity diameter. T-wave duration is the only superior electrocardiographic variable in untrained animals.

1. **Conclusions**

Training provides physiological, biochemical and morphofunctional adaptations of the heart in Criollo breed horses trained for the *Freio de Ouro*, identified through the association of the analysis of cardiac biomarkers with echo and electrocardiographic exams.

**Acknowledgments**

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), Brasília, Brazil, for the scholarship granted to Ribeiro, P.F. This study was funded in part by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financial Code 001 and also by the *Universidade Federal de Pelotas* (UFPel) – PIB-M/D Program. The authors thank the animal owners for allowing data collection in their establishments – *Centro de Treinamento Zeca Macedo*. The authors also thank the Clinical Pathology Laboratory of the Department of Veterinary Clinics, Veterinary College, belonging to the *Universidade Federal de Pelotas* for the processing and analysis of blood samples.

**References**

Albernaz, R. M., Dias, D. P. M., Junior, D. P., Pascon, J. P., Neto, A. Q., and Neto, J. C. L. (2011). Respostas eletrocardiográficas de equinos ao treinamento com base na curva velocidade-lactato determinada em esteira rolante. *Ciência Animal Brasileira/Brazilian Animal Science*, *12*(1), 163-171.

Al-haidar, A., Farnir, F., Deleuze, S., Sandersen, C. F., Leroux, A. A., Borde, L., ... and Amory, H. (2013). Effect of breed, sex, age and body weight on echocardiographic measurements in the equine species. *Research in veterinary science*, *95*(1), 255-260.

Associação Brasileira de Criadores de Cavalos Crioulos, 2019. Pelotas: História da Raça Crioula, 2019. Disponível em: http://www.cavalocrioulo.org.br/studbook/historia.

Ayala, I., Montes, A., Benedito, J. L., Castillo, C., Hernández, J., Gutierrez, C., and García‐Partida, P. (1998). Modifications of the Form and Amplitude of the Electrocardiographic QRS Complex During Growth in the Spanish‐Bred Horse. *Journal of Veterinary Medicine Series A*, *45*(1‐10), 309-317.

Ayala, I., Montes, A., Bernal, L. J., Sandoval, J. A., and Gutierrez, C. (1995). Electrocardiographic values in Spanish‐bred horses of different ages. *Australian veterinary journal*, *72*(6), 225-226.

Bakos, Z., and Lohne, L. (2009). Reference values of Holter electrocardiography in normal horses. *West Ind. Vet. J*, *9*, 3-7.

Bello, C. A. D. O., Vasconcelos, C. E. D. S., de Godoy, R. F., Teixeira-Neto, A. R., Borges, J. R. J., and de Lima, E. M. M. (2011). Echocardiography in Arabian horses after endurance exercise of different intensities/Ecocardiografia de equinos Puro Sangue Arabe apos exercicio de enduro de diferentes intensidades. *Ciência Rural*, *41*(1), 132-137.

Bello, C. A., Dumont, C., Souza, T. C., Palma, J. M., Lima, E. M., Godoy, R. F., ... and Meryonne, M. (2012). Electrocardiographic evaluation of horses after polo exercise. *Pesquisa Veterinária Brasileira*, *32*, 47-52.

Benito, M.; Boutigny, L. Cardiovascular Clinical Assessment in Greyster Dogs in Bikejöring Training. *Animals* **2020**, *10*, 1635.

Boon, J. A. (1998). *Manual of veterinary echocardiography*. Williams and Wilkins..

Brown, S. J., Child, R. B., Day, S. H., and Donnelly, A. E. (1997). Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *Journal of sports sciences*, *15*(2), 215-222.

Coenen, M. (2008). The suitability of heart rate in the prediction of oxygen consumption, energy expenditure and energy requirement for the exercising horse. *Nutrition of the exercising horse. Wageningen, the Netherlands: Wageningen Academic Publishers*, 139-146.

Cornelisse, C. J., Schott II, H. C., Olivier, N. B., Mullaney, T. P., Koller, A., Wilson, D. V., and Derksen, F. J. (2000). Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. *Journal of the American Veterinary Medical Association*, *217*(2), 231-235.

Costa, C. F. D. (2017). *Aquisição eletrocardiográfica em equinos: definindo uma nova e mais adequada metodologia para a espécie* (Doctoral dissertation, Universidade de São Paulo).

de Oliveira, G. I. V., Kuhawara, K. C., Laposy, C. B., and Melchert, A. (2011). Bioquímica sérica de equinos da raça puro sangue lusitano antes e após exercício. In *Colloquium Agrariae. ISSN: 1809-8215* (Vol. 7, No. 2, pp. 14-19).

Devereux, R. B., Alonso, D. R., Lutas, E. M., Gottlieb, G. J., Campo, E., Sachs, I., and Reichek, N. (1986). Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *The American journal of cardiology*, *57*(6), 450-458.

Di Bello, V., Riccardo Bellina, C., Molea, N., Talarico, L., Boni, G., Magagnini, E., ... and Giusti, C. (1996). Simultaneous dobutamine stress echocardiography and dobutamine scintigraphy (99m Tc-MIBI-SPET) for assessment of coronary artery disease. *The International Journal of Cardiac Imaging*, *12*, 185-190.

Diniz, M. P., Michima, L. E., and Fernandes, W. R. (2011). Electrocardiographic study in healthy showjumping horses. *Pesquisa Veterinária Brasileira*, *31*, 355-361.

Diniz, M. P., Muzzi, R. A. L., Muzzi, L. A. L., and Alves, G. E. S. (2008). Eletrocardiographic study in horses of the Mangalarga Marchador breed. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *60*, 536-542.

Dumont, C. B. D. S., Leite, C. R., Moraes, J. M. D., Alves, R. D. O., Godoy, R. D. F., and Lima, E. M. M. D. (2010). Parâmetros eletrocardiográficos de equinos Puro Sangue Árabe submetidos a exercício prolongado de enduro. *Ciência Rural*, *40*, 1966-1973.

Evans, D. L. (1991). T-waves in the equine electrocardiogram: Effects of training and implications for race performance. *Equine Exercise Physiology*, *3*(1), 475-481.

Evans, D. L. 1994. The cardiovascular system: anatomy, physiology, and adaptations to exercise and training. In The athletic horse: principles and practice of equine sports medicine (pp. 129-144). WB Saunders, Philadelphia

Evans, D. L. 2000. Training and fitness in athletic horses. Dydney: Rural Industries Redearch and Development Corporation. P. 1-64.

Fernandes, T., Baraúna, V. G., Negrão, C. E., Phillips, M. I., & Oliveira, E. M. (2015). Aerobic exercise training promotes physiological cardiac remodeling involving a set of microRNAs. *American Journal of Physiology-Heart and Circulatory Physiology*, *309*(4), H543-H552.

Fernandes, W. R., and Larsson, M. H. M. A. (1994). Alterações dos parametros do eletrocardiograma e da crase sanguínea em equinos das raças arabe e mangalarga, bem como de mestiços, submetidos a prova de enduro.

Fernandes, W. R., Larsson, M. H. M. A., Alves, A. L. G., Fantoni, D. T., and Belli, C. B. (2004). Características eletrocardiográficas em eqüinos clinicamente normais da raça Puro Sangue Inglês. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *56*, 143-149.

Filho, L. D. P. V., Apolinário, J. P. C., do Carmo, J. O., Pascoal, L. B., Figueiredo, L. P., Sousa, L. L., ... and Junior, W. D. J. F. (2020). Adaptações cardíacas fisiológicas induzidas pelo exercício físico em atletas amadores: revisão narrativa. *Revista Eletrônica Acervo Saúde*, (56), e3999-e3999..

Fregin, G. F. (1992). Medical evaluation of the cardiovascular system. *Veterinary Clinics of North America: Equine Practice*, *8*(2), 329-346.

Gama, J. A. N. D. (2018). Determinação dos parâmetros cardiovasculares eletrocardiográficos materno-fetal e neonatal em equinos da raça Mini-Horse.

Garcia, C. A. S. D. C., Martins, C. F., Silva, L. L., Brandi, R. A., Feijó, J. O., Barbosa, A. A., ... and Corrêa, M. N. (2020). Frequência cardíaca, lactacidemia e gasto energético de equinos da raça Crioula em provas credenciadoras ao Freio de Ouro. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *72*, 1631-1638.

Gehlen, H., Haubold, A., e Stadler, P. (2007). Reference values for echocardiographic parameters of trained and untrained Icelandic horses. *DTW. Deutsche Tierarztliche Wochenschrift*, *114*(10), 374-377.

Gehlen, H., Marnette, S., Rohn, K., Ellendorff, F., and Stadler, P. (2006). Echocardiographic comparison of left ventricular dimensions and function after standardized treadmill exercise in trained and untrained healthy warmblood horses. *Equine and Comparative Exercise Physiology*, *3*(1), 3-11.

George, K.P.; Wolfe, L.A.; Burggraf, G.W. The “athletic heart syndrome”. A critical review. *Sports Med.* **1991**, *11*, 300–330.

Gerche, André La; ROBERTS, Timothy; CLAESSEN, Guido. The response of the pulmonary circulation and right ventricle to exercise: exercise-induced right ventricular dysfunction and structural remodeling in endurance athletes (2013 Grover Conference series). *Pulmonary circulation*, 2014, 4.3: 407-416.

Ghorayeb, N., Batlouni, M., Pinto, I. M., and Dioguardi, G. S. (2005). Hipertrofia ventricular esquerda do atleta: resposta adaptativa fisiológica do coração. *Arquivos Brasileiros de Cardiologia*, *85*, 191-197.

Goldfeder, R. T. (2010). Comportamento da creatina kinase em participantes de provas de triatlo Ironman; triatlo.

Gonçalves, M. P., and Alchieri, J. C. (2010). Motivation to practicing physical activities: A study with non-athletes. *Psico-USF*, *15*, 125-134.

Gottdiener, J. S., Bednarz, J., Devereux, R., Gardin, J., Klein, A., Manning, W. J., ... and Weissman, N. J. (2004). American Society of Echocardiography recommendations for use of echocardiography in clinical trials: a report from the american society of echocardiography's guidelines and standards committee and the task force on echocardiography in clinical trials. *Journal of the American Society of Echocardiography*, *17*(10), 1086-1119.

Hamer, M. (2006). The anti-hypertensive effects of exercise: integrating acute and chronic mechanisms. *Sports medicine*, *36*, 109-116.

Hamlin, R. L., Smetzer, D. L., Senta, T., and Smith, C. R. (1970). Atrial activation paths and P waves in horses. *American Journal of Physiology-Legacy Content*, *219*(2), 306-313.

Hodgson, D. R., McGowan, C. M., and McKeever, K. (2013). *The athletic horse: principles and practice of equine sports medicine*. Elsevier Health Sciences.

Jaffe, A. S., Garfinkel, B. T., Ritter, C. S., and Sobel, B. E. (1984). Plasma MB creatine kinase after vigorous exercise in professional athletes. *The American journal of cardiology*, *53*(6), 856-858.

Li, Y., Schoufour, J., Wang, D. D., Dhana, K., Pan, A., Liu, X., ... and Hu, F. B. (2020). Healthy lifestyle and life expectancy free of cancer, cardiovascular disease, and type 2 diabetes: prospective cohort study. *bmj*, *368*.

Lisbôa, L. D. M. (2010). Marcadores bioquímicos na avaliação de lesão muscular associada ao treinamento físico.

Littlejohn, A. (1987). Exercise-related cardiovascular problems. *Current therapy in equine medicine*, *2*, 176.

Kavazis AN. Pathological vs. physiological cardiac hypertrophy. J Physiol. 2015 Sep 1;593(17):3767. doi: 10.1113/JP271161.

Melchert, A., Laposy, C. B., Guasi, V. H. B., Valle, H. F. D., and Santos, G. C. (2012). Eletrocardiografia computadorizada em cavalos Puro Sangue Lusitano submetidos a exercício físico. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *64*, 547-554..

Michima, L. D. S., Mirandola, R. M. S., and Fernandes, W. R. (2010). Study of serum creatine kinase isoenzyme CKMB in endurance horses after prolonged physical exercise. *Brazilian Journal of Veterinary Research and Animal Science*, *47*(1), 23-30.

Michima, L. E. D. S. (2007). *Influência do exercício físico prolongado sobre a concentração sérica de troponina I cardíaca e sobre a função cardíaca em cavalos de enduro* (Doctoral dissertation, Universidade de São Paulo).

Miranda, C. H., Gali, L. G., Marin-Neto, J. A., Louzada-Júnior, P., and Pazin-Filho, A. (2012). Coronary thrombosis as the first complication of antiphospholipid syndrome. *Arquivos Brasileiros de Cardiologia*, *98*, e66-e69.

Mitten, L. A. (1996). Cardiovascular causes of exercise intolerance. *Veterinary Clinics of North America: Equine Practice*, *12*(3), 473-494.

Morganroth, J., Maron, B. J., Henry, W. L., and Epstein, S. E. (1975). Comparative left ventricular dimensions in trained athletes. *Annals of internal medicine*, *82*(4), 521-524.

NRC [U.S. National Research Council]. 2007. Status of Pollinators in North America. The National Academies Press. Washington, D.C. USA.

Nyland, T. G., & Mattoon, J. S. (2002). *Small animal diagnostic ultrasound*. Elsevier health sciences

Oyama, M. A., and Sisson, D. D. (2004). Cardiac troponin‐I concentration in dogs with cardiac disease. *Journal of Veterinary Internal Medicine*, *18*(6), 831-839.

Pascon, J. P., Santos, F. P., Pereira, D. T., Mistieri, M. L., and Mozzaquatro, F. D. (2015). Estudo eletrocardiográfico de éguas da raça Crioula. *Pesquisa Veterinária Brasileira*, *35*, 319-323.

Pastore, C. A., Pinho, J. A., Pinho, C., Samesima, N., Pereira, H. G., Kruse, J. C. L., ... and Atanes, S. M. (2016). III Diretrizes da Sociedade Brasileira de Cardiologia sobre análise e emissão de laudos eletrocardiográficos. *Arquivos brasileiros de cardiologia*, *106*, 1-23..

Paterick T. E, Gordon, T., Spiegel D. 2014. Ecocardiografia: perfil do coração do atleta. Journal of the American Society of Echocardiography, 27 (9), 940-948.

Patteson, M. W. (1996). Cardiovascular pathology and pathophysiology. *Equine cardiology. Blackwell Science Ltd: Oxford, 1996a*, 27-37..

Pelliccia, A., Maron, B. J., Spataro, A., Proschan, M. A., and Spirito, P. (1991). The upper limit of physiologic cardiac hypertrophy in highly trained elite athletes. *New England Journal of Medicine*, *324*(5), 295-301.

Piccione, G., Assenza, A., Fazio, F., Giudice, E., and Caola, G. (2003). Electrocardiographic changes induced by physical exercise in the jumper horse. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *55*, 397-404.

Pinho, R. A. D., Araújo, M. C. D., Ghisi, G. L. D. M., and Benetti, M. (2010). Coronary heart disease, physical exercise and oxidative stress. *Arquivos brasileiros de cardiologia*, *94*, 549-555.

Restan, A. Z., et al. Conditioning program prescribed from the external training load corresponding to the lactate threshold improved cardiac function in healthy dogs. *Animals*, 2022, 12.1: 73.

Riedhammer, H.H.; Rafflenbeul, W.; Weihe, W.H.; Krayenbuhl, H.P. Left ventricle contractile function in trained dogs with cardial hypertrophy. *Basic Res. Cardiol.* **1976**, *71*, 297–308.

Rishniw, M., and Erb, H. N. (2000). Evaluation of four 2‐dimensional echocardiographic methods of assessing left atrial size in dogs. *Journal of Veterinary Internal Medicine*, *14*(4), 429-435.

Rovira C. S, Muñoz J. A, and Benito H. M. 2008. Valores ecocardiográficos em modo M em potros Pura Raza Española com edades comprendidas entre 1 y 12 meses. RecVet. 3:1.

Santilli, R. A., and Perego, M. 2009. Manuale di elettrocardiografia del cane e del gatto. Genesi ed interpretazione del ritmo cardiaco. Elsevier, pp.173

Schade, J., Schade, M. F., andFonteque, J. H. (2014). Auscultatory and electrocardiographic characteristics of Crioulo horses. *Pesquisa Veterinária Brasileira*, *34*, 281-289.

Soares, L. L., Pimenta, E. M., Barros, A. F. S., Lessa, L. B., and Pussieldi, G. A. (2012). Analysis of serum creatine kinase in athletes in college football after an intermittent session/Analise dos niveis sericos de creatina quinase em atletas de futebol universitario apos uma sessao intermitente. *Motricidade*, *8*(S2), SS439-SS439.

Teichholz, L. E., Kreulen, T., Herman, M. V., and Gorlin, R. (1976). Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. *The American journal of cardiology*, *37*(1), 7-11..

Toledo, P. S., Domingues Júnior, M., Fernandes, W. R., and Magone, M. (2001). Serum activities of aspartate aminotransferase, creatine kinase, gamma glutamyltransferase, lactate dehydrogenase and glycaemia in thoroughbred horses submitted to exercise of different intensities. *Revista Brasileira de Ciências Veterinárias*, *8*, 73-77.

Trachsel, L.D.; Ryffel, C.P.; De Marchi, S.; Seiler, C.; Brugger, N.; Eser, P.; Wilhelm, M. Exercise-induced cardiac remodeling in non-elite endurance athletes: Comparison of 2-tiered and 4-tiered classification of left ventricular hypertrophy. *PLoS ONE* **2018**, *13*, e0193203.

Valberg, S. J. (1996). Muscular causes of exercise intolerance in horses. *Veterinary Clinics of North America: Equine Practice*, *12*(3), 495-515..

Weiner, R.B.; Baggish, A.L. Exercise-induced cardiac remodelling: The need for assessment of regional myocardial function. *J. Physiol.* **2012**, *590*, 2829–2830.

Yonezawa, L. A., Machado, L. P., da Silveira, V. F., Watanabe, M. J., Saito, M. E., Kitamura, S. S., and Kohayagawa, A. (2009). Exame eletrocardiográfico em equinos da raça puro sangue árabe submetidos ao exercício em esteira de alta velocidade e à suplementação com vitamina E. *Archives of Veterinary Science*, *14*(3).

Young, L. E. (1999). Cardiac responses to training in 2‐year‐old Thoroughbreds: an echocardiographic study. *Equine Veterinary Journal*, *31*(S30), 195-198.