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EQUINE ELECTROCARDIOGRAPHIC RESPONSES TO TRAINING BASED ON SPEED-LACTATE CURVE PERMORMED ON TREADMILL

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ABSTRACT

The aim of this study was to evaluate, by electrocardiographic analysis, equine cardiovascular adaptations to training based on speed-lactate curve. For this evaluation, eight Arabian horses previously submitted to four months of rest were used. The animals performed a graded exercise test (ET) and samples were collected for blood lactate measurement. The speed where the blood lactate concentration reached 4.0 mmol / L (V_4) was calculated by regression analysis. The estimated training speed was 60% of V4 performed on a treadmill for 45 min three times a week on alternate days for eight weeks. The electrocardiogram (ECG) analysis was made before and after the beginning of the training. The training improvement is confirmed by the increase in both V_2 and V_4 of the second ET. After the second ECG analysis, a significant increase in the R wave amplitude was observed. Training improved the metabolic activity, promoted discrete changes in electrocardiogram; however, it was not adequate to result changes in cardiac parameters.

KEYWORDS: Training adaptation, cardiovascular, electrocardiogram, treadmill, equine.

RESUMO

EQUINE ELECTROCARDIOGRAPHIC RESPONSES TO TRAINING BASED ON SPEED-LACTATE CURVE PERMORMED ON TREADMILL

Neste trabalho, avaliaram-se, mediante exame eletrocardiográfico, as adaptações cardiovasculares de equinos ao treinamento com base na curva velocidade-lactato. Para tanto, foram utilizados oito equinos da raça Puro Sangue Árabe previamente submetidos a um período de quatro meses de destreinamento. Os animais realizaram inicialmente um teste de esforço progressivo (ET), durante o qual colheram-se amostras de sangue para determinação do lactato. A velocidade na qual a concentração de lactato atingiu 4,0 mmol/L (V_4) foi calculada por meio de análise de regressão. Utilizaram-se 60% de V_4 como velocidade de treinamento em esteira rolante, com duração de 45 minutos, três vezes por semana em dias alternados, durante oito semanas. Exames eletrocardiográficos (ECG) foram realizados antes e após o período de treinamento. O treinamento melhorou o condicionamento físico dos equinos, confirmado pelo aumento tanto de V_2 como de V_4 no segundo ET. Pelo exame ECG, observou-se aumento significativo na amplitude de onda R após o treinamento. Em que pese o treinamento ter melhorado a atividade metabólica, não foi suficiente para conferir modificações em parâmetros cardíacos, embora tenha promovido discretas mudanças no exame eletrocardiográfico.

PALAVRAS-CHAVES: Adaptações ao treinamento, cardiovascular, eletrocardiografia, esteira, equino.

INTRODUCTION

The heart is an organ located in the anterior and ventral portion of the thoracic cavity. In horses, it presentes its characteristical position, has the right cranial ventricle to the left and is displaced to the left of the thoracic midline. Its apical portion is in a caudal-ventral position in the thorax near the sternum, at the height of the dorsal portion of the olecranon, while its base is situated cranial-dorsallly. Radiographically, the heart occupies th extension of five ribs and four intercostal spaces, from the second to the seventh rib (MENDES, 2004; REEF, 1998).

The main cardiac function is to keep a good blood circulation, which should be adequate for the proper functioning of all organs and body tissues. If the circulatory demand is increased, the heart can compensate it by two basic ways which may provide greater blood volume per minute: increase in the heart rate and in the contraction force (MENDES, 2004).

The heart is a muscle and, as such, responds and adapts to training in an attempt to maintain homeostasis (MARLIN & NANKERVIS, 2002; BUGAISKY et al., 1992). Any change in cardiac demand, whether for athletic activity or pathologic changes, will increase or decrease cardiac mass, known as cardiac hypertrophy and atrophy, respectively (BUGAISKY et al., 1992).

According to GROSSMAN et al. (1975), there are two types of adaptations in response to training due to the type of myofibril produced. An increase of both myofibrils arranged in series (hyperplasia), enlarging the diameter of the heart chamber, or of myofibrils arranged in parallel, expanding the thickness of the left ventricle (hypertrophy) could occur. According to these authors, there is a chance that the increase of the diameter precedes the increase of heart thickness.

The increase of wall thickness facilitates the contraction strength of the heart chamber and provides an augmentation of blood stroke volume (volume percentage pumped at the end of systole in each cardiac beat). This contributes to a better animal performance, since more blood, thus more oxygen, can be sent to the organs and tissues (MARLIN & NANKERVIS, 2002; POOLE & ERICKSON, 2004).

In trained horses, the cardiac mass can reach 1.1% of body weight and is related to stroke volume, cardiac output and aerobic capacity. Cardiac output is defined as the volume of blood pumped from the ventricles and it can be measured in L / minute. When there is an increase in the cardiac output, oxygen demand to the tissues is improved, and this variable is the main determinant of the velocity of maximal oxygen distribution (VO_2 max).Trained animals can get cardiac output values exceeding 500 L / min due to the increase of cardiac mass (POOLE & ERICKSON, 2004).

The major complication related to this cardiac adaptation, studied in vivo, is the size of the heart compared to the weight of the animal. Cardiac hypertrophy may, in some cases, be accompanied by necrotic areas, leading to myofibrillar disruption, mitochondrial damage and proliferation of connective tissue with collagen accumulation (BUGAISKY et al., 1992).

The heart's electrical activity can be assessed by electrocardiographic exam. This is a non-invasive, inexpensive, easy to perform exam which can provide information on heart rate and rhythm and detect abnormalities or arrhythmias visible in the electrocardiographic tracing (REEF, 1998). In equine sports medicine, the electrocardiogram is used to investigate athletic underperformance related to fibrillation, complete atrioventricular block and atrial and ventricular premature contractions (EVANS, 1999).

The waves particular characteristics for each species is an important factor for its different format types. In horses, the P wave is often biphasic or polyphasic and has a "V" shape, which should not be interpreted as an abnormality. The form of the P wave is partly dependent on heart rate and, as the frequency increases, the space between the two peaks tend to be smaller or even disappear, the first part of the wave may be lost as the second section becomes larger. Changes in form, regardless of frequency, indicate the origin of the atrial contraction is changing (SPEIRS, 1999). When changes occur in ventilation or perfusion, we consider the increase of T waves, which should be differentiated in cases of hyperkalemia. Changes in T waves and in the ST segment can also be interpreted as a physiological variation related to training (SPEIRS 1999)

The T wave is the most variable parameter of an electrocardiogram in horses. When animals are submitted to stress or physical exhaustion, it is possible to observe reversal polarity and increase in amplitude that may exceed the QRS complex (STEEL et al., 1976; PICCIONE et al., 2003).

The establishment of a training program should consider the burden of effort to which the different organ systems will be submitted (POWERS & HOWLEY, 1997). The variables that reflect the typical overload include intensity, duration and frequency (ISLER et al., 1982), besides the obvious development of skills specific to each modality.

To guide the establishment of these principles, the use of some useful variables should be considered to assess the physical conditioning and determine the workload of the animals. Among these variables, we highlight the lactate, which has been the guide of numerous training programs, whether conducted in the field, track or trails (WITTKE et al., 1994; COROUCÉ et al., 1997), or treadmills, under controlled conditions (WERKMANN et al., 1996; ETO et al., 2004).

The curve established by blood lactate concentrations determined at increasing speed is called the lactate-velocity curve. The slope of this curve reflects the metabolic pattern prevalent in subsequent intervals of increasing speeds. At lower speeds, there is a predominance of aerobic metabolism and lactate concentrations remain relatively low. However, when the energy requirements to meet increasing speeds reach high values, the metabolism becomes predominantly anaerobic, with concomitant increase of lactate which is characterized by a sudden upward inflection of the curve. This inflection point is called the anaerobic threshold, or, what seems to be the most appropriate name, the start of blood lactate accumulation (BAYLY, 1986).

Thus, the aim of this study was to evaluate, by means of ECG, possible adaptations of the cardiovascular system of horses to physical training performed on a treadmill with an intensity determined by the values of blood lactate.

MATERIAL AND METHODS

Animals

We used eight adult Arabian horses, males and females, aged from five to six years, weighing between 339 and 422 kg, belonging to the experimental herd of the Faculty of Agricultural and Veterinary Sciences of UNESP, Jaboticabal. The animals were kept in Tifton paddocks and received commercial concentrate with 40% of energy requirements for moderate work (NRC, 1989).

Before the experimental period, the animals underwent a physical examination for healthiness assessment. Only healthy horses – in good nutritional condition that were protected against endo and ectoparasites and had undergone vaccination against respiratory viruses – were used. Before the beginning of the study, the animals remained at rest for at least four months. Trimming was performed prior to the experimental procedures and every five weeks, until the end of the experiment.

Electrocardiographic Examination

The examination was performed with the animal in standing position, on a rubber surface, contained in a squeeze chute, located in a calm and quiet environment.

The heart's electrical activity was evaluated by means of a 12-channel[1] electrocardiograph (ECG model - PC) of the base peak derivative at 25 mm / s and 2N sensitivity. The electrodes were fixed to the skin via 21G stainless steel needles in the region previously moistened with alcohol. The tracing was obtained according to technique described by FREGIN (1982).

A period of about ten minutes of the tracing was captured and recorded on computer hard drive. Heart rate, myocardial depolarization and repolarization sequency, morphology, duration in seconds (sec) of the P wave, polarity characteristic of the T wave, QT, PR

1 Model of ECG acquisition for computer (ECG – PC, Tecnologia Eletrônica Brasileira – TEB, Brazil)

and QRS intervals, and amplitude in millivolts (mV) of R, Q and T waves were assessed.

The same procedure was performed after eight weeks of training under these conditions.

Progressive cardiac stress test

After three days of adaptation to a high performance treadmill[2] designed for large animals, horses were considered able to undergo the progressive cardiac stress test, called exercise test (ET). Before testing, the animals were cannulated in the jugular vein using a 14G catheter[3] to enable the blood collection with the animal in motion. Once the horses were positioned on the treadmill, it was activated for the beginning of ET. The test consisted of a five minutes warm up at 1.7 ms^{-1} and five minutes at 3.5 ms^{-1} without tilting. After the warm up period, the treadmill was tilted to 6%, with an initial speed of 3.5 ms^{-1}, with 0.5 ms^{-1} speed addition every three minutes.The end of ET was determined by the moment in which each animal showed signs of fatigue, such as lack of focus, intense sweating, lowering of the head and tiredness, tending to not follow the treadmill and moving backwards. After this period, the animal was submitted to a cool-down exercise for 15 minutes at 1.7 ms^{-1}, aiming at its recovery.

Blood samples collected for blood lactate determination were placed in flasks[4] containing 1% sodium fluoride in a 1:2 ratio at the following occasions: before the test (basal), at the end of warm-up, 30 seconds before each speed increase, 30 seconds before and after the test and the cool-down and 60 minutes after the exercise. Blood lactate assessment was carried out by means of lactate oxidase with an automated analyzer[5] and expressed as mmol / L.

The calculations for determining the speed at which the concentrations of lactate corresponded to 2.0 and 4.0 mmol / L (V_4) were carried out by means of regression analysis, from the values obtained in ET (COUROUCÉ et al. 1997).

The calculation of V_4 was used in the establishment of the workload to be performed during training, as well as in evaluating the efficiency of the training protocol. The animals were submitted to two ET on treadmill: before starting the training (ET_1) and after eight weeks of training (ET_2).

2 Galloper Treadmill, Sahinco Ltda., Palmital, SP, Brazil.
3 Angiocatt, BD ind. Cirúrgicas Ltda., juiz de Fora, MG, Brazil.
4 Vacuttainer, BD ind. Cirúrgicas Ltda., juiz de Fora, MG, Brazil.
5 Lactate meter YSL, 1500 Sport, Yellow Springs, Ohio, USA.

Training

Once the V_4 was determined in exercise tests, it was established that the work intensity to be imposed on the animals would correspond to 60% of V_4, which lasted 45 minutes, in a frequency of three times per week on alternate days. The training program lasted eight weeks and within this period the horses performed a total of 18 sessions of treadmill workouts. The animals underwent weekly clinical examinations to assess their healthiness and the integrity of the locomotor system.

Statistical analysis

The different parameters were evaluated by analysis of variance (ANOVA), with the average values for the means comparison test (t test) with significance level of $P < 0.05$ (SAMPAIO, 2002) using SAS - Statistical Analysis System (SAS 2002).

RESULTS AND DISCUSSION

The values of V_2 and V_4 obtained during the cardiac stress tests performed on a treadmill, in the pre and post-training, are listed in Figure 1. The training improved physical conditioning of horses, which was confirmed by increases in both V_2 and V_4 in the second stress test. The training model used, based on the lactate values obtained in cardiac stress test, have also provided an increase of the variables studied in similar researches (GOMIDE et al. 2006).

Speeds corresponding to 2.0 (V_2) and 4.0 mmol / L of blood lactate (V_4), the value equivalent to 60% of V_4 and the speed required for the development of fatigue (maximum V) are presented in Table 1. V_2, V_4 and speed training (60% of V_4) increased significantly after the training period. $V_{Maximum}$ presented no significant difference in ET performed after training.

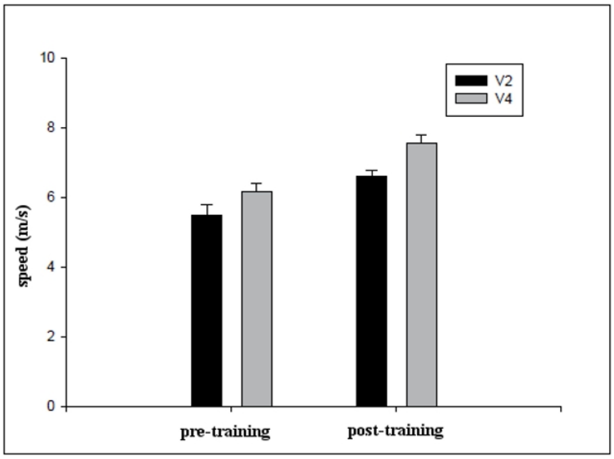

Figure 1. Means ± standard deviation of speeds corresponding to V_2 and V_4, obtained from Arabian horses in the pre and post-training, during progressive cardiac stress test performed on a treadmill (* and ** indicate higher values than in the pre training for the same variable, $p \leq 0.05$).

Table 1. Mean ± SEM for values determined for the speed at which the lactate concentration reached 2.0 mmol / L (V2), 4.0 mmol / L (V4), 60% of V4 and VMaximum during exercise test of Arabian horses before and after training

Variable (m / sec)	Before the training	After the training
V_2	5.4 ± 0.2	6.4 ± 0.3 *
V_4	6.3 ± 0.3	7.6 ± 0.2 *
60% of V_4	3.8 ± 0.2	4.4 ± 0.1 *
$V_{Maximum}$	7.7 ± 0.4	8.2 ± 0.3

* Value greater than that obtained before the training by the Tukey test ($p \leq 0.05$).

The training program designed for horses in this work with effort load corresponding to 60% of V4, produced a significant increase of both V2 and V4. Other studies show the importance of adopting speed where the blood lactate concentration reaches 4.0 mmol / L (V4) during cardiac stress test as a means of assessing the physical conditioning of horses (GERARD et al., 2002; ART & LEKEUX, 1993). In this experiment, there was an increase of 21.0% in V4 after training, higher than that reported by TRILK et al. (2002), who observed 17% increase in this variable in training also guided by the results of the speed-lactate curve and performed on a treadmill. Another study, using the speed-lactate curve to train horses on a treadmill, was unsuccessful in seeking increases in V4 after two months of training, when the exercise sessions lasted only five or 15 minutes (WERKMANN et al., 1996).

The V4 increase observed during the training is due to the metabolic changes from the program adopted. According to EATON et al.(1999), GERARD et al.(2002), HINCHCLIFF et al. (2002) and McGOWAN et al. (2002), increased V4 and the lowest concentrations of lactate under the same exercise intensity during the training indicate greater energy production by aerobic means. This comes from the better utilization of lactate, according to DONOVAN & BROOKS (1983), and fat, according to MUÑOZ et al. (2002), as energy sources, as well as increased activity of enzymes of aerobic metabolism and of the buffering system of muscle fibers (McGOWAN et al., 2002). Increased activity of citrate synthetase, the enzyme involved in the entry of acetyl-CoA in the citric acid cycle, was observed in horses after training (HODGSON et al., 1985). Whereas the enzymatic pathways of energy production present in muscle fibers are closely interrelated, the increased activity of citrate synthetase would imply increased activity of other enzymes involved in the mentioned cycle. Apparently, the progressive increases in the intensity of training constitute necessary stimulus to induce continuous increase of enzyme activity in muscle fibers. This increase would allow a greater proportion of energy to originate from aerobic mechanisms, which are more efficient than the anaerobic ones (EATON et al., 1999).

Training conducted at slower speeds and lasting 45 minutes caused major changes in muscle (MARTINS et al., 2007) and, consequently, more positive effects on the resistance ability than when the training has greater intensity and shorter duration (GANSEN et al., 1999). In this experiment, sessions with intensities based on V4 and lasting 45 minutes resulted in sufficient stimulus to increase horses´ resilience. Although TRILK et al. (2002) indicate periods of two weeks to adjust the intensity of training seeking to improve the increase in V4, in this case, even without performing the adjustment, training for eight weeks was enough to cause a significant increase in this variable.

Although McGOWAN et al. (2002) found larger concentrations of lactate in the plasma and lower in the muscles at moments of fatigue; after a training period, they did not observe changes in lactate concentrations determined at the end of the tests and after the gradual cool-down period, showing that this variable increases the capacity of buffering and removing lactate from the muscle. HINCHCLIFF et al. (2002) did not find significant differences in lactate concentration at the end of the test in ten weeks of training. The data would be justified, therefore, by the fact that the exercise intensity was not sufficient to induce significant changes (ART & LEKEUX, 1993). However, considering that the lactate values obtained during the cardiac stress tests showed great individual variability (RONÉUS et al., 1994), which was detected during this evaluation, this could be the factor that made it difficult to obtain significant results in statistical analysis.

The speed required for the expression of fatigue (VMaximum) during tests on treadmill increased by the end of 90 days of training. This data shows that the metabolism of the animals suffered adaptations that allowed the maintenance of exercise for a longer period, improving performance (LINDNER, 1997). The increased time required for the expression of fatigue due to training has been reported by other authors (GERARD et al., 2002; McGOWAN et al., 2002) and can be a parameter for evaluating physical conditioning improvement (McGOWAN et al., 2002).

TRILK et al. (2002) reported signs of psychological changes typical of overtraining and characterized both the lack of physical motivation and the food intake decrease as signs of the disorder at the final phase of a survey of treadmill-trained horses. However, the animals used in this study showed excellent physical motivation during the exercise sessions and kept the appetite. This response is attributed to the fact that the horses used in this work remained in the field, in groups, with the opportunity not only to maintain social relationships, so important to this species, but also to diversify the training routine through leisure activities in the paddock.

The data related to the ECG is shown in Table 2.

Table 2. Mean ± standard deviation of certain electrocardiographic variables in Arabian horses, at rest, before and after eight weeks of training on a treadmill

Variables	Before training	After training
Heart rate (bpm)	37.38 ± 1.66	35.13 ± 1.92
P duration (sec)	0.134 ± 0.005	0.137 ± 0.013
P amplitude (mV)	0.210 ± 0.020	0.310 ± 0.040
P-R interval (sec)	0.339 ± 0.011	0.319 ± 0.022
QRS duration (sec)	0.147 ± 0.002	0.146 ± 0.008
R amplitude (mV)	1.510 ± 0.100	2.040 ± 0.130 *
Q-T interval (sec)	0.531 ± 0.009	0.517 ± 0.009
Amplitude of Q (mV)	0.410 ± 0.110	0.310 ± 0.070

* Value greater than that obtained before the training by the Tukey test ($p \leq 0.05$).

The heart rate values observed are within the physiological ranges reported for not active equine animals (FERNANDES et al., 2004). The values obtained after training did not differ statistically from those recorded before training; however, there was a tendency to decrease, which supports the assertion that horses which are trained, thus well-conditioned physically, have lower heart rate than unconditioned horses (LITTLEJOHN, 1987).

The process of depolarization in the ventricular myocardium can be affected by intensive training and probably by direct action of diastole duration and by factors which interfere with this duration, including the autonomic effects and possibly the metabolic effects on myocardial fibers influenced by the coronary circulation (HOLMES & REZAKHANI, 1975). In this study, there was statistical difference in the R-wave amplitude, suggesting an increase in the time of ventricle depolarization, which may be associated with hyperplasia or hypertrophy of the heart chamber due to training.

SKARDA et al. (1976) evaluated the influence of training on heart rate, the duration of PQ and QT intervals and of QRS complex in Thoroughbred horses. The analysis was performed before and after six weeks of submaximal exercise on sand track. In this study, there were no significant changes in the electrocardiographic tracing that proved an improvement of the athletic state of the animal due to training.

The effect of training on ECG parameters was evaluated by YONEZAWA et al. (2009) using eight Arabian mares, submitted to 20 days of training on a high speed treadmill. Analysis and interpretation of electrocardiographic tracings were performed regarding rhythm, heart rate, duration and amplitude of the waves and intervals. In this assay, a decrease of heart rate was only observed during resting after training, which was attributed to the animals´management during the experimental period.

The T wave, according to SEVESTRE (1982), comprises a variable parameter; therefore, its interpretation can be difficult due to variations in shape and amplitude. In this study, as for the polarity characteristic, T wave remained biphasic at the beginning of the trial and eight weeks after training, in all animals evaluated in the exercise test. In electrocardiographic study of Mangalarga Marchador horses conducted by DINIZ et al. (2008), T wave was positive in 11 (18.3%), negative in 12 (20%) and biphasic in 37 (61.7%) cases. According to the authors, such distribution of the waves was considered compatible with the pattern described by PATTESON (1996) for this derivation, and therefore similar to that of other breeds.

It has been postulated that some types of arrhythmias, such as second-degree atrioventricular block detected in horses at rest, disappear during exercise (ALPS & HOLMES, 1966). In this work, one of the horses studied showed second-degree atrioventricular block on ECG performed before training, but this arrhythmia disappeared after training. The interruption of the beating observed in the second degree atrioventricular block is a response to the gradual increase of peak aortic pressure (HOLMES, 1982). Considering this is probably a baropressure response, the effort produced during the training associated with possible increases in blood pressure during higher intensity exercise, as in this study, would cause adjustments in pressure receptors sensitivity, and hence the disappearance of the previously mentioned blocking.

CONCLUSION

Although training on a treadmill under the conditions of this study has improved the variables associated with metabolic activity of the horses, it was not enough to cause changes in most electrocardiographic parameters, except for the R wave amplitude.

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