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Pre-race and race management impacts serum muscle enzyme activity in Australian endurance horses

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Abstract

Background: Marked increases in serum muscle enzyme activity can occur in endurance horses but the diagnostic certainty in predicting cases of myopathy is unclear. Improved understanding of horse management effects on serum muscle enzyme activity as markers of muscle health would assist interpretation of serum muscle enzyme activity and guide management to reduce myopathy risk.

Objectives: To investigate associations between serum muscle enzyme activity and management factors in endurance horses.

Study design: Cross-sectional study.

Methods: One hundred endurance horses competing in four endurance events (offering distances of 20-120 km) in south-eastern Australia were observed. Data were collected from official horse logbooks, pre-and post-race serum samples, an owner questionnaire of pre-race and race management of horses and the Australian Endurance Riders Association results database. Multivariable linear regression modelling tested associations between management factors and serum muscle enzyme activity.

Results: First leg speed, distance raced, number of rest days pre-race, and pre-race activity of aspartate aminotransferase (AST) and creatine kinase (CK) explained 47.3% of the variance in post-race CK. As first leg speed increased by 1 km/h, CK activity increased by 25.8% (95% CI 11%-35%). Race distances >80 km increased post-race CK activity by 124% (95% CI 116%-145%). Each additional pre-race rest day increased post-race CK activity by 30.5% (95% CI 11%-42%). Modelling a 10% increase in pre-race CK and pre-race AST activity was associated with post-race CK activity increasing by 7.3% (95% CI 3%-14.4%) and 8.5% (95% CI 0.3%-14.2%) respectively. Horses experiencing training distances >40 km and a greater number of rest days prior to race day developed increased pre-race AST and CK activity respectively.

Main limitations: Owner questionnaires may be subject to bias. Limited data were available to model ride terrain, horse fitness, ration detail and myopathy. Muscle biopsies were not used to confirm myopathy.

Conclusions: Nearly half of the variation in post-race CK activity observed can be attributed to management factors unrelated to myopathy, suggesting increased CK activity may not be pathognomonic for myopathy. We advise caution in relying solely on serum muscle enzyme activity for diagnosis of myopathy until the strength of association between CK and myopathy is further ascertained in future studies.

KEYWORDS
AST, CK, endurance horse, horse, management, muscle enzyme, risk factor

1 | INTRODUCTION

There is a considerable body of published literature reporting serum muscle enzyme activity in exercising horses.[1-10] Increased activities of serum muscle enzymes after exercise occur in horses of varying breeds with or without concurrent evidence of muscle pathology or genetic predisposition to muscle pathology.[9,11-15] In a number of studies, a strong association between increased serum muscle enzyme activities and muscle pathology has been described, including but not restricted to, exertional rhabdomyolysis (ER).[13,15-18] As a result, measurement of serum muscle enzyme activity is a common diagnostic tool, and the combination of serum muscle enzyme activity and histological examination of muscle biopsy samples strengthens the diagnostic evidence of myopathy.[18] However, due to the invasive nature of the procedure and expertise required, muscle biopsies are not performed routinely in clinical practice. Consequently, clinicians face the challenge of interpreting increased serum muscle enzyme activities in exercising horses that do not have convincing clinical signs of myopathy.

Many studies have reported effects of exercise on serum muscle enzyme activity in normal horses; however, the heterogeneity of study designs,[19] including variations in diets, exercise regimens and sample collection times, complicates comparisons between studies and interpretations of serum muscle enzyme activities in exercising horses.[1-5,9,10,19-22] Furthermore, there is inconsistency in the case definition of myopathy between studies, including the use of serum muscle enzyme cut-off levels, history of recurrent exertional rhabdomyolysis (RER), inclusion of clinical signs and use of biopsy data or ex vivo muscle contracture studies.[15,18,22-25] As a result, the serum muscle enzyme response to exercise and association with myopathy remains incompletely understood.

Exercising horses with inappropriate increases in serum muscle enzyme activity in the absence of clinical signs have been classified as subclinical myopathy cases.[18] Recent observations by Wilberger et al[25] support the longstanding concept that serum CK activity correlates poorly with clinical signs of ER in horses.[26] Anecdotally, there is concern that subclinical myopathy cases may be undetected by officiating veterinarians at endurance rides, risking more serious muscle injury if exercise continues. The cut-off level of serum muscle enzyme activity above which muscle damage is likely remains to be determined. Furthermore, it remains unknown how potentially modifiable risk factors, including training, diet and other horse management factors contribute to serum muscle enzyme activity in endurance horses. A clearer understanding of such factors could improve the welfare and performance of endurance horses.

The aim of this study was to determine associations between horse management factors and serum muscle enzyme activity in a population of competing Australian endurance horses.

2 | MATERIALS AND METHODS

2.1 | Participant recruitment

Permission to conduct the current study was obtained from the relevant endurance rider associations and ride organisers. Access to ride social media pages was gained to post information about the study ahead of the event. Information sheets were made available at the ride base and rider consent was obtained prior to data collection. Horses at four different endurance rides in Australia were enrolled into the study upon arrival at the ride base on the day before race start. The number of participating horses and total number of horses entered for each ride was obtained from the official Australian Endurance Riders Association (AERA) results database (https://www.aeraspace.com.au). The number competing and percentage of horses enrolled in the study varied between rides, with 28/78 (36%), 24/91 (26%), 31/166 (19%) and 18/123 (15%) enrolled for Table Top, Eldorado, Tumut and Gundagai respectively.

2.2 | Ride venue characteristics

All four venues offered a range of ride types (Weblink for PDF http://aera.asn.au/new-site/wp-content/uploads/S1-General-Rules-Current.pdf, see table 4) and took place under temperate climate conditions in New South Wales or Victoria, during autumn 2017 (Table Top, 80 and 120 km endurance rides), spring 2017 (Eldorado, 20 km introductory ride, 40 km intermediate ride and 80 km endurance ride) and autumn 2018 (Tumut, 20 km introductory ride, 40 km intermediate ride, 80 and 120 km endurance rides, and Gundagai, 40 km intermediate ride and 80 km endurance ride). Horses completing marathon rides only contributed data from the first of several rides. Daily maximum temperatures recorded on the day of each ride were 16, 28, 25 and 29°C respectively (http://www.bom.gov.au/climate/data/).

2.3 | Data collection

Data were collected from an owner questionnaire, horse official ride logbooks, laboratory analyses of pre- and post-ride serum samples and the official Australian Endurance Riders Association (AERA) results database (www.aeraspace.com.au).

The questionnaire (Data S1) involved 18 multiple choice questions (using rider-familiar terminology) about horse nutrition, exercise, ER history, transport and management in the week prior to the ride, and rider-reported horse behaviour and performance during the ride. Riders were asked to complete the questionnaire in the presence of a researcher at the time of the pre- and post-race blood samples and in a setting conducive to talking about their horse and ride. This provided an opportunity for riders to ask questions in order to clarify what was being asked.

The following data were obtained from the logbook: capillary refill time, gastro-intestinal sounds, muscle tone, gait, overall veterinary assessment score, target distance and placing and outcome (completion, veterinary elimination or withdrawal by the rider). The guide to scoring of the official clinical parameters is provided in Data S2. Clinical categories for recording veterinary elimination in the logbook are limited to pulse, lame, metabolic, back, gall or injury. A suspect ER horse would be coded as 'metabolic' without provision of additional clinical information.

Pre-ride blood samples were collected within 24 hours of ride commencement at the time of arrival at the ride base (this included the morning of the ride for local horses or the day before for horses that travelled from further afar). Post-ride blood samples were collected from all participating horses within a standardised time of 4-6 hours [26] of horse withdrawal or completion. All samples were collected into plain vacutainer tubes (BD Vacutainers, Becton Dickinson) and immediately stored at 4°C without centrifugation. Serum CK and AST activities were measured using the Kinetic UV test[27] within 72 hours of collection (Beckman Coulter AU 480).

Distance completed, first leg and average speeds were obtained from the official results database website (https://www.aeraspace.com.au/). Definitions of ride distance and ride timing are available in tables 2, 6 and 8 of the official AERA Rulebook (http://aera.asn.au/national-rules/). The first leg speed is an official record of the speed of each horse-rider combination around the first loop of the event course; the leg distance varies between venues and is not recorded. The distance completed in an eliminated or withdrawn horse is accurate only to the last timed check point (at the end of the previous leg).

2.4 | Data analysis

All data were entered and managed in Microsoft Excel. All analyses were performed in R (R Development Core team [2019] R Foundation for Statistical Computing. https//www.R-project.org). A multiple linear regression was used to model potential relationships between variables for horse demographics (horses age, sex, conditions score, height, weight), race logistics (first leg speed, average speed, distance completed, length of race, pre and post-race CK and AST, distance of race >80 km, heart rate and veterinary score), training (rest days prior to the race, kilometres trained in week before race), ER history (prior veterinary elimination, prior owner withdrawal, prior elimination), pre-race management (hours rest between travel to venue and race start, lucerne in ration, main hay type, feed energy density, main feed energy type) and the change observed in the pre- and post-race serum activities of CK and AST. The outcome variable, difference in CK between pre- and post-race, was log transformed to satisfy the normality assumptions of the linear regression. The normality of the log transformed pre- and post-race CK activity was tested using the Shapiro-Wilk test. The log transformation of pre-race CK was normally distributed ($W = 0.98$, $P = .2$). While there was evidence of non-normality in the log of the post-race CK ($W = 0.79$, $P < .001$) this deviation was accepted due to the difficulties in interpretation of more complex transformations and the large sample size. The final regression model was subjected to the standard regression model diagnostics.

To adjust for the widely varying pre-race CK activities, the natural log of the pre-race CK activity was included as a predictor variable.

To screen the large number of candidate variables a Best-Subsets regression model building process was utilised with the Akaike Information Criterion (AIC) as the selection criteria. Best-Subsets regression examines all combinations among all the candidate variables and chooses the model with the lowest AIC. The Best-Subsets regression was performed using the 'bestglm' package in R (https://cran.r-project.org/web/packages/bestglm/bestglm.pdf). Using the variables identified and other variables with a plausible biological basis, manual model fitting was then undertaken to identify a robust model consistent with all the assumptions of multiple linear regression. Variables were only retained in the model if they satisfied a threshold of $P < .05$.

A similar model building approach to that outlined was used to identify management variables associated with log transformed pre-race AST and pre-race CK activities. Variables considered in the Best-Subsets regression were rest days prior to the race, kilometres trained in week before race, prior veterinary elimination, prior owner withdrawal, prior elimination, hours rest between arrival at venue and start of race, rest days in week before the race, lucerne in ration, main hay type, feed energy density and main feed energy type.

An analysis of variance was used to test the difference in post-race CK for horses classified by the veterinarian as either 'normal' or 'abnormal' (representing the overall veterinary clinical score awarded after the race of 'A' for 'normal' and 'B', 'C' and 'D' for 'abnormal', respectively, Data S2).

All level one interactions were tested. Whilst the inclusion of the interactions created marginal improvement in the R^2 values of the models, the interactions are not included because of the complexity added to interpretation and communication of the models. To determine the relative importance of independent variables, the hierarchical approach of Chevan and Sutherland[28] was implemented in the R package *hier.part*. A flow chart of the numbers of horses available for each stage of data analysis is provided in Figure 1.

3 | RESULTS

3.1 | Horse details

A total of 100 horses were enrolled in the study, with a mean (±SD) age of 9.7 (±3.3) years. There were 51 geldings, 46 mares and three

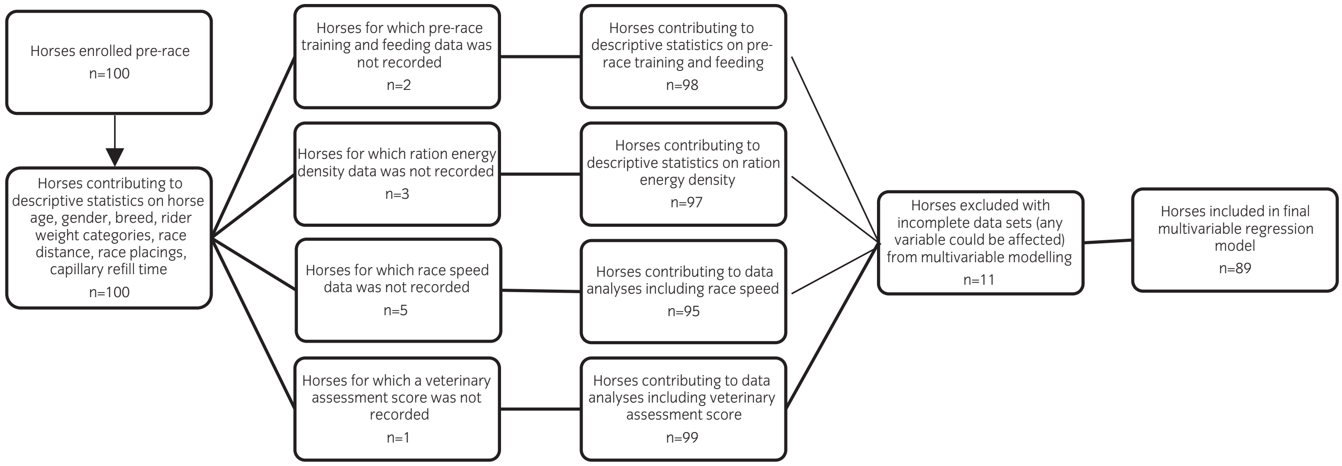

FIGURE 1 Flow chart of number of horses contributing to various data analyses

stallions. The median body condition score of the horses was 3/5 (range: 2-4). Most horses (92/100, 92%) were 14.2-16 hands tall. Ninety-three horses were of Arabian or part Arabian breeding, and there were three cross-bred horses, two Standardbred horses, one Australian stock horse and one Thoroughbred horse.

Fifty-two, 31 and 10 horses competed in medium (≥73 and <91 kg), light (≤72 kg) and heavy (≥91 kg) rider weight categories respectively. The remaining horses were ridden in ride types without weight categories.

Of 12 horses with a prior ER event, eight had been diagnosed by a veterinarian and four by the owners.

3.2 | Pre-race management

Of the 98 horses that trained in the 7 days prior to the race, 51 covered <40 km and 37 covered between 40-80 km, seven >80 km and three horses did not train at all. The median (range) number of rest days reported by the rider in the week prior to an event was 3 (0-7) days.

Information about pre-race feeding was recorded for 98 horses. Forty-two horses were fed the same amount or more on a rest day as was fed on a training day. Ration components were categorised into energy feeds (including concentrates and grains) and roughage (including a variety of hays and chaff). The main energy feed provided in the week prior to the race was either a commercial concentrate for 31 (31.6%) of horses or a mix of commercial concentrates and grains (either oats, barley or lupins) for 47 (47.9%) of horses. Commonly fed roughages included lucerne, meadow and oaten hay, with a combination of roughages preferred. Lucerne hay or chaff was part of the ration for 45 horses. Three horses were reported to be fed only roughage (meadow hay) and no additional energy feed (either concentrates or grains) provided. Quantities fed were not recorded.

Information for energy density of the concentrates and grain fed to horses before the race was obtained from public product information and recorded for 97 horses. The maximal energy density of any concentrate or grain provided was 18 MJ/kg while the mean energy density of the diets was 12.2 MJ/kg.

Duration of travel to the endurance ride varied considerably from <1 to >6 hours.

For all horses, the rest provided after travel and prior to racing was >1 hour. Most horses (72/100) were permitted >12 hours rest.

3.3 | Race results

Race results were reported for 100 horses. Seventy-four horses successfully completed the race and 26 horses were either eliminated (n = 18) or withdrawn (n = 8). Completion rates varied with target distance, 3/3 (100%) for 20 km, 20/28 (71.4%) for 40 km, 39/55 (70.9%) for 80 km and 12/14 (85.7%) for 120 km. Of the horses that were eliminated, 13 were lame, four had increased heart rates and one had a non-ER metabolic disturbance (elevated HR, dehydration and prolonged capillary refill time). No horse was eliminated due to a veterinary diagnosis of ER.

The most common distance over which horses competed was 80 km. Sixty-eight horses competed in either 80 or 120 km rides and 32 horses competed in 40 or 20 km rides. The mean first leg speed was 11.6 ± 2.5 km/h (range: 6.1-17.9). Of 95 horses for which these data were recorded, 55 were reported by their riders to be travelling willingly at good speeds (indicating owner opinion rather than a measurable entity), 34 horses travelled faster than asked by the rider, and two horses travelled slower than asked, with four horses travelling at varying speeds and not always as asked.

Several horse health or behaviour problems affecting horses during the ride were reported by riders, most commonly lameness, skin abrasions and nervousness (Table S1).

At the conclusion of the ride, capillary refill time was reported as <1 second in 60/99 horses, 1-2 seconds in 38/99 horses and ≥2-3 seconds in one horse. Gut sounds were recorded for 99 horses and classified as 'A' in 54 horses, 'B' in 41 horses and 'C' in four horses. Post-race overall veterinary assessment score was 'A' in

TABLE 1 Range, median values and 50% interquartile range (IQR) for pre- and post-race muscle enzyme activity (CK and AST)

Muscle enzyme	Range	Median	50% IQR	Number of horses
Pre-race CK (U/L)	105-1182	342	290-448	96
Pre-race AST (U/L)	231-4821	335	300-392	96
Post-race CK (U/L)	302-139 147	723	555-1828	100
Post-race AST (U/L)	261-5442	398	339-515	100

64/99, 'B' in 18/99 and 'C' in 17/99 horses. Post-race veterinary gait assessment was 'A' in 72/99, 'B' in 2/99, 'C' in 2/99 and 'D' in 13/99 horses. Post-race veterinary assessment of muscle tone was normal in 97/99 horses.

3.4 | Serum creatine kinase and aspartate aminotransferase activities

Descriptive statistics showed marked variability in pre- and post-race CK and AST activities (Table 1). Thirty-three horses had ≥4-fold increases in post-race CK activity ((post-race CK - pre-race CK)/pre-race CK level) considered by some to be indicative of ER.[17] Of these 33 horses, a further eight horses had post-race CK activity exceeding 10 000 U/L (Table 2). One of these eight horses had previously recorded a veterinary diagnosis of ER. The other 11 horses with a prior veterinary or owner diagnosis of ER or both, recorded post-race CK activity of <10 000 U/L. Of the eight horses with post-race CK activity >10 000 U/L, six scored an overall 'A' vet score, one scored a 'D', and one did not have the veterinary score recorded.

3.5 | Predictors of post-race creatine kinase activity

The results of the model building process identified a multiple linear regression model with five variables that explained 47.3% of the variance in post-race CK activity ($R^2 = 0.47$, $P < .001$). The model was significantly better than the null model ($F(5,83) = 14.87$, $P < .001$). First leg speed was the most important factor (Table 3), with ride distance >80 km, Log(pre-race CK), Log(pre-race AST) and number of rest days in the week prior to the race also retained in the best model. The model residuals were normally distributed on the Shapiro-Wilk test ((W) = 0.97, $P = .16$). To validate the model, a leave-one-out cross validation was conducted. The R^2 of the model decreased from 54% to 44%, the mean squared error increased from 1.05 to 1.28 and the mean absolute percentage error increased from 13.6% to 14.9%. All 89 horses with complete datasets contributed to the multivariable regression modelling (Figure 1).

The observed associations between management variables and serum muscle enzyme activity are depicted in a web of association in Figure 2.

The association between overall official veterinary score (clinical signs) and serum muscle enzyme activity (both the rise in CK from pre-race values and the actual post-race values) was tested using linear regression modelling (Table S2). The relationship between veterinary score and post-race CK was not statistically significant ($F_{(1,99)} = 0.406$, $P = .53$), as the overlap in the distribution of CK values between the horse assigned by veterinarians as 'normal' or 'abnormal' was such that the CK activity is unlikely to have the ability to accurately allocate horses to either group (Figures S1 and S2).

Examining interaction variables revealed that pre-race AST activity magnified the effect of first leg speed on post-race CK (Figure 2).

3.6 | Predictors of pre-race serum muscle enzyme activity

Two variables had an association with natural log of pre-race CK activity: prior elimination from race due to ER ($P = .02$) and the number of rest days prior to competition ($P = .06$). For every additional day of rest prior to the race, the pre-race CK activity increased by 4.5% (95% confidence interval [CI] 0.01%-9.17%).

On average, horses that trained ≥40 km had pre-race AST activities that were 229.75 U/L (95% CI 10.8-448.7) greater than horses that trained <40 km ($P = .04$).

4 | DISCUSSION

This is the first analysis of associations between management factors and serum muscle enzyme activities in endurance horses. As markers of muscle health, serum muscle enzyme activities are widely used by clinicians to aid examination of performance horses and our study results improve current knowledge of factors impacting serum muscle enzyme activity. Increased activities of CK post-race were significantly associated with race speed and distance, pre-race activities of CK and AST and number of rest days. Increased pre-race activities of AST were associated with training distance and those of pre-race CK activity with previous elimination due to ER and rest days in week prior to the race.

The speed of study horses around the first leg of the race was significantly associated with post-race CK activity. Our model predicted that as the speed on the first leg increased by one km/h, the increase in post-race CK activity was 25.8%. Previously, greater enzyme activities were associated with faster race times in endurance horses competing over 160 km.[29] Recently, horses failing to finish had greater initial race speeds than horses finishing successfully,

TABLE 2 Rider reported problems encountered during the ride in eight horses with post-race creatine kinase (CK) activities exceeding 10 000 U/L

Sex	Age (years)	Breed	Distance (km)	L1 speed (km/h)[a]	CK post U/L	Muscle tone, post ride	Gait, post ride	Overall Vet score	Race result	History	Performance during ride	Rider reported problem noted during ride
Mare	8	Arabian	120	17.7	10 177	A	A	A	Second place	Prior ER[b]	Good speed	
Mare	Not recorded	Arabian	80	14.3	135 053	A	A	A	Rider withdrawn		Too fast	Sore muscles, anxious behaviour
Gelding	7	Arabian Cross	80	11.9	20 658	A	A	A	Fourth place		Good speed	
Gelding	6	Arabian	80	16.5	139 147	A	A	A	Completed		Good speed	Fatigued last 20 km
Gelding	11	Arabian	40	Not recorded[c]	19 669	Log book not provided	Log book not provided	Log book not provided	Eliminated: heart rate L1[d]		Too fast	
Gelding	10	Arabian	80	Did not complete L1	58 402	A	A	A	Rider withdrawn L1	Prior ER	Good speed	'Horse not himself ' at 10km into ride[e]
Gelding	16	Arabian	80	15.6	10 282	A	A	A	Completed		Too fast	
Mare	8	Arabian	80	12.6	65 625	A	D	D	Eliminated: lame		Too fast	Skin abrasion

[a]Speed of the first leg (loop) of the race.
[b]Exertional rhabdomyolysis.
[c]Actual distance completed not recorded.
[d]Horse eliminated during leg 1.
[e]Rider comment about horse health not further clarified.

TABLE 3 Regression parameters for the final model of post-race increase in creatine kinase (CK) activity and relative importance as % of R^2 for 89 horses

Predictors	Log (Post race CK – Pre-race CK) Estimates	95% CI	*P*	Relative importance
(Intercept)	−7.05	−11.94, −2.16	.006	
Leg one speed	0.23	0.11, 0.35	<.001	36.1%[a]
Race length ≥80 Km	0.81	0.16, 1.45	.02	25.5%[b]
Log (Pre-race AST)	0.86	0.30, 1.42	.004	17.6%[c]
Rest days prior to race	0.27	0.11, 0.42	.001	11.9%[d]
Log (Pre-race CK)	0.74	0.03, 1.44	.04	8.7%[e]
R^2/R^2 adjusted	0.473/0.441			

[a]As the speed on the first leg rose by 1 km/h, the increase in post-race CK activity post-race was 25.8%.

[b]Race distances >80 km had an increase in post-race CK activity of 124% more than those <80 km.

[c]For each additional rest day prior to the race, post-race CK activity increased by 30.5%.

[d]A 10% increase in the pre-race activity of CK resulted in the post-race CK activity by 7.3%.

[e]A 10% increase in pre-race AST activity was associated with an 8.5% rise in the post-race CK activity.

[f]Variable modelled was target distance.

suggesting that pacing strategy may increase the chance of completion,[30] and possibly reduce the likelihood of subclinical muscle damage. Our results suggest that excessive first-leg speed increases the risk of high post-race CK activity. This highlights the need for riders to carefully manage factors impacting race speed such as horse excitement, horse fitness and rider competitiveness to match faster horses at race start.

Study horses competing over >80 km had greater increases in post-exercise CK activities than those covering shorter distances. The influence of ride distance on CK activities has been observed previously,[31] and was a risk factor for elimination in New Zealand endurance horses.[32] The effect of distance is likely mediated through a combination of normal physiological exercise effects on muscle membrane permeability as well as potential sub-clinical muscle damage.

In the current study, pre-race AST activity was a risk factor for post-race increases in CK activity. Sampling times of 4-6 hours post-race were chosen to ensure participating horses were sampled prior to departure from the event; however, this sampling time frame may not have captured peak activities of AST. While the delayed response dynamics of AST after muscle damage likely explains the modest increases in activities of this enzyme in samples obtained within 6 hours of the race, pre-race AST activities may reflect the effects of exercise in the week prior to sampling. Indeed, pre-race AST activity was influenced by training distance in the week prior to race day. On average, horses that covered >40 km in the week before racing had pre-race AST activities 229.75 U/L greater than horses that did not. Further investigation is necessary to ascertain if pre-race AST activities could be a longer term indicator of muscle health in endurance horses. Resting muscle enzyme activities have received little attention as risk factors for ER in endurance horses. In one study, daily increases in pre-exercise AST activities over a training season were reported and may reflect cumulative training stress.[5] In young Thoroughbred racehorses, small influences on resting serum muscle enzyme activity were observed due to animal age and sex.[2] No such association was observed in the current study.

Pre-race (resting) CK activity was a risk factor for post-race CK activity in the current study. This association has not been documented previously. CK activity increases more rapidly in response to changes than AST and is more likely to reflect short term influences on muscle, such as exercise, diet and rest days. How previous elimination due to ER affects resting CK activity is unclear but may indicate failure to recover fully resulting in more marked serum muscle enzyme responses compared to horses without pre-existing myopathy.

An association between rest days and changes in muscle enzyme activities of endurance horses has been previously described[19,26] but not been subjected to multivariable modelling. In our study, the number of rest days predicted both increases in pre-race and post-race serum muscle enzyme activity, indicating that this management factor has important implications for muscle health in endurance horses. Similarly, Thoroughbred racehorses with RER have greater serum CK activities after 1 or more rest days compared to exercise days.[19] The impact of rest days on muscle health in endurance horses and risk of ER requires consideration and further investigation.

Post-race CK activity varied considerably among study horses, rising >4-fold and/or reaching >10 000 U/L in approximately a third of the horses. Such increases in post-exercise CK activity in asymptomatic horses are considered to represent subclinical cases of myopathy by some experts.[17] If we applied these diagnostic criteria to our study horses, and presumed that the eight horses with post exercise CK activity of >10 000 U/L were indeed subclinical ER cases, then the prevalence of ER would increase to 8%. Two of these horses had owner-reported signs that could be considered suggestive of

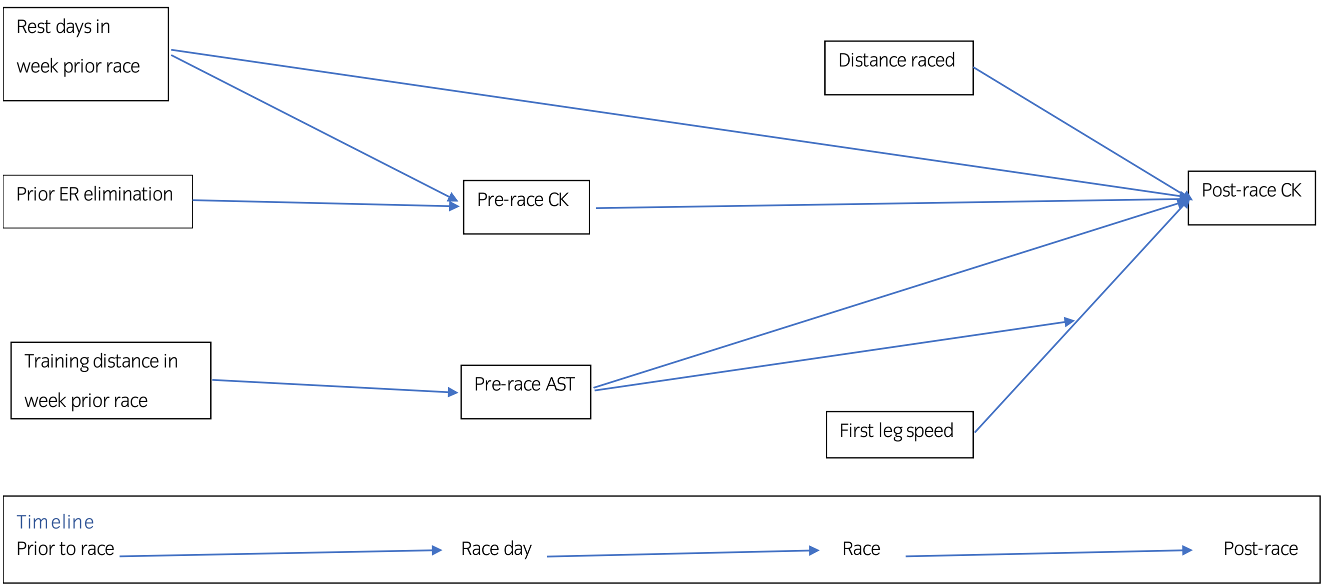

FIGURE 2 Web of associations between observed risk factors and post-race CK activity

myopathy, and one further horse with an official vet score of 'D' was eliminated. In the current study, any association between serum muscle enzyme activity and myopathy remains uncertain and outside the current dataset, as no muscle biopsies were performed to confirm myopathy. Furthermore, there was no association between veterinary scores and serum muscle enzyme activity in participating horses suggesting that clinical signs would be a poor predictor of changes in serum muscle enzyme activity. No study horses were diagnosed with ER by officiating veterinarians; however, undetected myopathy in some horses cannot be discounted. Officiating veterinarians face considerable challenges when assessing endurance horse health under time constraints and without the benefits of serum muscle enzyme activities or biopsy data and misclassification of myopathy cases is possible. The potential welfare implications of missing cases of ER during veterinary inspections at endurance rides have been raised previously. The current study supports findings by Wilberger et al,[25] who suggested that real or perceived muscle pain was not a reliable indicator of muscle necrosis, and reliable, practical methods for ER detection are lacking. One horse in the current study was subsequently diagnosed with ER on the following day (author-owner communication) after competing in the second of two rides (marathon style event).

The observed range of post-race CK activities was marked compared to pre-race values. This finding suggests that considerable variation in CK activity between horses occurs, likely representing a complex set of factors, confounding the determination of an optimal cut point value for CK for the detection of myopathy. Any undiagnosed cases with high serum muscle enzyme activity may be at increased risk of myopathy if exercise persists, particularly if fluid/metabolic derangements develop. The contribution of rest, training, race speed and distance to serum muscle enzyme activity, based on our findings, is considerable, and should be considered in the interpretation of enzyme activities and diagnostic evaluation of each horse. Our results suggest that additional factors contribute to serum muscle enzyme activity and the observed increases in enzyme activities may not reflect myopathic damage alone.

While nervous temperament and female sex have been reported as risk factors for ER in Thoroughbred and Standardbred racehorses,[33] the current study did not find an association of sex and rider-reported horse behaviour with serum muscle enzyme activity. Similarly, in a previous study of endurance horses, pre- and post-race CK activities did not differ with horse signalment or temperament.[25] It is possible that animal-level factors may not be as important for predisposition to myopathy in endurance horses compared to racehorses with RER.

In the current study, feed type was not associated with changes in serum muscle enzyme activities. Dietary effects on serum muscle enzyme activity in endurance horses have not been described, in contrast to horses affected by RER and polysaccharide storage myopathy (PSSM), where diet is an important influence on serum muscle enzyme activities.[13,19] Similarly, no association between diet and CK activities in endurance horses was found in a previous study.[25] The commercial concentrate diets fed to horses in our study had similar energy densities, and most horses had access to roughage and pasture. Despite the lack of an association with changes in serum muscle enzyme activity, 43% of study horses did not have a reduction of the amount of concentrate feed on a rest day. In Thoroughbred racehorses, excessive concentrate feed on a rest day is associated with increased risk of RER.[23] It is possible that inadequate detail on ration quantities fed to our horses may have precluded determination of any association with serum muscle enzyme activity. The potential influence of feeding regimens on risk of myopathies in endurance horses requires further investigation.

This study has limitations. One hundred study horses provided a cross sectional insight but should not be considered a representative sample of all Australian endurance horses. No cases of clinical ER were diagnosed, precluding investigation of risk factors for ER in endurance horses. The lack of biopsy assessment of muscle health precluded a more accurate diagnosis of subclinical myopathy.

Blood samples were immediately refrigerated at the endurance ride and underwent centrifugation for harvesting of serum at the laboratory within 72 hours of collection. While some haemolysis may have occurred in transit to the laboratory, any effect on serum muscle enzyme activity in the chilled samples is likely to be minimal.[27,34,35]

Caution is advised in the interpretation of results from the owner-reported horse problems and owner questionnaire, potentially subject to recall bias, misclassification of horse problems and personal opinion. The current study also did not consider or measure genetic factors in study endurance horses reported in other breeds.[36]

With respect to the risk factors identified, the regression models cannot explain all the changes in the observed serum muscle enzyme activities, and this suggests that other factors not studied, had an impact. Modelling of data of the diverse cohort of horses competing over varying distances was not adjusted for fitness or horse/rider experience. In future studies, these factors need to be considered, as they may attenuate increases in serum muscle enzyme activities mediated through changes in cell membrane permeability or susceptibility to oxidative stress with conditioning.[1,3]

For the first time, management factors have been implicated in serum muscle enzyme activity in endurance horses. Nearly half of the variation in post-race CK activity observed can be attributed to management factors unrelated to myopathy, suggesting increased CK activity may not be pathognomonic for myopathy. Consideration of such factors could assist in evaluating muscle health, attenuating increases in serum muscle enzyme activities and potentially identify at risk horses during ride vet checks. Future inclusion of horses with ER in similar studies, along with detail on horse fitness and ration fed, is required.

ETHICAL ANIMAL RESEARCH

The current project was approved by the Charles Sturt University Animal Care and Ethics Committee (A16088) and by the Charles Sturt University Ethics in Human Research Committee (400/2017/14).

OWNER CONSENT

Written owner consent was obtained at the time of enrolment into the study, which occurred at the ride base the day before race start and prior to data collection.

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CONFLICT OF INTERESTS

No competing interests have been declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

AUTHORSHIP

P. Buckley contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript. D. Buckley contributed to data analysis and interpretation, and preparation of the manuscript. R. Freire contributed to preparation of the manuscript. K. Hughes contributed to study design, study execution and preparation of the manuscript. All authors gave their final approval of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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