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Influence of Cavalcade of Different Distances on the Serum Concentrations of the Cardiac Biomarkers Troponin I (Ctni) and Creatine Kinase Myocardial Band (CK-MB) in Horses

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Abstract

Cardiac biomarkers are potentially useful to identify primary or secondary cardiac muscle damage in horses and higher values of cardiac troponin have also been seen after high intensity exercise. The objective of this study was to investigate the effect of cavalcade of different distances on the serum concentrations of cardiac troponin I (cTnI) and creatine kinase myocardial band (CK-MB) in Mangalarga Marchador horses. Fifteen clinically normal geldings (age 6.2 ± 1.2 years, weight 420 ± 58 kg) were distributed into three groups of five animals each. The groups were submitted to cavalcade along 4 km (G4), 8 km (G8), and 20 km (G20) at mean speeds of 15 km h^{-1}, 12 km h^{-1}, and 12 km h^{-1}, respectively. From each horse, blood samples were collected after and post-exercise. Serum cTnI and CK-MB concentrations were determined with a solid-phase chemoluminescence immunoassay. The assay has an analytical sensitivity of 0.02 ng mL-1. The concentration of blood lactate was analyzed using an i-STAT portable clinical analyzer. After exercise, increases ($P < 0.01$) in cTnI and CK-MB concentrations were observed in G4 and G8. No changes ($P > 0.05$) in lactate concentration were observed post-cavalcade in all groups. Power tests values were 1.0 for cTnI and CK-MB, 0.63 for lactate. Positive correlations ($0.4 < r < 0.5$) were observed between cTnI and CK-MB. The changes in cTnI and CK-MB concentrations post-exercise is indicative of myocardial cell injury in healthy Mangalarga Marchador horses, which could be considered as an adaptation to physical exercise.

Keywords: Cardiac Biomarkers; Cardiac Disease; Troponins; Creatine Kinase; Equine Exercise; Lactate

Introduction

Myocardial injury is difficult to diagnose in horses. Traditionally, electrocardiography and echocardiography have been used to detect myocardial injury, but with limitations. Biochemical tests available for detection of myocardial damage in horses include the isoenzymes creatine kinase (CK-MB) and lactate dehydrogenase (LDH 1). These enzymes also pose limitations, due to the short half-life in serum, low specificity and sensitivity, and the occurrence of cross-reactions with skeletal muscle injury [1,2]. Actually, in equine medicine, cardiac Troponin I (cTnI) has been reported to be the best biomarker to detect myocardial disease [3,4,5]. cTnI is a protein found in the myocardial cell, it is linked to tropomyosin and contributes to the muscular contraction in the presence of calcium. Damage to cardiac myocytes increases serum concentration of Troponin [4]. High serum concentrations of cTnI occur in horses with ventricular tachyarrhythmias, monensin toxicosis, atypical myopathy, atrial fibrillation, structural heart disease, and amyloidosis [3,6,7,8]. cTnI is also increase with non-cardiac diseases that induces secondary cardiac involvement, as example with babesiosis and endotoxaemia [9,10]. Release of cTnI during and after exercise also has been described in equine athletes after racing or race training, endurance rides or treadmill exercise [11,12,14,13]. Creatine Kinase-Myocardial Band (CK-MB) is another cardiac biomarker that has been reported to increase with exercise and with chest pain in humans [15]. However, it was reported that CK-MB is found in equine heart, but at insignificant quantities [1]. The Mangalarga Marchador horse is the most important and numerous Brazilian equine breed, and its natural gait is marcha rather than trot [16]. The marcha is a comfortable four-beat lateral and diagonal gait with moments of triple support and no suspension [17]. In recent years, cavalcades have gained sport status, with an increasing number of adopters. The effort is predominantly aerobic and of moderate-intensity for long duration exercises, similar to endurance [18]. The combination of good physical resistance and comfortable riding features may explain why the Mangalarga Marchador breed has gained popularity in this kind of event. Nevertheless, the effects of cavalcade exercises on cardiac integrity in these animals have not been investigated. Therefore, the aim of this study was to determine if cavalcade exercise of various intensities induced myocardial damage, defined by changes in the cardiac biomarkers cTnI and CK-MB.

Materials and Methods

Horses

This study was approved by the ethical committee of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (approval number 304). The study population consisted of 15 healthy Mangalarga Marchador geldings (age 6.2 ± 1.2 years, weight 420 ± 58 kg). All gait horses included in the study were privately owned and were prepared to participate in cavalcade by the owners. These horses were trained two to four times a week for 30-60 min. The horses underwent a clinical examination with specific focus on the cardiovascular system. Body temperature, heart rate, and respiratory rate were recorded, and cardiac and pulmonary auscultation was carried out. Horses included had a normal clinical examination, and complete blood count, biochemical parameters, electrocardiogram, and echocardiography were performed.

Groups and Blood Sampling

The horses were divided into three groups with five animals each according to the distance covered, namely, G4 (4 km, 15 km h^{-1}), G8 (8 km, 12 km h^{-1}), and G20 (20 km, 12 km h^{-1}). Data were collected at a cavalcade held in Campos dos Goytacazes, Rio de Janeiro, Brazil on May 8, 2010 (20^{0}48'21"S, 40^{0}38'52"W, altitude 13 m a.s.l.). The previously marked loops had a length of 20 km, and were taken in a single day, without rest. Mean environmental temperature was 30.7^{0}C and relative humidity was 79.3%. Blood samples (5 mL) were collected in plain tubes Vacutainer (BD) from the jugular vein of each horse 24 h prior to the cavalcade and 2 h post-cavalcade, when animals were resting. After collection, the samples were stored in ice. The tubes were centrifuged 2 h after collection for 10 min at 1500 g and the serum samples obtained were transferred to tubes and immediately stored at - 20^{0}C for 2 days. The serum samples were thawed and immediately analyzed for cTnI and CK-MB. Heparinized blood samples was collected anaerobically from the jugular vein and stored on ice for lactate analyses. The samples were obtained before the start the cavalcade and 30 minutes afterward.

cTnI, CK-MB, and Lactate Assays

Cardiac troponin I and CK-MB concentrations were analyzed in serum using a solid-phase chemoluminescence immunoassay analyzer (ImmuliteTurbo®, Siemens Healthcare, USA). The detection limit of this assay is 0.02 - 20 ng mL^{-1}, with analytical sensitivity of 0.02 ng mL^{-1}. All results are expressed as ng mL^{-1}. This test has already been validated for horses [12,19]. The concentration of blood lactate was analyzed using an i-STAT portable clinical analyzer (VetScan i-STAT 1; Abaxis, CA, CG^{4+} cartridges) according to the manufacturer's instructions. The results are expressed as mmol L^{-1}.

Statistical analysis

Normality of data (means ± SD) was verified using the Shapiro-Wilk test. The data obtained were submitted to One-Way Analysis of Variance using the Sigma Plot 12.0 statistical program, and means were compared by the Tukey test ($P < 0.05$). Power of performed test was determined. Relationships between the variables CK-MB and cTnI were determined using the Pearson correlations coefficient r. Values of $P \leq 0.05$ were considered statistically significant.

Results

All horses from G4, G8, and G20 managed to complete the cavalcade without abnormal signs. Mean values of blood cardiac biomarkers and lactate concentration in horses are presented in Table 1. Pre-cavalcade cTnI, CK-MB and lactate concentrations did not differ between groups or distances. After the exercise, the cTnI and CK-MB concentrations in G4 and G8 were significantly higher ($P < 0.01$) than the pre-exercise values. The values of cTnI and CK-MB in G20 horses also increased; however, the differences were not statistically significant (Table 1). There was no increase in lactate concentration after exercise in G4, G8, and G20 ($P > 0.05$). Power tests values were of 1.0 to cTnI and CK-MB; 0.63 to the lactate. Positive correlations ($0.4 < r < 0.5$) were observed between cTnI and CK-MB (Figure 1).

Discussion

The present study is the first to investigate the influence of cavalcade on the serum concentrations of cTnI and CK-MB in an apparently healthy population of Mangalarga Marchador horses using the solid-phase chemoluminescence immunoassay. The range of serum cTnI concentrations observed in the present study is similar to the ranges reported using the same immunoassay in normal pasture and race-training thoroughbred horses [12,19]. In order to quantify the intensity of effort carried out by horses during physical activity, it becomes necessary to determine the genesis of the physiological response that depends on the kind, speed, distance, and duration of gait. In all experimental groups, the kind of gait observed may be characterized as extended marcha. In addition, the evaluation of the intensity of exercise using physical and physiological parameters may be a non-subjective criterion that can be used for the sake of comparison with other studies. We used lactatemia to determine physical effort, and observed that the parameter did not vary for any of the distances covered (G4, G8, and G20). Therefore, in order to cover the predetermined distances, the horses used predominantly the oxidative metabolic route, which characterizes this effort as submaximal and aerobic.

Table 1: Means (± SD) of serum levels of cTnI, CK-MB and lactate in Mangalarga Marchador horses before and after cavalcade that included 4-km, 8-km, and 20-km runs.

Parameters	Groups	Times	
		before	after
Troponin I (ng mL^{-})	G4	0.04 ± 0.01 Aa	0.17 ± 0.01 Ba
	G8	0.05 ± 0.04 Aa	0.17 ± 0.02 Ba
	G20	0.06 ± 0.03 Aa	0.10 ± 0.02 Ab
CK-MB (ng mL^{-1})	G4	4.72 ± 0.21 Aa	4.74 ± 0.42 Aa
	G8	4.04 ± 0.49 Aa	5.54 ± 0.21 Ba
	G20	5.14 ± 0.39 Aa	5.62 ± 0.42 Ab
Lactate (mmol L^{-1})	G4	0.63 ± 0.02 Aa	0.68 ± 0.01 Aa
	G8	0.82 ± 0.09 Aa	0.89 ± 0.09 Aa
	G20	0.80 ± 0.07 Aa	0.86 ± 0.05 Aa

G4, G8, and G20 are groups of 5 animals each that ran 4 km, 8 km, and 20 km at mean speed of 15 km h^{-1}, 12 km h^{-1}, and 12 km h^{-1}, in that order;
Means followed by different capital letters in columns differ significantly between times (Tukey test at $P < 0.05$);
Means followed by different lowercase letters in rows differ significantly between groups (Tukey test at $P < 0.05$).

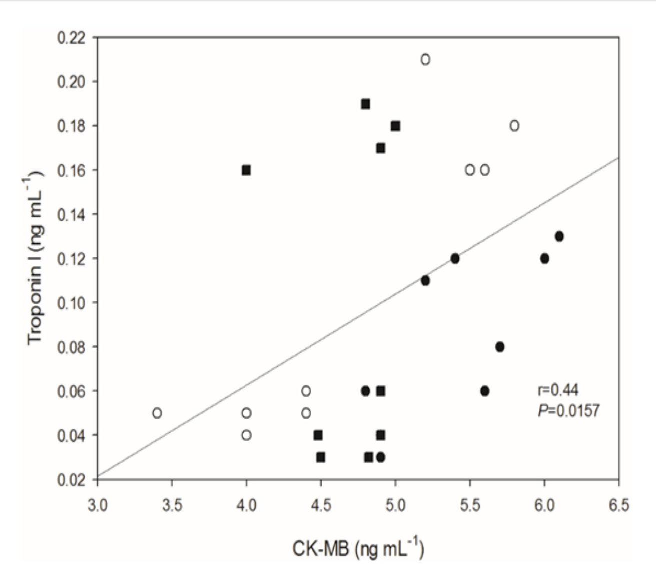

Figure 1: Serum concentration of cTnI vs. CK-MB activity of Mangalarga Marchador horses before and after 4-km (black squares) 8-km (black circles), and 20-km (white circles) cavalcade runs correlated positively. r: Pearson correlation coefficient.

These results are in accordance with other studies that analyzed the Mangalarga Marchador breed [20]. The results obtained show that horses generally have low cTnI concentrations at rest, and that moderate exercise such as a cavalcade induces mild increases in cTnI concentrations, consequently increasing the risk of some degree of myocardial injury. Unfortunately, factors that may predispose individual horses to myocardial injury associated with exercise such as fitness status, electrolyte imbalances, and dehydration could not be determined from the results of this study. Increased concentrations of cTnI have also been reported in connection with high-intensity activity, short and moderate-duration exercise, and in endurance horses without signs of cardiac disease [11,12,13,14]. The causes of exercise-induced cTnI concentrations are not clear. One theory suggests that the increases in cTnI concentrations may be explained by mild hypoxia during exercise. The hypoxia could cause a change in the permeability of myocardium, resulting in the leakage of macromolecules into blood flow [11,12,13]. found that short periods of hypoxia can induce release of cTnI without cell death in rats Piper, et al. [20]. However, it did not become clear whether high cTnI values had any clinical relevance. In other words, it remains to be established whether cardiac damage triggered by hypoxia due to exercise or any other cause may bring about cardiac problems.

For, temporary cardiac lesions caused by exercise may be similar to the muscle lesion induced by exercise, which does not have the potential to cause permanent damage or even affect performance in sports Rifai, et al. [21]. It may be presumed that the release of cTnI in equines after exercise originates form cytosol pools within myocytes, representing approximately 3% of the total level of cTnI [12]. The increase in cTnI concentration after exercise indicates reversible cell lesions, which could be considered as an adaptation to physical exercise [22]. As a rule, the increased in cTnI values observed in humans, dogs, camels, and horses after exercise are mild or moderate, as opposed to the concentrations found in cardiac disease such as myocardial ischemia, aortic rupture and myocardial necrosis [3,6,23]. Marked elevations of cTnI alone could be considered as a strong indication of myocardial disease [22]. Correlations between the degree of myocardial infarction size and the release of cTnI have been shown in humans and dogs, and can be used as a prognostic indicator [23,24]. However, in horses, there is not a defined range for values considered modest vs. those considered severe, or what clinical diseases or mechanisms cause mild, modest and severe elevations [13]. In humans, serum cTnI concentrations increase after prolonged intense exercise such as marathon and ironman triathlon training [25,21]. Increased levels of troponins after a cycling ultramarathon were also found [26]. However, the values were lower on the following day, suggesting that increases were transient and no echocardiographic evidence of myocardial dysfunction was observed. In the preset study, evaluations were not repeated at later dates to determine if the alterations in cardiac biomarkers were more permanent.

The increase in CK-MB concentration observed in this study was similar to that detected in Arabian horses after prolonged physical exercise [27]. One of the hypotheses that could explain this finding is the occurrence of cardiac hypertrophy due to training, similarly to what takes place in skeletal muscles [2]. Yet another important aspect is the fact that, despite the comparatively higher levels in cardiac tissues, CK-MB is also present in skeletal muscles (roughly only 0.4%) [1]. For the relevance of CK-MB in the diagnosis of cardiac lesion may be higher in resting animals, when the effect of exercise is not present Michima ,et al. [27]. It should be warned that CK-MB levels alone do not produce important information on the cardiac integrity of horses submitted to exercise. On the other hand, studies have suggested that the increase in the serum enzyme activity occurs due to the transitory increase in the permeability of the sarcolemma in the absence of histological lesions [28,29]. Positive correlations were observed for cTnI and CK-MB in the present study. In contrast, there was no correlation between cTnI and CK-MB concentrations in endurance athletes after prolonged strenuous exercise [30]. For in humans, the exercise-induced increases in cTnI concentrations and CK-MB activity seem to be independent, and probably underlie different mechanisms Scharhag, et al. [30]. Our results indicated that, in the equine, the elevation mechanisms these cardiac biomarkers may be similar. The standardization of the cardiac biomarkers through kinetic study after exercise, training or clinical evaluation, considering their half-life, is the more technical procedure, because it improves the accuracy of significance of the response [31].

In the present study, factors probably related with individual physical conditioning of each horse seemed to have more effect on myocardial injury than travelled distance, as shown by the significantly increased cTnI concentrations in horses of G4 and G8, compared with G20. Three other studies also showed an inverse relationship between exercise time or distance and post-exercise troponin concentration [13,21,32]. The researchers postulated that the younger, faster athletes competed harder and closer to their cardiovascular limits than other competitors and, therefore, may have been more likely to induce minor cardiac injury [21,32]. Some limitations were identified in this study and need to be considered. One limitation is the difficulty in determining that the horses included did indeed have healthy hearts. Although all the horses included had normal physical examinations, ECG, and echocardiograms, early myocardial disease might not have been detectable by these tests. Similar considerations were made by in horses in pastured and race training thoroughbred horses Phillips, et al. [19]. Histopathology of the heart tissues would have been the gold standard for detection of myocardial cell injury, but it was not possible in the present study [19]. The present study was based on a small sample size (n 5/15) of a single breed (Mangalarga Marchador). The small number of horses reflects the difficulty of experimenting with these animals due to the high cost involved in issues like handling, and, mainly, standardization of subjects concerning gender, age, and fitness condition. This reality led to varied test power values for the variables assessed: 0.63 (lactate) to 1.00 (cTnI and CK-MB). Because of the relatively small number of horses used in this report, additional studies involving a large population of horses of various breeds and ages will be required to determine the effects of these factors on cTnI concentrations. Further studies are needed to evaluate the serum cTnI concentrations in athletic horses with signs of cardiac disease in order to establish the correlation between elevated concentrations of cTnI and pathological findings. Future standardized exercise trials are recommended to further elucidate the potentially differential effects of training status, exercise time, and intensity on post-exercise increases in cTnI.

Conclusions

The increase in cTnI and CK-MB concentrations post-cavalcade of 4 km and 8 km is indicative of myocardial cell injury in healthy Mangalarga Marchador horses, which could be considered as an adaptation to physical exercise.

Author's contributions

PADF, GCF and CBC participated in the design of the study, supervised the experiments, revised and submitted the final manuscript. JML, PMB, RVBA, and SRBB participated in the study from the early phase up to the end of data collection. FSF and JML carried out the biochemistry studies. PADF and GCF performed statistical analysis and prepared the final draft of the manuscript. All authors were involved in discussing and interpreting the data, critically read the manuscript, and approved the final version.

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