Full Length Article

Validity of an established metabolic disorder index as a predictor for metabolic eliminations in endurance horses

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ABSTRACT

Endurance horses are usually eliminated from the race due to lameness, metabolic ailments or technical reasons. The purpose of the study was to determine the validity and reliability of the metabolic disorder index (MDI) in predicting metabolic eliminations in endurance horses during an endurance race. Fifty-four endurance horses competing on two local endurance tracks were involved in the study. Blood samples were collected a day prior to the event to determine packed cell volume (PCV), chloride (Cl^-), interleukin-6 (IL-6), creatine kinase (CK) and glutathione reductase (GR) concentrations from all participating horses. These parameters were used for the determination of metabolic disorder index in endurance horses at rest (one day before the competition). All data were statistically analysed. In 40, 80 and 120 km race distances, the successfully completed horses had a significant lower serum concentration of CK and a significant higher serum concentration of Cl^- than the eliminated horses ($P < .05$). There were no significant differences in PCV, serum concentrations of IL-6 and GR between the successfully completed and eliminated horses in the 40, 80 and 120 km race distances. The MDI at the rest one day before the race could predict potential of metabolic eliminations in endurance horses with at least 78.26%, 80% and 83.33% accuracies in 40, 80 and 120 km race distances. The sensitivity of the MDI was 81.82%, 80% and 100% in the 40, 80 and 100 km race distances. The specificity of the MDI was 80% for the 80 km race distance and 75% for the 40 and 120 km race distances. In conclusion, the metabolic disorder index is a reliable method for the prediction of metabolic eliminations in endurance horses participating in endurance races.

1. Introduction

Endurance horses are usually eliminated from the race due to lameness, metabolic ailments or technical reasons, including failure to reach veterinary gate within the stipulated period, optional withdrawal, and concern over the welfare of the horse. The Féderation Équestre Internationale (FEI) defines endurance race as a long-distance race that tests the speed and endurance of horse across all kinds of terrain and the skill of riders in controlling pace of the horse using their knowledge to manage the capabilities of their horses [1,2]. In endurance rides, each rider must safely manage the race with total consideration for the fitness and stamina of their horse [1]. Organization of endurance races puts high emphasis on the welfare of the horses [1,3,4]. To ensure the horse competes safely, the competitions are punctuated with compulsory halts for a veterinary inspection or 'vet gate' at the end of each

predetermine phase of the race [3]. This exercise is to ensure that only fit horse will be allowed to continue with the race, while unfit horses are eliminated and subjected to treatment for their ailments [1].

Metabolic disorder is defined as a complex of abnormal physical changes that occur in horses either in excess or short of body system requirements during the strenuous long-distance races [3,5]. Metabolic disorder may lead to organ and tissue damages resulting in metabolic eliminations of endurances horses from the races [6]. In endurance race, the metabolic status of the horses is accessed by examination of mucous membrane and determination of capillary refill time, hydration status, intestinal activity, cardiac recovery index, and demeanor [3,7].

The horse is one of the strong animal among mammals with an inherent characteristics for endurance, the horse had a large splenic blood reserve of as much as 50% of the total red blood cells (RBC) number [8]. Thus, horses can release 4 to 12 L of splenic blood into

circulation in response to excitement (handling, venipuncture, pain) and intense exercise, although the number of RBC is higher in splenic than in circulating blood [9], the packed cell volume (PCV) increases to as much as 0.02 to 0.13 L/L [5,10].

In normal situation, exercise under the influence of catecholamine can result in mobilization of splenic blood [8]. Exercise-induced fluid shift also plays a role in the increase of hematocrit value [8]. Horse performing moderate short-term incremental exercises may experience 5 to 10% decrease in plasma volume because loss of fluid will lead to a subsequent increase in PCV, erythrocyte count, and hemoglobin concentration [11]. The increase of these blood parameters will increase the oxygen-carrying capacity that is beneficial in the support of high aerobic capacity of these horses [11]. On the other hand, excessive increase in PCV leads to high blood viscosity [11] that will impair transportation of the blood to the organs, thus, adversely affecting horse performance [12].

Creatine kinase (CK) is a useful indicator in the assessment of training progress [13]. Janicki et al. [14] reported a higher serum CK activity in untrained than trained horses. Serum CK activity is useful to assure that the training intensity is not producing muscle damage/significant changes in muscle membrane permeability [15]. Exercise, depending on type, intensity, and duration, can cause increases in serum CK activity in horses [14]. It is believed that the increase in serum CK is due to increase in muscle cell membrane permeability because of tissue hypoxia during exercise rather than tissue damage [16].

The blood sodium, calcium, chloride (Cl^-) and potassium are low in disqualified endurance horses in some cases [14]. The decrease in the blood electrolytes is due to losses of fluid through sweat [14]. The low blood potassium in these horses is also as a result of loss through urine [14–18]. Even though another study has described an increase in sodium [10] and Chloride [17]. Electrolyte loss during an endurance race has a proclivity towards type of terrain, the length of a ride, humidity, and temperature [10].

The increased in physical activity leads to high oxygen consumption and thus increasing the production of "reactive oxygen species" (ROS) [14]. The increased production of ROS causes the peroxidation of cell membrane lipids and damage to the nucleic acids, proteins, and carbohydrates [19]. The ROS activity is reflected by the total antioxidant status (TAS) that can be measured as an assessment of protective capacity towards damage caused by the oxidative radicals [14]. There are small increases in blood TAS with exercise load due to the redistribution of antioxidant compounds from the tissue [14] or as the result of hemoconcentration [20]. However, a moderate training load causes a non significantly increase the oxygen radical absorbance capacity (ORAC), another measure of oxidative stress in horses even after long-term exercise [21]. In fact, regular exercise can stimulate the antioxidant mechanism in the body to prevent stimulation of tissue peroxidation [21,22]. The lower disseminating quantity of glutathione reductase (GR) in endurance horses is suggestive of lower ability to overcome oxidative stress. In extreme physical task, there is an increase in the cellular response of inflammatory moderators, mainly IL-6 generated by monocytes [23]. Lower quantities of GR signify risk and influencing factors for contagions in equine, the commencement of long-lasting infections, oxidative stress, and the onset of metabolic disorders [23]. Oxidative stress and abnormalities in antioxidant prominence have been specified during strenuous endurance isometrics [23].

In horses that develop metabolic disorders during an endurance race, the PCV, CK, and IL-6 tend to increase and GR and Cl^- tend to decrease [23]. Thus, using these parameters and evidences collected from eliminated and completed horses during endurance races, the metabolic disorder index (MDI) was developed. The MDI is used as a predictor for metabolic elimination of horses during the races [23].

The study suggested that several markers for metabolic disorder, PCV, CK, Interleukin-6 (IL-6), Cl^- and glutathione reductase (GR) change from resting value to those during races, according to the fitness of the horse [23]. This formula was based on products of PCV, CK, and

IL-6 divided by the products of chloride and GR [23]. The formula suggested that a resting MDI value less than 5.5 is suggestive of a horse be likely free from metabolic disorder and between 5.5 and 14.4 is on the borderline for the horse to develop metabolic disorder. Horses with MDI values between 14.5 and 17.5 are prone to develop metabolic disorder and those with MDI ≥ 17.5 have higher probability of developing metabolic disorders [23] and subsequently eliminated from the races. Therefore, the main purpose of the study was to determine the reliability of MDI in the prediction of metabolic eliminations during endurance races.

2. Materials and methods

2.1. Ethical approval

The study was performed according to the guidelines of the care and use of animals provided by Institutional Animal Care and Use Committee at Universiti Putra, Malaysia. This work was supported by Universiti Putra, Malaysia [grant number GPI-9440000].

2.2. Study design

Fifty-four endurance horses were recruited in this study. Horse selection did not take into consideration breed, age, sex, weight, or height. The horses were those competing in 40, 80 or 120 km race distance held in Malaysia. The races were held on two tracks used by the Terengganu International Endurance Park (TIEP), at Lembah Bidong, Terengganu and the Zahra Arabian Park at Tok Bali, Kelantan. Both of the tracks were under rubber plantation and near beaches. Water points were also provided at strategic positions along the track at an interval of 5-km distance. All horses were ridden by experienced riders, registered under *Fédération Équestre Internationale* (FEI) with the body weight ranging between 52 and 70 kg.

The status of the horses were determined according to the post-ride physical examination and results of the race; rank, average speed, total recovery period, and total riding time as recorded in the physical examination form. The rank of the horse was established by taking account of all the results, including speed, recovery time, riding time, and ability of the horse and rider to finish the race successfully. Horses that finished the race in fastest and splendid condition were given the first rank, hence considered as successfully completed the race. Thus, a horse that could not attain to this level and developed metabolic problems and eliminated from the race was considered as eliminated for metabolic reasons. Average speed was one of the indicators to determine horse status in the current study. Horses that were able to complete the race with the fastest speed while maintaining good health were also considered as successfully completed. The total recovery period was the time taken from the time of arrival of the horse at the vet gate to the time it was presented for examination at the vet check. In endurance race, each horse was given 20 min from arrival time to attain heart rate less than 60 bpm before it was presented to the vet check. Longer time taken indicated that the horse may have a health problem that could compromise their continued participation in the competition. Horses recovered well were considered as successfully completed. The total ride time was the time taken by a horse to complete the race, according to the distance of the race. Thus, the data were collated according to the distance of the race 40, 80 or 120 km for comparison.

The endurance events of 40, 80, and 120-km races were conducted under tropical climate (the average ambient temperature and environmental humidity for the various races was 29.06 $\pm$ 1.1 °C and 71.73 $\pm$ 4.05% respectively). Under this condition, the rate of elimination was anticipated to be high. The ambient temperature and humidity were measured using portable thermohygrometer H1936440N, Hanna instruments Romania, and were recorded at an interval of 30 min throughout the race in the premises of the event.

The horses performed journeys of different distances to the premises

of the events, and the horses were rested sufficiently for at least 3 to 4 days before the event. The initial (pre-race) blood samples were collected at 8:30 AM from the participating horses a day prior to the event. The environmental conditions of the competitions, places were not adverse at the time of sampling and when the race commenced which was usually at 5.30 PM in the evenings. All the three categories of endurance (40, 80 and 120 km) were in their stables at the time of sample collection. The blood was collected from apparently healthy horses at rest, and a comprehensive clinical investigation was carried out to establish their health status immediately after the blood collection on the same day. The samplings were conducted prudently to certify that the horses were not enflamed by the procedure which could conceivably upset the hematological and biochemical parameters. The pre-race blood sample was analyzed instantaneously in the laboratory located within the premises of the event before the race. The selected parameters for MDI were computed for the pre-race blood samples and those horses that showed values greater than 17.5 were recorded. The horses were kept at rest until 5.30 PM the next day when the endurance race started, and during the endurance race of the (40, 80 and 120 km) and on each loop of the various races and after a 20-minute recovery period, the carefully chosen parameters used for the determination of metabolic disorder index (MDI) for each horse were computed, and those horses with MDI values greater than 17.5 before the race were mostly eliminated during the procession of the event.

Blood samples were obtained through the jugular vein by using 21G venipuncture needles in 5 mL ethylene diamine tetraacetic acid (EDTA) vacutainer tubes to obtain plasma for determination of hematological parameters and in heparin vacutainer tubes for biochemical parameters. Blood samples were taken one day prior to the race at 8.30 AM and processed immediately in the laboratory located within the premise of the competition. Blood samples in EDTA tube were analyzed for PCV only by centrifuging in the hematocrit centrifuged (Hettich-Hematocrit 210) for 5 min and measuring using Hawksley microhematocrit reader®. Blood in a heparin tube was centrifuged for 10 min at $300 \times g$ to obtain plasma. The plasmas were analyzed instantaneously in the laboratory after the collection. The plasma biochemical parameters were determined with a chemistry analyzer (Hitachi 920®) using a standard diagnostic kit (Roche®). The parameters analyzed were interleukin–6 (IL-6) and glutathione reductase (GR), and were determined using (Cusabio ®) horse IL-6 and GR ELISA kit according to the manufacturer's instruction.

MDI were determined using; PCV, Cl⁻, CK, IL-6 and GR by adopting the method of Adamu et al. [23]

$$\text{Metabolic Disorder Index (MDI)}$$
$$= \frac{\text{PCV}\,(\text{L}\,\text{L}^{-1}) \times \text{CK}\,(\text{U}\,\text{L}^{-1}) \times \text{IL-6}\,(\text{ng}\,\text{mL}^{-1})}{\text{GR}\,(\text{ng}\,\text{mL}^{-1}) \times \text{Cl}^{-},\,(\text{mmol}\,\text{L}^{-1})} \times 100$$

where PCV is the packed cell volume (hematocrit), CK, IL-6, GR, Cl⁻, are the plasma creatine kinase, interleukin-6, glutathione reductase and chloride. Prediction of the horse as a result of metabolic elimination was based on Table 1.

2.3. Statistical analysis

The sensitivity of the MDI and its positive and negative predictive

Table 1
Prediction of metabolic elimination based on MDI values in endurance horses.

MDI values	Prediction for Metabolic Disorder
5.5–14.5	Borderline
14.5–17.5	Prone
17.5 and above	High probability

Adamu et al. [23].

Table 2
Average speed of horses competing in endurance races.

Parameter	Average Speed (km h⁻¹)
Sucessfully completed (n = 33)	15.72ᵃ ± 2.9
Eliminated due to metabolic disorder (n = 21)	13.91ᵇ ± 2.6
Race distance (km)	
40 (n = 23)	12.65ᵃ ± 1.8
80 (n = 25)	15.99ᵇ ± 3.5
120 (n = 6)	12.86ᵃ ± 0.2

All values are expressed as mean ± SE. [a, b] means with different superscripts within column are significantly different at $P < .05$ for the parameters,

values were determined using MedCalc Statistical Software version 18.5 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) as described by Michael [24]. The data were also analyzed using Student's *t*-test for horse status (successfully completed and eliminated horses) and ANOVA was used for the three different race distances using Tukey Kramer (HSD) for comparison as post hoc test with JMP version 11 software (SAS Institute Inc, Cary, NC). Analyses were considered significant at a P value of $< .05$.

3. Results

There was significant ($P < .05$) differences between successfully completed and eliminated speeds of horses (Table 2). The average speed for successfully completed and eliminated horses was 15.72 ± 2.9 and 13.91 ± 2.6 km h⁻¹, respectively. The eliminated horses due to metabolic disorder had a lower average speed which was 13.91 ± 2.6 km h⁻¹. In the 80 km race distance, the average speed was significantly ($P < .05$) higher compared to that in the 40 and 120 km races at 12.65 ± 1.8, 15.99 ± 3.5 and 12.86 ± 0.2 km h⁻¹, respectively.

The blood parameters of both successful and eliminated horses in endurance races for the 40 km race distance are presented in Table 3. Generally, all the blood parameters were within the normal ranges for both groups of horses. However, there were significant differences in blood CK and Cl⁻, concentrations between successfully completed and eliminated endurance horses. In the successfully completed horses, the CK concentration (147.5 ± 2.6 U L⁻¹) was significantly ($P < .05$) lower compared to that in eliminated horses (219.0 ± 5.3 U L⁻¹), while the Cl⁻, concentration was significantly ($P < .05$) higher (105.4 ± 3.2 mmol L⁻¹) in the successfully completed horses than the eliminated horses (97.6 ± 4.3 mmol L⁻¹).

The blood parameters of both successful and eliminated horses in endurance races for the 80 km race distance are presented in Table 4. Largely, all the blood parameters were within the normal ranges for both groups of horses. However, there were significant differences in blood CK and Cl⁻ concentrations between successfully completed and

Table 3
Blood parameters of successfully completed and eliminated endurance horses in 40 km race distance.

Blood parameters	Horse status	
	Successfully completed (n = 14)	Eliminated (n = 9)
PCV (L L⁻¹)	0.34ᵃ ± 0.05	0.32ᵃ ± 0.04
CK (U L⁻¹)	147.5ᵃ ± 2.6	219.0ᵇ ± 5.3
Cl⁻ (mmol L⁻¹)	105.4ᵇ ± 3.2	97.6ᵃ ± 4.3
IL-6 (ng mL⁻¹)	4.3ᵃ ± 2.3	4.0ᵃ ± 2.5
GR (ng mL⁻¹)	29.1ᵃ ± 6.5	21.2ᵃ ± 3.1

All values are expressed as mean ± SE. [a, b, c] Means with different superscripts within rows are significantly different at $P < .05$. PCV = packed cell volume; CK = Creatine kinase; Cl⁻ = Chloride; IL-6 = Interleukin-6; GR = Glutathione reductase.

Table 4
Blood parameters of successfully completed and eliminated endurance horses in 80 km race distance.

Blood parameters	Horse Status	
	Successfully completed (n = 15)	Eliminated (n = 10)
PCV (L L^{-1})	0.37^a ± 0.06	0.35^a ± 0.07
CK (U L^{-1}	150.7^a ± 2.9	216.0^b ± 6.5
Cl$^-$ (mmol L^{-1})	103.7^b ± 3.3	99.8^a ± 5.6
IL-6 (ng mL^{-1})	5.3^a ± 4.6	5.0^a ± 2.8
GR (ng mL^{-1})	32.1^a ± 7.8	23.4^a ± 3.3

All values are expressed as mean ± SE. [a, b, c] Means with different superscripts within rows are significantly different at $P < .05$. PCV = packed cell volume; CK = Creatine kinase; Cl$^-$ = Chloride; IL-6 = Interleukin-6; GR = Glutathione reductase.

eliminated endurance horses. In the successfully completed horses, the CK concentration (150.7 ± 2.9 U L^{-1}) was significantly ($P < .05$) lower than that in the eliminated horses (216.0 ± 6.5 U L^{-1}), while the Cl$^-$, concentration was significantly ($P < .05$) higher (103.7 ± 3.3 mmol L^{-1}) in the successfully completed horses than that in the eliminated horses (99.8 ± 5.6 mmol L^{-1}).

The blood parameters of both successful and eliminated horses in endurance races for the 120 km race distance are presented in Table 5. Mainly, all the blood parameters were within the normal ranges for both groups of horses. However, there were significant differences in blood CK and Cl$^-$ concentrations between successfully completed and eliminated endurance horses. In the successfully completed horses, the CK concentration (152.2 ± 2.4 U L^{-1}) was significantly ($P < .05$) lower compared to that in the eliminated horses (224.0 ± 5.5 U L^{-1}), while the Cl$^-$ concentration was significantly ($P < .05$) higher (101.4 ± 2.9 mmol L^{-1}) in the successfully completed horses than that in the eliminated horses (92.7 ± 4.7 mmol L^{-1}).

At the end of the endurance race, we gathered comprehensive results of 54 horses that participated in the endurance race (Table 6). Twenty three horses participated in the 40 km race, 14 horses were completed the race successfully while 9 horses were eliminated from the race. Twenty-five horses were involved in the 80 km endurance race, 15 horses completed the endurance race successfully whereas 10 horses were eliminated from the race. Furthermore, 6 horses participated in the 120 km race distance, 4 horses completed the race successfully and 2 horses were eliminated from the endurance race.

Out of 54 horses (Table 7), 23 horses participated in the 40 km race distance, 12 horses of them had MDI values greater than 5.5 prior to the race. Among the 12 horses, 9 horses were eliminated from the race while 3 finished the race successfully. Eleven horses had MDI value lower than 5.5 prior to the race and 2 horses of them were eliminated while 9 horses finished the race successfully.

Out of 54 horses (Table 8), 25 horses participated in the 80 km race

Table 5
Blood parameters of successfully completed and eliminated endurance horses in 120 km race distance.

Blood parameters	Horse Status	
	Successfully completed (n = 4)	Eliminated (n = 2)
PCV (L L^{-1})	0.36^a ± 0.04	0.30^a ± 0.05
CK (U L^{-1}	152.2^a ± 2.4	224.0^b ± 5.5
Cl$^-$ (mmol L^{-1})	101.4^b ± 2.9	92.7^a ± 4.7
IL-6 (ng mL^{-1})	5.1^a ± 3.8	4.9^a ± 2.6
GR (ng mL^{-1})	30.9^a ± 5.7	24.5^a ± 2.5

All values are expressed as mean ± SE. [a, b, c] Means with different superscripts within rows are significantly different at $P < .05$. PCV = packed cell volume; CK = Creatine kinase; Cl$^-$ = Chloride; IL-6 = Interleukin-6; GR = Glutathione reductase.

Table 6
Intact results of 54 horses that participated in the endurance race of 40, 80 and 120 km race distances.

Status of horses	40 km	80 km	120 km	Total
Successfully completed	14	15	4	33
Eliminated from race	9	10	2	21
Total	23	25	6	54

Table 7
Sensitivity, Specificity, Positive and Negative predictive values of MDI in 40 km endurance horses.

Parameter	MDI		
	Eliminated	Successfully completed	Total
> 5.5 (Positive)	9 True Positive	3 False Positive	12
< 5.5 (Negative)	2 False Negative	9 True Negative	11
Total number of horses	9	14	23
Statistics	**Values**	**Lower and Upper 95% confidence interval**	
Sensitivity	81.82%	48.22% to 97.72%	
Specificity	75.00%	42.81% to 94.51%	
Positive predictive value	75.00%	51.99% to 89.26%	
Negative predictive value	81.82%	55.20% to 94.27%	
Accuracy	78.26%	56.30% to 92.54%	

Table 8
Sensitivity, Specificity, Positive and Negative predictive values of MDI in 80 km endurance horses.

Parameter	MDI		
	Eliminated	Successfully completed	Total
> 5.5 (Positive)	8 True Positive	3 False Positive	11
< 5.5 (Negative)	2 False Negative	12 True Negative	14
Total number of horses	10	15	25
Statistics	**Values**	**Lower and Upper 95% confidence interval**	
Sensitivity	80.00%	44.39% to 97.48%	
Specificity	80.00%	51.91% to 95.67%	
Positive predictive value	72.73%	48.06% to 88.49%	
Negative predictive value	85.71%	62.87% to 95.51%	
Accuracy	80.00%	59.30% to 93.17%	

distance, 11 horses of them had MDI values greater than 5.5 prior to the race. Among the 11 horses, 8 horses were eliminated from the race while 3 horses finished the race successfully. Fourteen horses had MDI value lower than 5.5 prior to the race and 2 horses of them were eliminated while 12 horses finished the race successfully.

Out of 54 horses (Table 9), 6 horses participated in the 120 km race distance, 3 horses had MDI values greater than 5.5 prior to the race. Among the 3 horses, 2 horses of them were eliminated from the race while 1 horse finished the race successfully. Three horses had MDI value lower than 5.5 prior to the race and none of them was eliminated while 3 horses finished the race successfully.

Based on the data of Table 7, the sensitivity of the test was 81.82%, the specificity of the test was 75% and the accuracy of the test was

Table 9
Sensitivity, Specificity, Positive and Negative predictive values of MDI in 120 km endurance horses.

Parameter	MDI		
	Eliminated	Successfully completed	Total
> 5.5 (Positive)	2 True Positive	1 False Positive	3
< 5.5 (Negative)	0 False Negative	3 True Negative	3
Total number of horses	2	4	6
Statistics	**Values**	**Lower and Upper 95% confidence interval**	
Sensitivity	100.00%	15.81% to 100.00%	
Specificity	75.00%	19.41% to 99.37%	
Positive predictive value	66.67%	26.81% to 91.61%	
Negative predictive value	100.00%	18.71% to 100.00%	
Accuracy	83.33%	35.88% to 99.58%	

78.26% for the 40 km race distance. Furthermore, the data of Table 8, showed the sensitivity and specificity test of 80% respectively and the accuracy of the test was 80% for the 80 km race distance whereas, the data of Table 9, showed the sensitivity test of 100%, the specificity test of 75% and the accuracy of the test to be 83.33% for the 120 km race distance.

Sensitivity was the ability of the test to correctly identify the horses with metabolic disorder (true positive rate), whereas specificity was the ability of the test to correctly identify the horses without the metabolic disorder (true negative rate). The positive predictive value for the 40 km race distance was 70%, while the negative predictive value was 84.62%. Moreover, the positive predictive value for the 80 km race distance was 72.73%, while the negative predictive value was 85.71%, the positive predictive value for the 120 km race distance was 66.67%, whereas the negative predictive value was 100%. Positive predictive value was the probability that horses identified as metabolic disordered horses before the races with a positive screening test were truly eliminated from the race due to metabolic disorder during the race. Negative predictive value was the probability that horses identified as successfully completing before the race with a negative screening test truly completed the race successfully without developing metabolic disorder during the race.

4. Discussion

In the present study, we only compared five blood parameters of successfully completed and eliminated horses because these parameters are essential for calculation of the MDI. The main purpose of the study was to determine the reliability of MDI in the prediction of metabolic eliminations during endurance races. The MDI developed by Adamu et al. [23] used PCV, CK, Cl^-, IL-6, and GR as components of the formula. The present study showed that the pre-ride values of these parameters in both successfully completed and the eliminated horses due to metabolic reasons were within the normal range. This was expected, because all horses registered to compete in the races were apparently healthy and fit. Furthermore, blood sampling was done on rested horses in their stables. After the race, these horses were grouped into successfully completed and eliminated horses due to metabolic reasons', according to the criteria described earlier. Except for CK that showed an increase and Cl^-, that decreased, others parameters did not vary significantly between these groups of horses.

It is believed that the higher plasma CK concentration in the eliminated horses due to metabolic reasons might be due to poor training that could have caused dehydration, exertional

rhabdomyolysis, and excessive damage. The successfully completed horses were well-adapted and well-trained, thus, did not experience as much muscle wear and tear as the inadequately trained eliminated horses. This supports an earlier observation that showed the plasma CK activity in untrained is higher than in trained horses [14].

Creatine kinase was found to be high in concentration in working muscles [16]. The enzyme facilitates phosphorylation to meet the energy demand of the working muscles [16]. This serum enzyme is also one of the most useful parameters for the assessment of training progress in endurance horses. This enzyme increases in concentration during isometrics [13]. In a study conducted in 100-day training of stallions, it was found out that the CK activity gradually decrease with training because of the adaptation of the muscles to increase exercise load [14,15].

In the current study, the serum Cl^- concentration in the eliminated horses was significantly lower than the successfully completed horses. This finding agrees with that of other studies [25]. During endurance races, blood chloride is lost in large quantities through sweat. Prolonged sweating during endurance races will lead to electrolyte depletion, particularly chloride and sodium [26], causing muscle cramp, weakness, acid-base imbalance and eventually affecting the horse performance [27]. Loss of chloride and sodium also affect the muscle contractibility, a predisposing factor to the development of metabolic problems and colic-causing reduced gastrointestinal motility in endurance horses [26]. Horse with hypochloremia will also eventually develop metabolic alkalosis, if they persist in the heavy workload endurance races [28,29]. In Malaysia, the warm and humid environment, further increases sweating and cause greater chloride loss in the endurance horses than in the cool temperate climate [4]. Thus, horses in endurance races in Malaysia have a greater proclivity to develop metabolic disorder. In the current study, it was found that the speed decreases significantly in horses that developed metabolic disorder and eliminated from the race compared to those eliminated due to lameness and for technical reasons. This finding concurred with the study conducted by Marlin and Williams [2].

In a previous study, Adamu et al. [23], tested the validity of using the MDI to predict metabolic elimination in endurance horses on two occasions. The first occasion involved 20 endurance horses, was retrospective conducted on horses competing at Padang Besar, Perlis, Malaysia in 2013. The study showed that MDI showed that 95% of horses predicted to develop metabolic disorder did develop the disorder. The test was able to correctly predict horses that will be eliminated and complete races. On the second occasion, on 12 horses, 6 horses had MDI values of greater than 14 were eliminated from the race whereas those who had MDI values less than 5.5 completed the race successfully. However, the sampled number of both surveys was small to probably conclude on the practicality of MDI.

In the current study, 54 endurance horses were used, the study was a prospective study, better designed, and conducted with proper and controlled samples a day before the race. The horses were well-rested under controlled conditions. The study showed that horses at rest with MDI less than 5.5 performed well and completed their races. Horse with MDI greater than 5.5 were subsequently eliminated from the races. The present study showed that accuracy of the MDI for the 40, 80 and 120 km were 78.26%, 80% and 83.33% respectively in the prediction of metabolic eliminations in endurance races.

With the development of MDI as a predictor of metabolic elimination in endurance horses, the frequency of horses with the potential for development of metabolic disorders could be reduced. This is also a means of forefending horses from participating in life-threatening heavy-load work. The main benefit of using MDI is in the protection of horses through their withdrawal from events that may cause life-threatening outcomes.

5. Conclusions

The present study used the MDI to determine the potential of horses to be eliminated due to metabolic disorder in endurance races. The MDI at rest during the pre-ride period could predict potential of metabolic eliminations in endurance horses with at least 78.26%, 80% and 83.33% accuracies in the 40, 80 and 120 km race distances. The sensitivity of the MDI was 81.82%, 80% and 100% in the 40, 80, and 120 km race distances. The specificity of the MDI was 80% for the 80 km race distance and 75% for the 40 and 120 km race distances, respectively. Thus, the MDI is reliable for the prediction of metabolic elimination in endurance horses.

Acknowledgments

Competing interests

The authors declared no conflict of interest.

References

[1] Fédération Equestre Internationale. Rules for endurance events. 9th ed. Effective 1 January 2017, Updates effective 1 January 2017. http://www.fei.org/.

[2] Marlin D, Williams J. Equine endurance race pacing strategy differs between finishers and non-finishers in 120 km single-day races. Comp Exerc Physiol 2018:1–8. https://doi.org/10.3920/CEP170027.

[3] Nagy A, Dyson SJ, Murray JK. A veterinary review of endurance riding as an international competitive sport. Vet J 2012;194:288–93.

[4] Adamu L, Noraniza MA, Rasedee A, Bashir A. Effect of age and performance on physical, hematological, and biochemical parameters in endurance horses. J Equine Vet Sci 2013;33:415–20.

[5] Adamu L, Noraniza MA, Rasedee A, Bashir A. Alterations in biochemical, hematological and physical parameters of endurance horses with metabolic crises. J Anim Vet Adv 2012;11:4108–14.

[6] Trigo P, Castejon F, Riber C, Muñoz A. Use of biochemical parameters to predict metabolic elimination in endurance rides. Equine Vet J 2010;42:142–6.

[7] Adamu L, Noraniza MA, Rasedee A, Bashir A. Physical parameters and risk factors associated with the elimination of Arabian and crossed Arabian endurance horses during a 120-km endurance race. J Equine Vet Sci 2014;34:494–9.

[8] McGowan C. Clinical pathology in the racing horse: the role of clinical pathology in assessing fitness and performance in the racehorse. Vet Clin North Am Equine Pract 2008;24:405–21.

[9] Persson SGB, Iréne Ö. Racing performance in red blood cell hypervolemic Standardbred trotters. Equine Vet J 1999;31:617–20.

[10] Larsson J, Pilborg PH, Johansen M, Christophersen MTA, Holte L, Roepstorff L, et al. Parameters of endurance horses pre-compared to post-race, correlated with performance: a two race study from Scandinavia. ISRN Vet Sci 2013:1–2. https://doi.org/10.1155/2013/684353. 684353.

[11] Satué K, Hernández A, Muñoz A. Physiological factors in the interpretation of equine hematological profile. In Hematology-science and practice. InTech 2012:574–96. https://doi.org/10.5772/38961.

[12] Funkquist P, Nyman G, Persson SGB. Haemodynamic response to exercise in Standardbred trotters with red cell hypervolaemia. Equine Vet J 2000;32:426–31.

[13] Szarska E. Wykorzystanie badan diagnostycznych krwi do oceny stanu zdrowia i zaawansowania treningowego koni wyczynowych [The use of blood diagnostic test to assess the state of health and progress of training performance of racing horses]. Ed. SGGW, Warszawa 2002;250:1–4.

[14] Janicki B, Kochowicz A, Buzała M, Krumrych W. Variability of selected biochemical parameters in young stallions during the 100-day performance test. J Equine Vet Sci 2013;33:1136–41.

[15] Cywińska A, Szarska E, Górecka R, Witkowski L, Hecold M, Bereznowski A, et al. Acute phase protein concentrations after limited distance and long distance endurance rides in horses. Res Vet Sci 2012;93:1402–6.

[16] Buzala M, Krumrych W, Janicki B. Usefulness of creatine kinase activity determination for assessing the effects of physical effort in horses. Pak Vet J 2015;35:267–73.

[17] Adamu L, Noraniza MA, Rasedee A, Bashir A. Responses of serum biochemical parameters, electrolytes and heart rate in an 80 km endurance race. J Vet Adv 2014;4:329–37.

[18] Viu JE, Jose-Cunilleras L, Armengou C, Cesarini I, Taracón J, Rios L. Acid-base imbalances during a 120 km endurance race compared by traditional and simplified strong ion difference methods. Equine Vet J 2010;38:76–82.

[19] Kinnunen S, Atalay M, Hyyppä S, Lehmuskero O, Oksala N. Effects of prolonged exercise on oxidative stress and antioxidant defense in endurance horse. J Sports Sci Med 2005;4:415–21.

[20] McArdle F, Pattwell Vasilaki DMA, McArdle A, Jackson MJ. Intracellular generation of reactive oxygen species by contracting skeletal muscle cells. Free Radic Biol Med 2005;39:651–7.

[21] Kinnunen S, Hyyppä S, Lehmuskero A, Oksala N, Mäenpää P, Hänninen O, et al. Oxygen radical absorbance capacity (ORAC) and exercise-induced oxidative stress in trotters. Eur J Appl Physiol 2005;95:550–6.

[22] Navarro A, Gomez C, López-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol-Regulatory. Integr Comp Physiol 2004;286:505–11.

[23] Adamu L, Noraniza MA, Rasedee A, Bashir A. The use of a metabolic disorder index as a predictor for metabolic eliminations in endurance horses. J Equine Vet Sci 2017;51:113–21.

[24] Michael T. Veterinary epidemiology. Blackwell Science 2013:1–24.

[25] Barnes A, Kingston J, Beetson S, Kuiper C. Endurance veterinarians detect physiologically compromised horses in a 160 km ride. Equine Vet J 2010;42:6–11.

[26] McKeever KH. Body fluid and electrolytes: responses to exercise and training. In: Hinchcliff KW, Kanep AJ, Geor RJ, editors. Equine sports medicine and surgery. Philadelphia: WB. Saunders; 2004. p. 854–71.

[27] Muñoz A, Riber C, Trigo P, Castejón-Riber C, Castejón FM. Dehydration, electrolyte imbalances and renin-angiotensin-aldosterone-vasopressin axis in successful and unsuccessful endurance horses. Equine Vet J 2010;42:83.

[28] Dumont CBS, Okiyama FTM, Barreto-Vianna ARC, Lima EMM. The distance run is not the determining factor on the occurrence of electrolyte change for equine athletes disqualified in endurance competitions. Pak Vet J 2014;34:239–42.

[29] Robert C, Goachet AG, Fraipont A, Votion D, van Erck ME, Leclerc JL. Hydration and electrolyte balance in horses during an endurance season. Equine Vet J 2010;42:98–104.