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Research Article

EFFECT OF EXERCISE ON HAEMATO-BIOCHEMICAL AND ELECTROCARDIOGRAPHIC PARAMETERS IN INDIAN THOROUGHBRED HORSES

PRAVEEN V. MATHAPATI, NEETU SAINI*, S.K. UPPAL and ARUN ANAND[1]
Department of Veterinary Medicine, [1]Department of Veterinary Surgery and Radiology
Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana- 141004, India

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ABSTRACT

The present study was conducted in twenty clinically healthy Thoroughbred horses (15 males and 5 females) presented to Teaching Veterinary Clinical Complex, GADVASU, Ludhiana. Electrocardiography was performed in all horses using ECG machine with the deflection of 10 mm/mV and paper speed of 25 mm/sec.Various electrocardiographic parameters were evaluated by Base apex lead system and Lead II was used for interpretation of results. The ECG recording was carried out at rest, immediately after exercise (walk, trot and canter) and twenty minutes after exercise i.e. in recovery period. Haemato-biochemical parameters and cardiac biomarkers were estimated before and after exercise. Heart rate was significantly increased immediately after exercise however, significantly reduced in the recovery period *i.e.* 20 min after exercise. There was significant effect of exercise on P-wave morphology. Bifid P-waves were observed in all the animals (n=20) examined before exercise and most of P waves were single immediately after exercise. There was significant effect of exercise on T wave morphology and duration. Serum Lactate and NT-Pro-BNP concentrations were also significantly ($pd \leq 0.05$) increased after exercise. ST-segment and QT-interval were significantly($pd \leq 0.05$) decreased after exercise.

Key words: Electrocardiography, Exercise, Heart rate, Thoroughbred horses.

Electrocardiography (ECG) is a complementary, non-invasive, low-cost examination easily performed in the field conditions (Fregin, 1982). ECG is the ultimate tool for the diagnosis and classification of arrhythmias. Horses have a higher incidence of cardiac arrhythmias at rest than any other domestic species due to their high vagal tone (Young, 2004). These physiological arrhythmias are usually abolished when the vagal tone decreases and the sympathetic tone increases such as during exercise or excitement (Senta *et al.*, 1970). Cardiac arrhythmias play an important role among cardiovascular causes of poor performance in horses (Zucca *et al.*, 2003). Arrhythmias occurring during maximal intense exercise or immediately after exercise are of clinical importance and are a potential cause of reduced performance in horses (Martin *et al.*, 2000). Arrhythmias are frequently observed in healthy Thoroughbred race horses during training (Ryan *et al.*, 2005). ECG of horses at rest is of limited value because cardiac diseases and disturbances in cardiac rhythm leading to decreased performance rarely manifest themselves during rest (Young, 2007). Moreover, some cardiac arrhythmias tend to appear in the recovery period after exercise only (Reef, 1999).

Hematobiochemical analysis plays an important role in assessing fitness and changes in hematobiochemical profile can also affect performance in horses (Burlikowska *et al.*, 2015). Cardiac troponin I (cTnI) has been reported to be the best biomarker to detect myocardial diseases in horses (Schwartzwald *et al.*, 2003). In the present study, electrocardiographic and hematobiochemical examination were conducted to evaluate cardiovascular adaptations occurring after exercise in Thoroughbred horses.

*Corresponding author: sainin26@gmail.com

MATERIALS AND METHODS

The present study was conducted in 20 clinically Healthy Thoroughbred horses (15 males and 5 females) presented to Teaching Veterinary Clinical Complex, GADVASU, Ludhiana. Electrocardiography was performed in all horses using ECG machine (BPL CARDIART 108T-DIGI) with the deflection of 10 mm/mV and paper speed of 25 mm/sec. Standard base apex lead system was used as described by Smith (2014). The positive electrode was placed on the left thorax in the fifth intercostal space at the level of the elbow or at the location where the apex beat is most readily palpable. The negative electrode is attached to the skin of the right jugular furrow two thirds of the way from the ramus of the mandible to the thoracic inlet or at the top of the right scapular spine. The ground electrode can be attached to any site remote from the heart. Electrical contact is improved by wetting the skin with alcohol. Lead II was used for interpretation of results. The ECG recording was carried out at rest, immediately after 20 minutes of exercise (walk, trotand canter) and 20 min after exercise i.e. in recovery period.

Following ECG parameters were measured on each recording: Heart rate, P-wave duration and amplitude, PR interval, QRS duration, ST segment, QT interval, T-wave amplitude and duration at rest, immediately after exercise and in the recovery period. All the recordings were analyzed for rhythm and morphology of the different waves. Duration and amplitude of different waves were

recorded and then statistically analyzed for significant difference.

6 ml of blood was collected in EDTA vial (Poly Medicure Ltd.) for hematology and in clot activator vial for biochemical analysis both before exercise and immediately after exercise. Fully Automatic Laser Based Hematology Analyzer (ADVIA ® 2120 Hematology system, Siemens Healthcare diagnostics Inc., USA) was used. Hematological examination included hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC), platelet count and Differential leucocyte count (DLC) was done using Giemsa stain by the method described by Jain (1986). Biochemical parameters included creatinine kinase (CK), total protein (TP), lactate (LAC), aspartate amino transaminase (AST), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) and CKMB. Cardiac biomarkers e.g. Cardiac troponin-I (Calbiotech) and NT Pro BNP (Raybion) were analyzed by ELISA. Microplate ELISA reader (BIO-RAD I Mark) was used. Electrocardiographic parameters were analyzed by Equal variance ANOVA (One Way) and hematobiochemical parameters and cardiac biomarkers were analyzed by paired t-test by using SAS software version 9.3. The significance level was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The physiological parameters before exercise and immediately after exercise and in the recovery period are given in table 1. Heart rate was significantly ($p \leq 0.05$) increased immediately after exercise however, significantly ($p \leq 0.05$) reduced in the recovery period i.e. 20 min after exercise. The increase in heart rate is mainly attributed to the increase in the sympathetic tone required to satisfy the increase in cardiac output resulting from physical effort (Piccione *et al.*, 2003). Respiration rate and pulse rate also followed the same trend. Reef (1985) also observed that at rest, the horse's heart rate ranges between 24 to 50 beats per minute (bpm) and increased to a maximum of 220-240 bpm during exercise.

The haematological parameters before exercise and immediately after exercise are given in the table 2. TEC and PCV were increased after exercise although non-significantly. TLC was not significantly increased after exercise, however, absolute lymphocytes and eosinophil count significantly ($Pd \leq 0.05$) increased after exercise that may be indicating that exercise of low intensity is helpful in improving acquired immunity due to temporary enhancement of cellular immune function (Ali *et al.*, 2003) Increase in TEC and PCV may be attributed to the fact that adrenergic splenic contraction adds red blood cells to the circulation (Longhurst *et al.*, 1986).

Table 1
Effect of exercise on Physiological parameters (Mean ± S.E.) in healthy Thoroughbred horses (n=20)

Physiological Parameters	Before Exercise	After Exercise	Recovery
Heart rate (min)	33.4±0.95[a]	53.55±1.77[b]	42.6±1.59[c]
Respiration rate (min)	21.6±1.09[a]	52.9±3.83[b]	30.1±1.87[c]
Pulse (min)	33.15±1.53[a]	58.4±3.64[b]	38.5±1.18[c]

Values with different superscript lower case alphabets in same row differ significantly ($Pd \leq 0.05$)

Table 2
Effect of exercise on Hematological parameters (Mean ± S.E.) in healthy Thoroughbred horses (n=20)

Parameters	Before exercise	After exercise
Haemoglobin (×10 g/L)	10.38±0.46	10.96±0.2
PCV (×10-2 L/L)	33.8±1.24	35.69±0.49
TEC ((×1012/L)	6.39±0.27	6.88±0.18
TLC ((×109/L)	7.16±0.37	7.75±0.61
PLT ((×109/L)	134.17±9.63	136.75±5.63
Absolute Neutrophil count (×109/L)	61.17±3.3	54.17±2.34
Absolute Lymphocyte count (×109/L)	35±3.33[a]	44.5±2.15[b]
Absolute Eosinophil count (×109/L)	3.67±0.92[a]	1±0.52[b]

Values with different superscript lower case alphabets in same row differ significantly ($Pd \leq 0.05$)

The biochemical parameters before exercise and immediately after exercise are given in the table 3. Lactate was significantly ($p \leq 0.05$) increased after exercise. However chloride, sodium and potassium decreased non-significantly after exercise. Increase in the lactate concentration may be attributed to the fact that lactate is a product of carbohydrate metabolism in muscles. Blood lactate concentration is linearly correlated to lactate content of muscle tissue (Gauvreau *et al.*, 1996).

The cardiac biomarkers before exercise and immediately after exercise are given in table 4. NT Pro-

Table 3
Effect of exercise on biochemical parameters (Mean ± S.E.) in healthy Thoroughbred horses (n=20)

Biochemical Parameters	Before exercise	After exercise
AKP (U/L)	272.42±56.77	248.92±54.42
AST (U/L)	714±56.31	649.5±60.3
CK (U/L)	144.75±24.57	157.5±23.22
TP (g/dL)	7.7±0.77	7.32±0.56
Na (mEq/L)	161.33±10.03	155.25±8.83
Cl (mEq/L)	112.25±5.15	107.5±5.01
K (mEq/L)	5.46±0.59	4.2±0.4
LAC (mg/dl)*	1.69±0.11[a]	2.22±0.21[b]

Values with different superscript lower case alphabets in same row differ significantly ($Pd \leq 0.05$)

BNP was significantly ($p \leq 0.05$) increased after the exercise. Physical exercise can induce acute alterations in plasma NT-proBNP concentrations in healthy athletes. Similarly, Scharhag *et al.* (2005) also documented higher NT-proBNP concentrations after prolonged and strenuous endurance exercise. Divers *et al.* (2009) and Kraus *et al.* (2010) documented that cardiac troponin was increased with induced cardiac necrosis in horses, the increase appears to be positively associated with the degree of cardiac damage at necropsy. Contrary to this, Troponin I was not significantly affected in present study.

The ECG recordings before exercise, immediately after exercise and 20 min after exercise (recovery period) are given in table 5. P-wave morphology was significantly affected by exercise and there were bifid P-waves in all the animals (n=20) examined before exercise. After exercise, 90% (18) P-waves were bifid and 10% (2) were changed to single P-wave. In the recovery, 70% (14) horses had bifid P-waves and 30% (6) had single P wave. The results obtained in the present study showed that P wave morphology was influenced by alterations in heart rate as observed by Schade *et al.* (2014) in Crioulo horses at rest. Variations in morphology of the P wave in the ECG tracing has been attributed to the fact that with increase in HR, the P wave changed their bifid positive form to single positive form due to the fusion of the P1 and P2 components of P-wave. A similar finding was observed by Senta *et al.* (1970) in trotting equines during exercise. However, no significant effect of exercise on P-wave amplitude and duration was observed. In the present study, PR interval and QRS duration were also not significantly affected by exercise. Training does not seem to bring about appreciable variations in duration, morphology and voltage of the ECG waveforms (Nakamoto, 1969). Contrary to our findings, Piccione *et al.* (2003) found decrease in PR duration after the exercise. They also found decrease in the QRS complex duration after the warm up as compared to at rest.

T-wave morphology was significantly altered after exercise as well as in recovery period. Before exercise i.e. at rest, 60% (12) horses had negative T-wave, 20% (4) horses had biphasic and 20% (4) horses had positive T-wave. However, after exercise, 50% (10) horses had biphasic and 50% (10) horses had positive T waves and in the recovery 50% (10) biphasic, 20% (4) negative and 30% (6) positive T- waves were found. The T wave is the most variable parameter of electrocardiographic tracing in horses. The most common electrocardiographic abnormalities include inversion of polarity and increase in the T wave amplitude especially related to stress and to strenuous training (Piccione *et al.*, 2003). They further observed that under strenuous training, T-wave becomes wider and more pointed, even sometime sexceeding the QRS complex voltage. In present study, T-wave duration was reduced immediately after the exercise which was further increased in recovery period although non-significantly. Piccione *et al.* (2003) also reported that duration and amplitude of the T-waves were significantly increased in the recovery phase than immediately after the exercise.

Table 4
Effect of exercise on cardiac biomarkers (Mean ± S.E.) in healthy Thoroughbred horses (n=20)

Cardiac biomarkers	Before exercise	After exercise
NT-proBNP (ng/ml)	0.17±01[a]	0.23±0.01[b]
cTrI (ng/ml)	0.8±0.02	0.88±0.03
CKMB (U/L)	283.67±6.38	295.08±3.15
LDH (U/L)	584.08±48.56	605.83±48.32

Values with different superscript with lower case alphabets in same row differ significantly ($P d \leq 0.05$)

Table 5
Effect of exercise on electrocardiographic parameters (Mean ± S.E.) in healthy Thoroughbred horses (n=20)

ECG parameters	Before exercise	After exercise	Recovery
HR (bpm)	33.4±0.95[a]	53.55±1.77[b]	42.6±1.59[c]
P amplitude (mV)	0.37±0.02	0.35±0.03	0.34±0.02
P duration (sec)	0.13±0.01	0.13±0.01	0.13±0.01
PR interval (sec)	0.35±0.02	0.32±0.01	0.33±0.01
QRS duration (sec)	0.11±0.01	0.1±0	0.1±0
T amplitude (mV)	1.18±0.07[a]	0.89±0.07[b]	0.93±0.07[b]
QT interval (sec)	0.53±0.01[a]	0.47±0.01[b]	0.5±0.01[c]
T duration (sec)	0.13±0.01	0.12±0.01	0.13±0.01
ST segment (sec)	0.27±0.01[a]	0.23±0.01[b]	0.24±0.01[b]

Values with different superscript with lower case alphabets in same row differ significantly ($P d \leq 0.05$).

Significant ($p \leq 0.05$) decrease in the ST segment both after exercise and in recovery period might be because of the increase in heart rate after the exercise. Significant ($p \leq 0.05$) decrease in the QT interval was observed immediately after exercise. However, it was significantly ($pd \leq 0.05$) increased in the recovery period. Piccione *et al.* (2003) also observed significant decrease in QT interval in warm up and immediately after training however significant increase in the QT interval 30 min after the end of the training.

CONCLUSIONS

The study has proved that there is a significant effect

of exercise on electrocardiographic parameters and biochemical parameters like lactate and cardiac biomarkers like NT-proBNP. So the results of the present study can prove to have potential application in planning and monitoring of training program for athletic horses.

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