

Changes in Biochemical, Hematological and Cytokine in Endurance Horses with Metabolic Crises

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Abstract: Metabolic crises are the convoluted and injurious physiological changes observed in eliminated endurance horses during endurance races. Therefore, this study aims to evaluate the changes in biochemical hematological and cytokine in endurance horses with metabolic crises. The 32 endurance horses between the ages of 6-20 years and weighing between 350-450 kg were examined to be clinically healthy pre-ride. The 16 horses were eliminated post-race as a result of metabolic crises. Blood sample were collected at pre and post ride. The blood samples were analyzed for biochemical, hematological and cytokine. The parameters were assessed using one-way analysis of variance. There were significant increases in RBC, Hb and PCV ($p<0.0001$). There were significant increases in MCV ($p<0.0104$) and a significant decrease in MCHC ($p<0.0039$). There were significant increases in WBC segmented neutrophil and monocytes ($p<0.0001$). Band neutrophil ($p<0.0003$) and lymphocytes ($p<0.0043$). There was a significant increase in IL-6 ($p<0.0004$). Significant decreases were observed in sodium and potassium ($p<0.0001$) and chloride ($p<0.0004$) while significant increases were also assessed in total protein, urea, lactate and creatine kinase ($p<0.0001$) AST ($p<0.0049$) glucose ($p<0.0207$) plasma protein and uric acid ($p<0.0014$). In conclusion metabolic crises are the major causes of eliminations and poor performance in endurance horses. Thus, this study aims to evaluate the biochemical, hematological and cytokine as indicative of metabolic crises and poor performance in endurance horses.

Key words: Endurance horses, metabolic crises, biochemical, hematological, cytokine

INTRODUCTION

Under strenuous prolonged aerobic or anaerobic endurance events a larger proportion of horses subjected to endurance race developed metabolic problems with resultant damage to organs and tissues post race. Metabolic crises are the convoluted and injurious physiological changes occurring in horses either in excess or in short of the body system requirement during strenuous races leading to poor performance and subsequent eliminations from the races (Trigo *et al.*, 2010). Increased levels of RBC, Hb and PCV are due to splenic contraction releasing large number of erythrocytes into the circulation. This homeostatic adaptation induces an increase in the oxygen carrying capacity of blood to the ailing muscle tissues, increased fluid shift from the plasma, an elevated aerobic ability and a decrease in

lactate production (McKeever *et al.*, 1993; Munoz *et al.*, 1999; Kearns *et al.*, 2002; Kim *et al.*, 2005; Piccione *et al.*, 2010; Adamu *et al.*, 2010; Zobba *et al.*, 2011). Additionally, during strenuous maximal endurance races there were slight increases in MCV and decreases in MCHC (Smith *et al.*, 1989). During endurance races, leucocytosis is as a consequences of neutrophilia and lymphopenia due to redistribution of neutrophils from the peripheral pool into the blood circulation and by the splenic contraction this depends on the intensity and nature of the sporting event and associated stress factors (Snow *et al.*, 1983; Rose and Hodgson, 1994; Kastner *et al.*, 1999; Piccione *et al.*, 2010; Zobba *et al.*, 2011). Large volume of sweat loss and electrolytes during maximal strenuous endurance trials at elevated rates subsequently leads to metabolic alkalosis (Rose *et al.*, 1979; Poso-Reeta *et al.*, 2004; McKeever, 2004;

Piccione *et al.*, 2010) and causes fatigue and muscle weakness decreases the thirst response to dehydration and performance (Francesca *et al.*, 2007).

Many studies have indicated increased production of Interleukin-6 (IL-6) after a strenuous exercise in people and horses (Donovan *et al.*, 2007; Capomaccio *et al.*, 2011). Donovan *et al.* (2007) and Capomaccio *et al.* (2011) in their studies found that strenuous exercise induced a pro-inflammatory state in horses and man with an expression of mRNA for IL-6 and significantly increased circulating leucocytes. The increase in IL-6 is related to the intensity of exercise (Donovan *et al.*, 2007; Robson-Ansley *et al.*, 2009). Interleukin-6 is produced in larger amount compared to other cytokines it has surmountable biological activities and is largely produced by the contracting excitable muscle tissues (Pedersen, 2005; Robson-Ansley *et al.*, 2009).

An elevated level of plasma proteins and total proteins are indicative of dehydration status in endurance horses with metabolic crises (Castejon *et al.*, 2006). Creatine kinase is found in high concentration in working muscles in need of high energy (Rose and Hodgson, 1994) and is associated with muscular damage (Irving *et al.*, 1990). Increases in uric acid as a result of prolonged lopsided energy distribution could lead to metabolic crises during endurance events in horses (Castejon *et al.*, 2006). Horses with rhabdomyolysis revealed increased muscle enzymes, Creatine Kinase (CK), Aspartate aminotransferase (AST) and lactate (Hodgson *et al.*, 1994). In some studies, it was indicated that strenuous exercise elevates glucose transporters which mediate insulin-responsive utilization of glucose in the skeletal muscle tissues (Hirshman *et al.*, 1988; Prenen *et al.*, 2005). Insufficient levels of the glucose transporters in the skeletal muscle tissues causes poor performance of susceptible horses (Al-Qudah and Al-Majali, 2008).

Metabolic crises are the major cause of eliminations and poor performance in endurance horses. Thus, this study aims to evaluate the biochemical hematological and cytokine as indicative of metabolic crises and poor performance in endurance horses.

MATERIALS AND METHODS

Thirty two Arabian horses participated in an endurance race of 120 km out of this number 16 horses were eliminated from the race and 16 completed the race successfully and were used for this study. The horses were selected randomly in order to have an unbiased representation of the two groups. The age and body weight of the horses ranged between 6-20 years and

350-450 kg, respectively. Veterinary inspection was conducted after each loop of the races on all competing horses.

At the end of the endurance race the horses were categorized as successfully completed the race and eliminated from the race. The eliminated endurance horses are those with metabolic crises post-race while the successfully completed endurance horses were those that completed the race without any metabolic crises.

Therefore, the standard for assessing a horse as successfully completed the race rests on the horse's capability to maintain normal cardiac respiratory gastrointestinal or musculoskeletal activity and a heart rate of equal to or <64 beats/min and with good hydration status after 20-30 min of recovery period. Metabolic endurance horses are those that could not attend to the above mentioned criteria and were subsequently eliminated from the endurance race (Al-Qudah and Al-Majali, 2008).

The ambient temperature and humidity were recorded at an interval of 30 min from the beginning of the race to the finish. The mean temperature (°C) and humidity (%) were $29.06 \pm 1.1^{\circ}\text{C}$ and $71.73 \pm 4.05\%$, respectively during the period of the endurance race. The geographical terrain was good and conducive water points were also provided at specific places along the track which was generally flat and lack debris of stones. The ambient temperature and humidity were measured using portable thermohygrometer H1936440N Hanna instruments Romania.

Blood samples were obtained from all the horses via jugular veinipuncture into Ethyl Diaminotetra-Acetic Acid (EDTA) vacutainer tubes for hematological and into heparinized vacutainer tubes for biochemical analysis. The blood sample collection was performed immediately after 20-30 min of the recovery period and analyzed immediately in the laboratory which is located within the premises of the event.

The erythrocyte, leucocyte, thrombocyte, differential leucocyte count hemoglobin concentration Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were analyzed using Cell DYN 3700 Abbot®. While the Packed Cell Volume (PCV) was analyzed using Hettich-Hematocrit 210 and hawksley microhematocrit reader® and the plasma electrolyte and biochemical, sodium, potassium, chloride, calcium, urea, creatinine, bilirubin, Aspartate Transaminase (AST), Creatine Kinase (CK), glucose, lactate, total protein, albumin and globulin concentrations were determined with chemistry analyzer (Hitachi 920®) using standard diagnostic kits (Roche®). The pro-inflammatory cytokine Interleukin-6 (IL-6) was determined using high sensitivity and specificity (Cusabio®) horse

Interleukin-6(IL-6) ELISA kit. The data were analyzed using one way analysis of variance and pairwise correlations using the Statistical Software package JMP 9 (SAS). Analyses were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

All the 32 endurance horses completed the race. However, 16 had various metabolic disorders and were eliminated from the race and classified as metabolic endurance horses. Sixteen horses completed the race successfully and did not show any metabolic derangement and were classified as successfully completed the endurance race. The pre and post-race hematology, cytokine, plasma/serum electrolytes and biochemical parameters are presented in Table 1-4.

Table 1: Pre and post-endurance race erythrocytes parameters of horses

Parameters	Completed		Metabolic	
	Pre	Post	Pre	Post
Erythrocyte ($\times 10^{12} \text{ L}^{-1}$)	8.99 \pm 1.020 ^B	11.10 \pm 1.190 ^A	8.36 \pm 1.250 ^B	11.54 \pm 1.340 ^A
Hb (g L ⁻¹)	132.38 \pm 15.79 ^B	162.75 \pm 16.03 ^A	119.00 \pm 18.50 ^B	171.50 \pm 12.64 ^A
PCV (L L ⁻¹)	0.36 \pm 0.030 ^C	0.44 \pm 0.020 ^B	0.34 \pm 0.040 ^C	0.49 \pm 0.040 ^A
MCV (fL)	39.88 \pm 2.170 ^B	40.13 \pm 2.530 ^B	41.00 \pm 2.780 ^{AB}	43.25 \pm 2.310 ^A
MCHC (g L ⁻¹)	370.25 \pm 18.12 ^A	368.25 \pm 19.77 ^A	347.88 \pm 16.59 ^B	344.88 \pm 9.450 ^B

All values are expressed as mean \pm SD. ^{A-C}Within each row; means with different superscripts are significantly different at $p < 0.05$. Hb = Hemoglobin concentration; PCV = Packed Cell Volume; MCV = Mean Corpuscular Volume; MCHC = Mean Corpuscular Hemoglobin Concentration

Table 2: Pre and post-endurance race leukocyte counts and Interleukin-6 (IL-6) of horses

Parameters ($\times 10^9 \text{ L}^{-1}$)	Completed		Metabolic	
	Pre	Post	Pre	Post
Leucocytes	6.74 \pm 1.09 ^C	11.58 \pm 2.44 ^B	5.62 \pm 1.08 ^C	13.86 \pm 3.22 ^A
Band neutrophils	0.15 \pm 0.05 ^B	0.42 \pm 0.23 ^A	0.13 \pm 0.03 ^B	0.39 \pm 0.12 ^A
Segmented neutrophils	4.34 \pm 0.77 ^B	9.03 \pm 2.27 ^A	3.69 \pm 0.79 ^B	10.71 \pm 2.46 ^A
Lymphocytes	1.43 \pm 0.33 ^{AB}	1.25 \pm 0.23 ^B	1.24 \pm 0.27 ^B	1.76 \pm 0.47 ^A
Monocytes	0.34 \pm 0.09 ^C	0.52 \pm 0.15 ^B	0.27 \pm 0.06 ^C	0.71 \pm 0.16 ^A
IL-6 (ng mL ⁻¹)	0.35 \pm 0.27 ^B	2.73 \pm 2.52 ^A	0.12 \pm 0.11 ^B	3.63 \pm 2.37 ^A

All values are expressed as mean \pm SD. ^{A-C}Within each row; means with different superscripts are significantly different at $p < 0.05$. IL-6 = Interleukin-6 (Cytokine)

Table 4: Post-endurance race blood biochemical parameters of horses

Parameters	Completed		Metabolic	
	Pre	Post	Pre	Post
Urea (mmol L ⁻¹)	4.54 \pm 0.470 ^B	6.65 \pm 1.260 ^A	5.33 \pm 1.4200 ^B	7.22 \pm 0.77 ^A
Uric acid (μL^{-1})	9.83 \pm 3.940 ^B	30.60 \pm 11.98 ^A	5.63 \pm 3.4900 ^B	31.09 \pm 25.66 ^A
AST (U L ⁻¹)	293.18 \pm 8.690 ^B	447.58 \pm 80.86 ^{AB}	430.43 \pm 130.78 ^{AB}	705.63 \pm 512.66 ^A
CK (U L ⁻¹)	175.13 \pm 90.97 ^B	770.63 \pm 338.6 ^B	377.63 \pm 233.06 ^B	2487.00 \pm 1164.21 ^A
Glucose (mmol L ⁻¹)	5.58 \pm 0.610 ^C	8.70 \pm 2.370 ^A	6.06 \pm 0.2300 ^B	7.06 \pm 1.05 ^B
Lactate (mmol L ⁻¹)	1.71 \pm 0.210 ^C	2.98 \pm 0.920 ^B	1.15 \pm 0.1500 ^C	4.33 \pm 1.14 ^A
Total protein (g L ⁻¹)	67.86 \pm 3.410 ^B	80.75 \pm 8.660 ^A	66.39 \pm 6.1700 ^B	85.00 \pm 8.6 ^A
Plasma protein (g L ⁻¹)	64.63 \pm 3.740 ^B	81.00 \pm 6.760 ^A	68.00 \pm 4.4400 ^B	79.25 \pm 9.0 ^A

All values are expressed as mean \pm SD. ^{A-C}Within each row; means with different superscripts are significantly different at $p < 0.05$. AST = Aspartate Transaminase; CK = Creatine Kinase

There were significant differences between the completed and metabolic endurance horses in the pre and post-race RBC, PCV and Hb ($p < 0.0001$), MCV ($p < 0.0104$) and MCHC ($p < 0.0039$) (Table 1). Similarly, there were significant differences between the completed and metabolic endurance horses in the pre and post-race leukocyte, segmented neutrophils and monocytes, ($p < 0.0001$), band neutrophils ($p < 0.0003$), lymphocytes ($p < 0.0043$) and interleukin-6 ($p < 0.0004$) (Table 2).

Pre and post-race potassium and sodium concentration were significantly lower ($p < 0.0001$) and chloride ($p < 0.0004$) in the metabolic endurance horses (Table 3). There were significant differences between the successfully completed and metabolic endurance horses in the pre and post-race lactate, urea, total protein and CK ($p < 0.0001$), AST ($p < 0.0049$), glucose ($p < 0.0207$) plasma protein and uric acid ($p < 0.0014$) (Table 4). There were strong positive correlation between IL-6 and WBC ($r = 0.7049$; $p < 0.0001$), IL-6 and B and neutrophil ($r = 0.6983$; $p < 0.0001$), IL-6 and Segmented neutrophil ($r = 0.7220$; $p < 0.0001$), IL-6 and lactate ($r = 0.5018$; $p < 0.0034$) and a weak positive correlation is found between IL-6 and lymphocytes ($r = 0.3493$; $p < 0.05$) and IL-6 and RBC ($r = 0.3977$; $p < 0.0242$).

There were medium positive correlation between IL-6 and urea ($r = 0.4321$; $p < 0.0135$), IL-6 and total protein ($r = 0.4568$; $p < 0.0086$), IL-6 and Hb ($r = 0.4326$; $p < 0.0134$) and IL-6 and PCV ($r = 0.4890$; $p < 0.0045$). Furthermore, there was a strong positive correlation between total proteins and PCV ($r = 0.8307$; $p < 0.0001$) and between plasma proteins and PCV ($r = 0.6972$; $p < 0.0001$). There were also strong positive correlations among all the biochemical variables in the present study.

Table 3: Pre and post-endurance race plasma electrolyte concentrations of horses

Parameters (mmol L ⁻¹)	Completed		Metabolic	
	Pre	Post	Pre	Post
Sodium	140.06 \pm 1.29 ^B	143.29 \pm 2.77 ^A	142.44 \pm 3.36 ^{AB}	136.13 \pm 4.16 ^C
Potassium	3.96 \pm 0.96 ^B	3.19 \pm 0.29 ^C	5.53 \pm 0.72 ^A	3.04 \pm 0.87 ^C
Chloride	98.61 \pm 1.93 ^{AB}	95.11 \pm 2.83 ^{BC}	102.04 \pm 3.35 ^A	94.08 \pm 6.36 ^C
Calcium	3.37 \pm 0.09 ^A	3.19 \pm 0.12 ^A	3.21 \pm 0.13 ^A	3.18 \pm 0.52 ^A

All values are expressed as mean \pm SD. ^{A-C}Within each row; means with different superscripts are significantly different at $p < 0.05$

Metabolic crises are serious debilitating condition affecting health status and fitness level of endurance horses. In the present study, biochemical, hematological and cytokine perturbations were observed at large scale during the endurance race which subsequently resulted in poor performance and were probably induced by the strenuous ride. These and other similar findings were reported by numerous studies in endurance horses (Rose *et al.*, 1983; Foreman, 1998; Trigo *et al.*, 2010).

In the present study there were significant differences in the hematological parameters of the successfully completed and metabolic endurance horses. There were increases in RBC, PCV, Hb, MCV and a decrease in MCHC in the metabolic endurance horses compared to the successfully completed endurance horses these differences could be as a result of increased splenic contraction in response to catecholamines. This homeostatic adaptation induces an increase in the oxygen carrying capacity of blood to the excitable muscle tissues, increased fluid shift from the plasma and an elevated aerobic capability. Other possibilities are inclined towards the intensity of the race, type of sporting events, fitness and training levels and also the environmental condition during the event. These findings from the present study were similar to the findings of McKeever *et al.* (1993), Munoz *et al.* (1999), Funkquist *et al.* (2000), Kearns *et al.* (2002), Kim *et al.* (2005), Adamu *et al.* (2010) and Zobba *et al.* (2011).

Increases in PCV total protein, plasma protein and the strong positive correlations between these variables in the present study could be due to dehydration and stress levels observed in the metabolic endurance horses during the competition, these findings agree with the findings of Friend (2000), Munoz *et al.* (2010) and Trigo *et al.* (2010). Grosskopft and van Rensburg (1983) indicated PCV values $>55\%$, plasma protein $>99 \text{ g L}^{-1}$ and lactate $>3 \text{ mmol L}^{-1}$ as risk indicators of metabolic crises these findings were also similar to the findings of the present study. Trigo *et al.* (2010) also suggested in their study that higher concentrations of plasma proteins are predictors of metabolic changes in endurance horses during races, this finding also agrees with the result obtained from the present study which indicated high concentrations of plasma proteins in the metabolic endurance horses. Thus, in the present study there were increases in MCV and a decrease in MCHC in the metabolic endurance horses and this could be attributed to the alterations in the RBC which becomes more resistant to osmotic stress during endurance events (Smith *et al.*, 1989). Reduced RBC deformability was indicated by Geor *et al.* (1992).

In the present study there were significant increases in the values of leucocytes, band neutrophils, segmented

neutrophils, lymphocytes and monocytes in the metabolic endurance horses compared to the successfully completed endurance horses. These differences could be attributable to leucocytosis during endurance events as a result of neutrophilia and lymphopenia and could also be the result of the redistribution of neutrophils from the peripheral pool into the blood circulation from the splenic contraction this could also depend on the aerobic or anaerobic nature of the race and the associated stress factors as indicated by Snow *et al.* (1983), Rose and Hodgson (1994), Kastner *et al.* (1999), Piccione *et al.* (2010) and Zobba *et al.* (2011). These increases observed in the leukon of the metabolic endurance horses in the present study could be due to exhaustion in the metabolic endurance horses which had a left shift in the neutrophils and a significant lymphopenia as suggested by Trigo *et al.* (2010).

Furthermore, in the present study there was a significant increase in the value of Interleukin-6 (IL-6) in the metabolic endurance horses as compared to the endurance horses that successfully completed the race. There were also strong positive correlations between interleukin-6, WBC, band neutrophils, segmented neutrophils and lactate while weak positive correlations were found between interleukin-6 lymphocytes and RBC. Furthermore, medium positive correlations were found between interleukin-6, total protein, Hb and PCV. The findings in the present study could be associated with increased expression of IL-6 from leucocytes, band neutrophils, segmented neutrophils and lymphocytes during the race as a pro-inflammatory induced response which was similar to a comparative study conducted by Capomaccio *et al.* (2011). The association between IL-6 and lactate in this study could be as a result of exercise-related metabolic changes and their common origin of leakage from the contracting muscles tissues this finding was similar to the reports of Pedersen (2005), Donovan *et al.* (2007) and Robson-Ansley *et al.* (2009). Moreso, the association between IL-6 total protein, Hb and PCV could be as a result of dehydration and stress factors during strenuous endurance races. Therefore, IL-6 expression during endurance events could be a possible predictor of metabolic crises in endurance horses (Pedersen, 2005).

In the present study there were significant decreases in the concentrations of sodium, potassium and chloride while calcium concentration remains relatively unchanged. Large quantities of chloride loss through sweating could lead to reduced performances. Loss of these electrolytes leads to fatigue muscle weakness, decreased performance and also causes dehydration (Francesca *et al.*, 2007). It is a vital risk factor for metabolic disorders and the development of medical problems during and after

endurance races (Francesca *et al.*, 2007). In another study, it was found that an ambient temperature >20°C was accompanied by a decrease in chloride concentration in endurance horses (Rose, 1986).

Significant increases in plasma protein, glucose, lactate, creatine kinase, uric acid, urea, total protein and AST were assessed in the endurance horses with metabolic crises compared to the successfully completed the race in the present study. These alterations could be as a result of severe dehydration, exertional rhabdomyolysis, excessive release of damaging muscles enzymes, extended disproportionate energy levels and insufficient levels of glucose transporters in the skeletal muscle tissues of susceptible horses are other possibilities of poor performance and metabolic crises similar findings were reported by Irving *et al.* (1990), Tullson and Terjung (1991), Hodgson *et al.* (1994), Foreman (1998), Castejon *et al.* (2006), Al-Qudah and Al-Majali (2008). In the present study there were strong correlations between all the biochemical variables and these could be as a result of their common origin and similar effects on tissues and organs and similar findings were reported by Castejon *et al.* (2006) and Zobba *et al.* (2011).

CONCLUSION

The alterations in biochemical, hematological and interleukin-6 of endurance horses with metabolic crises could be used as indicators of metabolic crises and poor performance in endurance horses.

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