The Veterinary Journal 256 (2020) 105433

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Pre- and post-race serum cardiac troponin T concentrations in Standardbred racehorses

I.R. Hellings[a,*], R. Krontveit[b], M. Øverlie[c], A. Kallmyr[c], T. Holm[d], C. Fintl[a]

[a] Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences, Department of Companion Animal Clinical Sciences, Post Box 8146 Dep., 0033 Oslo, Norway
[b] Norwegian Medicine Agency, Post Box 240 Skøyen, 0213 Oslo, Norway
[c] Rikstotoklinikken Bjerke, Post Box 194 Økern, 0510 Oslo, Norway
[d] Evidensia Lørenskog Dyreklinikk, Solheimveien 56, 1473 Lørenskog, Norway

ARTICLE INFO

Keywords:
Cardiac troponin T
Equine
Exercise
Horse
Reference interval

ABSTRACT

Elevated cardiac troponin T (cTnT) concentrations may provide evidence of myocardial injury but physiological post-exercise release also occurs. Reference intervals are not fully established in horses making interpretation difficult. The aims of this study were to establish an upper reference limit for serum cTnT, compare pre-and post-race serum cTnT concentrations, and to evaluate factors that may influence these in a population of healthy, race-fit Standardbred racehorses. Serum samples were collected pre- (n = 108) and 1–2 h post-racing (n = 101) and analysed using a high sensitivity-cTnT assay. Reference limits with 90% confidence intervals (CI) were calculated by non-parametric methods using the bootstrap method. Effects of sex, age, racing speed, distance, placings and track surface were assessed by fitting generalized linear models with an identity link function and inverse Gaussian distribution.

The upper reference limit for serum cTnT concentration was 27.4 ng/L (90% CI 13.1–32.0). The median serum cTnT concentration was significantly higher 1–2 h post-racing compared to pre-racing ($P < 0.001$). Age and sex did not significantly affect serum cTnT concentrations pre-racing (P = 0.5 and P = 0.11). Cardiac troponin T concentrations were significantly higher post-racing in females (P = 0.018). Racing speed and placings had no effect on serum cTnT concentrations post-race (P = 0.71 and P = 0.66). The study contributed towards establishing an upper reference limit for serum cTnT concentrations in a population of race-fit Standardbreds and evaluated factors that may have influenced the results obtained.

Introduction

Cardiac disease is increasingly recognized as a cause of poor performance as well as of sudden death in horses (Brown et al., 1988; Boden et al., 2006; Lyle et al., 2011; Reef et al., 2014). While echocardiography and exercising electrocardiography are widely available in equine practice, these techniques are not sensitive in detecting subclinical cardiac disease including myocardial injury (Reef et al., 2014).

Cardiac troponins (cTn) are a group of cardiac regulatory proteins unique to the myocardium (Sharma et al., 2004). In humans, both cardiac troponin I (cTnI) and troponin T (cTnT) have been shown to be useful markers in diagnosing myocardial injury (Sharma et al., 2004; Thygesen et al., 2010). However, release of cTn during and after exercise in apparently healthy humans is also well documented (Vilela et al., 2014; Klinkenberg et al., 2016) and vary with fitness level, type and duration of exercise and certain physiological factors (Shave et al., 2010). Exercise-associated increases in cTnT typically peak within few hours (2–5 h) (Gresslien and Agewall, 2016) post-exercise and return to baseline within 24 h and differ from the prolonged elevated concentrations typically seen in humans with myocardial injury (Shave et al., 2010; Donnellan and Phelan, 2018). After a marathon, 78% of runners had cTnT above 0.01 ug/L (10 ng/L) while 36% had cTnT concentrations above the cut off values for acute myocardial infarct in humans of 5 ng/L (Shave et al., 2007).

The use of cTnI in equine clinical practice is well established with normal reference intervals (RIs) available (Nostell and Haggstrom, 2008; Slack et al., 2012; Van Der Vekens et al., 2015a). Furthermore, cTnI concentrations have been shown to increase or remain unchanged after exercise (Nostell and Haggstrom, 2008; Slack et al., 2012; Rossi et al., 2019). However, different assays for measuring cTnI exist, and reference values indicative of myocardial injury vary between assays (Sharma et al.,

* Corresponding author.
E-mail address: ingunn.risnes.hellings@nmbu.no (I.R. Hellings).

http://dx.doi.org/10.1016/j.tvjl.2020.105433

2004; Rossi et al., 2014; Van Der Vekens et al., 2015b). Also, different cTnI assays have different sensitivities and may therefore be unsuitable to detect subtle myocardial injury necessary in clinical sports medicine (Phillips et al., 2003; Begg et al., 2006; Rossi et al., 2014; Van Der Vekens et al., 2015a).

To address these limitations, a high sensitivity troponin T (cTnT-hs) assay has been developed and was recently validated for use in horses (Shields et al., 2016). Using this assay, cTnT concentrations from healthy leisure horses (Van Der Vekens et al., 2015b; Shields et al., 2016) and racing Thoroughbreds (Shields et al., 2016) have been published. Using the same assay, cTnT concentrations in racing Thoroughbreds were significantly higher at all sampling points between 2 and 6 h post-race compared to pre racing, peaked at 3 h and returned to baseline between 12 to 24 h (Shields et al., 2017). There is currently no published data exploring the normal RI for cTnT in Standardbred racehorses, and the effect of racing on cTnT concentrations in this breed.

The specific aims of this study were to establish an upper reference limit for serum cTnT, to compare pre-and post-race serum cTnT concentrations, and to study the potential effect of sex, age, racing speeds and distance, placings and track surface conditions on serum cTnT concentrations in a population of healthy race-fit Standardbred racehorses using a cTnT-hs assay.

Materials and methods

Study design

The study was a prospective, observational clinical study with convenience blood sampling of race-fit Standardbred racehorses pre- and post-racing collected at a single racetrack in Oslo, Norway during official races held by the Norwegian Racing Authority. Data were collected between December 2017 and March 2018 and the study was approved by the ethics committee of The Norwegian Food Authority Approval number, 14,190; Approval date, 24 November 2017 and by the Norwegian Racing Authority Approval date, 24 October 2017.

Horses

Informed consent was obtained from the owners or owner representatives prior to examination and sample collection. Healthy and actively racing Standardbred racehorses of any age and sex were eligible to enter the study.

To be included, horses had to be deemed fit to race by the trainer based on their training performance and general health status. Cardiac auscultation and assessment of quality and regularity of the peripheral pulse by palpation of the facial artery was performed prior to and after the race in all horses by experienced equine veterinarians. Written records were made on heart rate, rhythm and characterization of any cardiac murmurs present. Horses with murmurs of grade $\geq$3/6 were excluded. Normal cardiac rhythm was defined as a regular rhythm at rest or as a regularly irregular rhythm that disappeared following exercise. Horses with arrhythmias other than those defined were removed from the study and further evaluation recommended.

Racing speed (average time/km), distance (1609 m or 2100 m) and track surface condition at the day of racing (firm or heavy) as well as placing in the race were recorded for each horse. The track surface condition was defined based on the official description given by the racetrack authorities on any given race day.

Sample collection

Blood samples were collected by venepuncture of the jugular vein into serum tubes with separation gel before racing, 1–2 h following racing and in a subset of horses, 24 (22–24) h following racing. Following collection and coagulation of the samples, tubes were centrifuged at 3000 g for 10 min. The tubes were immediately refrigerated and transported chilled to the local hospital for same day analysis.

Laboratory methods

Samples were analysed using the Roche Elecsys troponin T-high-sensitivity (TnT-hs) assay (Roche Diagnostics) on the Cobas e801 Analyser (Roche Diagnostics) complying with the manufacturer's instructions and the Clinical and Laboratory Standards Institute (CLSI) as previously evaluated in horses (Shields et al., 2016). The degree of haemolysis was assessed using the serum indices on the Roche Cobas e801 Analyser (Roche Diagnostics). No samples reached threshold levels for exclusion. Daily calibration and quality control procedures were performed prior to sample analysis. Using this method (9 min application), the lowest level of quantification (LoQ) was 5.0 ng/L and the lowest level of detection (LoD) was 3.0 ng/L.

Statistical analysis

As the data was non-Gaussian distributed and not symmetrical a non-parametric method was used to determine the upper 97.5 percentile reference limit for serum cTnT. The 90% CI around the upper reference limit was calculated using the bootstrap method by Reference Value Advisor freeware v2.1[1] . in accordance with recommendations (CLSI (Clinical and Laboratories Standards Institute), 2010; Friedrichs et al., 2012; Geffre et al., 2011; Ozarda, 2016). Sera with cTnT concentrations <5.0 ng/L were for the purpose of statistical analysis set as 4.9 ng/L. Visual inspection of histograms was used to assess distribution. Histograms were also used along with clinical experience and the statistics Tukey and Dixon to assess outlying observations. As the data were not normally distributed, medians are presented.

Differences in median serum cTnT concentrations pre- and post-race were assessed using Wilcoxon matched–pairs signed–ranks tests. Any effects of sex, age, racing speed, racing distance, track surface and race placings (first, second, third and $\geq$ fourth place) on serum cTnT concentrations were univariably evaluated fitting generalized linear models with an identity link function and an appropriate distribution. Evaluation of the distribution of the Anscombe residuals and the distribution of the cTnT concentrations were both applied in the selection of distribution. Inverse Gaussian distribution was selected. Entire males and geldings were grouped together and compared to females. Continuous variables were assessed using lowess curves and categorized if deviation from linearity was detected. Age, racing speed and timing of the post-race sample (minutes passed from race to sampling) were evaluated as continuous variables. Statistical significance was set at $P < 0.05$.

The variables were tested for collinearity by Goodman and Kruskal's gamma for ordinal and dichotomous variables and pairwise correlations for continuous variables. Associations >0.7 or < − 0.7 were considered evidence of collinearity. Variables with a $P \leq 0.20$, provided there were no collinearity between them, were then considered for further analysis in a multivariable generalized linear model to assess the effect on post-race serum cTnT concentration. Biological plausibility of significant findings was evaluated by priori knowledge and causal diagrams. The multiple Wald test was used to evaluate differences between categories of categorical variables. Variables were kept in the model if $P < 0.05$.

Statistical analyses were performed using Stata 15 (StataCorp).

Results

One hundred-and ten horses from 54 different training yards primarily located in the south-east of Norway were enrolled in the study. One horse was excluded due to a clinical history of recurrent rhabdomyolysis and above normal RI concentrations for aspartate aminotransferase and creatinine kinase (CK) concentrations. One horse was defined as an outlier and removed from the statistical analysis because it had a pre-race serum cTnT concentration (76 ng/L) more than twice the maximum value of other samples from the group.

The pre-race samples therefore included serum from one-hundred and eight Standardbred racehorses of which 40 were females and 68 males, thereof 53 geldings. The median age for all horses was 6 (range 3–11), for females 6 (range 3–11) and for males 6 (range 3–11) years, respectively.

One-hundred and one horses, thereof 37 females and 64 males were sampled 1–2 h after racing and 18 horses were additionally sampled at 22–24 h post-racing. Of the 101 horses sampled post-racing, 15 horses raced over the shorter (1609 m) distance, while 86 raced over the longer (2100 m) distance. Sixty-eight horses raced when track surface conditions were classified as firm, while the remaining 33 horses raced on a heavy track surface. Descriptive statistics for serum cTnT concentrations pre-race, 1–2 h post-race and 22–24 h post-race and upper limit for RI with 90% CI for the pre-and post-race concentrations are shown in Table 1.

Overall, median serum cTnT concentrations were significantly higher 1–2 h post-racing compared to pre-racing ($P < 0.001$) (Fig. 1). The median serum cTnT concentration 22–24 h post-racing was not significantly different to the pre-racing concentrations

[1] See: Reference Value Advisor freeware v2.1 direction insert. http://www.biostat.envt.fr/reference-value-advisor/ (Accessed January 15, 2020)

Table 1
Descriptive statistics for cardiac troponin T (cTnT) in a sample of race-fit Standardbred racehorses pre-race, 1–2 h post-race and 22–24 h post-race including the 97.5% upper reference limit with 90% confidence interval (90% CI) for the pre-race and post-race cTnT concentrations.

cTnT				
Time sampled	(*n*)	Median ng/L	Maximum ng/L	97.5 % upper reference limit (90% CI) ng/L
Pre-race	All horses (108)	4.9	32.0	27.4 (13.1–32.0)
1–2 h post-race	All horses (101)	6.0	24.0	23.5 (18.4–24.0)
	Males (64)	6.0	20.0	–
	Females (37)	7.5	24.0	–
22–24 h post-race	All horses (18)	4.9	75.0	–

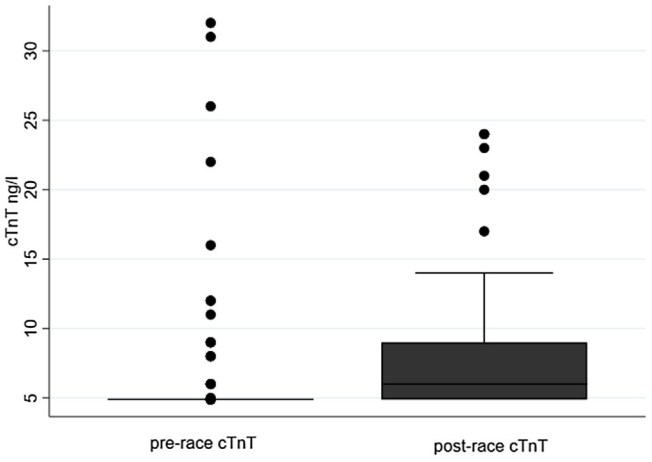

Fig. 1. Box plot showing concentrations of cardiac troponin T (cTnT) in a sample of race-fit Standardbred racehorses pre-race and 1–2 h post-race.

($P = 0.50$). One horse with a serum cTnT concentration of 75 ng/L 24 h post-race had pre-race and 2 h post-race serum cTnT concentrations of <5.0 and 9.0 ng/L, respectively.

Table 2 shows the univariable results from the generalized linear models for pre- and post-race serum cTnT concentrations. Sex and age did not have a significant effect on serum cTnT concentrations pre-racing (P = 0.20 and P = 0.38 respectively) (Table 2). Sex had a significant effect on post-racing measurements, with increased serum cTnT in females compared to males (P = 0.018). Cardiac troponin T concentrations were not significantly higher post-racing when racing on a heavy compared to on a firm surface track (P = 0.14), and when racing over 1609 m compared to 2100 m (P = 0.11) (Table 2). Racing speed or place position had no effect on serum cTnT concentrations post-racing ($P = 0.71$ and $P = 0.66$ respectively). Serum cTnT concentration was not significantly affected by the timing of sample collection within the 1–2 h interval following racing ($P = 0.82$; Table 2). Only sex was significant in multivariable modelling and hence a multivariable model was not built.

Table 2
Results from univariable generalized linear models of pre–race and post–race cardiac troponin T (cTnT) concentrations in a sample of race-fit Standardbred racehorses.

Variable	Coefficient (SE)	*P*	95% CI[a]
Pre–race cTnT (n = 108)			
Sex			
male	baseline	–	–
female	1.22	0.20	−0.64, 3.08
Age (years)	−0.18	0.38	−0.59, 0.22
Post-race cTnT (n = 101)			
Sex			
Male	Baseline	–	–
Female	2.18	0.018	0.38, 3.99
Age (years)	0.19	0.39	−0.25, 0.63
Race distance			
1609 m	Baseline	–	–
2100 m	−0.01	0.11	−0.01, -0.001
Track surface conditions			
Firm	Baseline	–	–
Heavy	1.42	0.14	−0.47, 3.31
Racing speed (min/km)	−0.07	0,71	−0.40, 0.27
Race position		0.66[a]	
First place	Baseline	–	–
Second place	0.88	0.51	−1.75, 3.511
Third place	1.91	0.22	−1.14, 4.95
≥ Fourth place	0.92	0.38	−1.17, 3.04
Minutes passed from race to sample	−0.004	0.82	−0.04, 0.03

95% CI, 95% confidence interval.
[a] Overall *P* value for categorical variable, Wald test.

Discussion

The current study has provided data which contributes towards establishing an upper reference limit for serum cTnT concentrations in an apparently healthy population of race-fit Standardbred racehorses. Significant post-race increases in serum cTnT concentrations also occurred in apparently healthy horses.

The 97.5th percentile upper reference limit for serum cTnT concentrations was 27.4 ng/L and was thus higher than previously published 95th and 99th percentile upper reference limits in plasma from non-competition horses (6.8 and 16.2 ng/L), but more similar to those reported from chuckwagon racing Thoroughbred geldings (14.0 and 23.2 ng/L) (Shields et al., 2016). This indicates that it may be appropriate to apply different reference intervals in different populations of horses. In humans, the absolute serum concentrations pre-and post-exercise depend on individual factors such as age and sex as well as fitness level, training, type and duration of exercise, timing of sampling and laboratory analytical factors (Shave et al., 2010; Rossi et al., 2014) and may apply to horses as well.

Cardiac troponin T concentrations were significantly higher 1–2 h post-racing compared to pre-racing and indicates release of cTnT into the circulation during, or immediately following racing This is in agreement with a previous study in racing Thoroughbreds documenting significantly higher serum cTnT concentrations in horses sampled 2–6 h post-race (Shields et al., 2017).

A recent kinetic study utilising a high-sensitivity cTnI assay in Standardbred racehorses found peak plasma cTnI concentrations

also occur 2–6 h post-exercise (Rossi et al., 2019). These results indicate that cTnT and cTnI may have similar release kinetics post-exercise in horses, in agreement with a previous study in humans comparing cTnT and cTnI post-exercise (Sou et al., 2016). Further, post-exercise release of both cTnT and cTnI returned to pre-race concentrations within 12–24 h in healthy horses (Shields et al., 2017; Rossi et al., 2019). Although the current study did not include a sufficient number of horses to perform statistical analysis for the 24 h samples, median serum cTnT concentration was not different to the median pre-race serum cTnT concentration. Together, this may suggest that post-race increases in serum cTn may be expected to be short-lived in the majority of horses, although individual variations may occur. Post-exercise physiological cTn release in humans typically peak within 2−5 h (Gresslien and Agewall, 2016) post-exercise and return to baseline within 24 h and moderate release of cTn may be seen as early as 30 min into sustained endurance exercise (Donnellan and Phelan, 2018). In acute coronary syndrome on the other hand, an initial rapid increase in serum cTn is followed by a delayed and sustained release of cTn occurring 12−24 h (Sharma et al., 2004; Shave et al., 2010; Rossi et al., 2014) and typically remain elevated for at least 4–7 days(Donnellan and Phelan, 2018).

It is currently unknown whether exercise–induced cTn release reflects reversible or irreversible changes in the cardiac myocytes (Wu, 2017). However, the rapid clearance of Tn post-exercise suggests a mechanism unrelated to myocyte injury or necrosis (Shave et al., 2010) being a normal physiological response (Sabatine et al., 2009; Weippert et al., 2016). During exercise, catecholamine release may induce transient myocardial ischemia, while increased heart rates and blood volume may cause mechanical stretching of the cardiac myocyte and dehydration and acid-base imbalances may cause cell damage (Weippert et al., 2016) resulting in a short-lived elevated circulating cTn concentrations.

The post-race serum cTnT concentrations in the current study were significantly higher in females compared to males. The population of horses in the previous study of cTnT (Shields et al., 2017) comprised geldings only and therefore no comparisons were made between the different sexes. However, in human athletes, both age (Tian et al., 2012) and sex (Kong et al., 2017) influence post-exercise cTnT concentrations; male runners having larger increases in cTnT concentrations following exercise compared to females. The reason for the difference in exercise-induced cTnT release between sex has not been established, but differences in hormones and cardiac muscle mass has been suggested (Kong et al., 2017). Confounders such as race-distance and track surface were suspected but only weakly changed (reduced) the effect of female. Whether the difference in post-race serum cTnT concentrations between females and males in the current study was caused by sex-related differences in response to exercise or due to some other unidentified exercise-related confounding factor, is not known.

In the current study, there was no correlation between serum cTnT concentration and age, in agreement with previously reported cTnI values in the horse (Slack et al., 2012). However, the horses participating in Standardbred races are typically within a narrow age bracket, and to investigate whether there is a correlation between age and cTnT concentrations a study including a wider range of ages is needed. This could be a challenge given the natural wastage of horses in training as they get older and also the upper age limit set by most racing jurisdiction for welfare reasons.

In the current study, no significant difference in serum cTnT concentrations was found between the two race-distances tested (1609 and 2100 m). Both distances involve short bursts of sprint exercise typically lasting 80–140 s. There is conflicting evidence in humans regarding which type of exercise provokes the largest increase in cTn and most studies have been performed after long distance running such as half or full marathons (Vilela et al., 2014). As racehorses typically exercise at maximum capacity for only a few minutes, direct comparison to humans may not be applicable (Slack et al., 2012).

One horse with a markedly elevated 24 h post-race serum cTnT concentration of 75 ng/L nevertheless performed according to the trainer's expectations. The owner declined further diagnostic work up and it is not possible to rule out underlying cardiac pathology causing the increase in serum cTnT 24 h post-race. However, the horse subsequently won a race only 7 days later making this less likely. Prolonged increased concentrations of cTnT in humans are generally attributable to irreversible myocardial damage, particularly ischemic myocardial injury (Wu, 2017). However, human patients with myocardial ischemia had significantly higher concentrations of blood cardiac troponins both before and after exercise compared to patients without ischemia (Sou et al., 2016) and it is recommended that sequential blood samples are taken to fully elucidate underlying causes of increases in cTnT in response to exercise as a sustained elevated cTnT concentrations may be expected in cardiac patients (Shave et al., 2010; Donnellan and Phelan, 2018; Thygesen et al., 2018). The above-mentioned horse had a pre-race cTnT below LoD of 5.0 ng/L and below the published recommended cut-off level of 6.6 ng/L (Van Der Vekens et al., 2015b) proposed to differentiate primary myocardial disease from normal myocardium in the horse (Van Der Vekens et al., 2015b). However, this cut-off value is within the reference range described in the current study and may not be appropriate for the current population. Further, using this cut-off value, 14 horses in the current population would have been classified as having primary myocardial disease. The horses included in the original study by Van der Vekens et al. (2015b), were not in active race-training, and factors known to increase cTnT concentrations in humans, such as fitness levels and dehydration, were not evaluated and may have influenced the results. Importantly, 21 of the 23 horses defined as primary myocardial disease had increased CK and suspect atypical myopathy (Van Der Vekens et al., 2015b). Although cTnT reportedly is cardiac specific in mammals (Katrukha, 2013), cross-reactivity to skeletal troponin T (sTnT) has been reported with first generation cTnT assays but use of more specific antibodies in the new generations cTnT-hs assays should limit this effect. However, in humans with nonspecific muscle pathology, damaged skeletal muscle expressed cTnT using the detection antibodies in the hs-cTnT assay (Jaffe et al., 2011). The authors therefore concluded that increased cTnT values in non-suspect cardiac cases should be interpreted with caution especially when occurring simultaneously with skeletal muscle pathology. It is possible that underlying skeletal muscle pathology may have interfered with interpretation in the referenced study above (Van Der Vekens et al., 2015b).

The current study was limited by the number of animals included in the study. According to recommended guidelines for establishing RIs (CLSI (Clinical and Laboratories Standards Institute), 2010; Friedrichs et al., 2012), sampling of more than 120 individuals is recommended. While the majority of horses in the current study (89/108) had serum cTnT pre-race concentrations below the LoQ of the assay (5.0 ng/L), four horses had serum cTnT concentrations above 20 ng/L (32.0, 31.0, 26.0 and 22.0 ng/L respectively) and were initially regarded as outliers However, as there were no obvious reasons for the high serum cTnT concentrations other than probable random variation in this field study, they were included in the final analysis following previously published guidelines (Geffre et al., 2011). This resulted in skewed data and a degree of uncertainty around the upper reference limit. The upper reference limit for the

serum cTnT concentrations therefore needs to be interpreted with caution. A complete screening for underlying cardiac disease or other health concerns were not within the scope of this study and it is possible that individuals with subclinical disease may have been included. Physiological factors such as fitness levels and dehydration status influence cTnT concentrations (Shave et al., 2010) and may also have influenced the results in the current investigation. A larger study and with a more extensive pre-race screening may help eliminate such animals and help firmly establish the serum cTnT upper reference limits.

Being a field study, it was not possible to standardise the timing of post-race blood sampling since it was often not possible to persuade owners/trainer to stay behind after racing had finished. However, the actual timing in minutes did not significantly affect post-race serum cTnT concentrations within the maximum predefined interval of 2 h, but this may have been too short to detect peak serum cTnT concentrations according to human studies (Donnellan and Phelan, 2018). Further studies are necessary to determine the kinetics of serum cTnT release and elimination post-exercise in different populations of horses.

Different cut-off values for reporting minimum cTnT concentrations are applied by different laboratories and studies in horses (Van Der Vekens et al., 2015a, b; Shields et al., 2017). The LoQ denotes the concentration above which quantitative results may be obtained with a specific degree of confidence and should be higher than the lower limit of detection (LoD; CLSI (Clinical and Laboratories Standards Institute), 2010; Shields et al., 2016). In the current study, a LoQ of 5.0 ng/L was applied based on the manufacturer's standards on human samples, while the LoQ of 3.0 ng/L reported by Shields et al. (2016) was validated in horses. In the validation study, 43.2 and 11.2% of non-competitive and competitive horses respectively had cTnT concentrations below the LoQ of 3.0 ng/L (Shields et al., 2016). This implies that many horses have a cTnT concentration that is below the LoQ of this assay of 3.0 ng/L since lower concentrations could not be reported due to manufacturer's defined limits of the assay. In the current study, 89 horses (82%) had serum cTnT concentrations below the LoQ of 5.0 ng/L pre-race and a true lower reference limit cannot be determined. In addition, use of anti-coagulants in plasma may result in lower concentrations of cTn measured while incomplete coagulation and free fibrin in serum may non-specifically bind assay-antibodies and falsely elevate cTn measured (Rossi et al., 2014). Correct sample collection and handling are therefore paramount.

Conclusions

The current study has contributed towards establishing an upper reference limit for serum cTnT concentrations in a population of race-fit Standardbreds and evaluated factors that may influence these. The findings may provide clinical guidelines when collecting and interpreting serum cTnT concentrations in horses.

Conflict of interest

None.

Acknowledgements

The authors would like to acknowledge Laila Fure for assistance with the laboratory analysis. Preliminary results were presented as an Abstract at the 11th International Congress of European College of Equine Internal Medicine Congress, Ghent 7-10 November 2018.

References

Begg, L.M., Hoffmann, K.L., Begg, A.P., 2006. Serum and plasma cardiac troponin I concentrations in clinically normal Thoroughbreds in training in Australia. Australian Veterinary Journal 84, 336–337.
Boden, L.A., Anderson, G.A., Charles, J.A., Morgan, K.L., Morton, J.M., Parkin, T.D., Slocombe, R.F., Clarke, A.F., 2006. Risk of fatality and causes of death of Thoroughbred horses associated with racing in Victoria, Australia: 1989-2004. Equine Veterinary Journal 38, 312–318.
Brown, C.M., Kaneene, J.B., Taylor, R.F., 1988. Sudden and unexpected death in horses and ponies: an analysis of 200 cases. Equine Veterinary Journal 20, 99–103.
CLSI (Clinical and Laboratories Standards Institute), 2010. Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-third Edition, 3rd ed Wayne, PA, USA.
Donnellan, E., Phelan, D., 2018. Biomarkers of cardiac stress and injury in athletes: what do they mean? Current Heart Failure Reports 15, 116–122.
Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovits, B., Walton, R.M., Barnhart, K.F., Blanco-Chavez, J., 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Veterinary Clinical Pathology 41, 441–453.
Geffre, A., Concordet, D., Braun, J.P., Trumel, C., 2011. Reference value advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Veterinary Clinical Pathology 40, 107–112.
Gresslien, T., Agewall, S., 2016. Troponin and exercise. International Journal of Cardiology 221, 609–621.
Jaffe, A.S., Vasile, V.C., Milone, M., Saenger, A.K., Olson, K.N., Apple, F.S., 2011. Diseased skeletal muscle: a noncardiac source of increased circulating concentrations of cardiac troponin T. Journal of the American College of Cardiology 58, 1819–1824.
Katrukha, I.A., 2013. Human cardiac troponin complex. Structure and functions. Biochemistry (Mosc) 78, 1447–1465.
Klinkenberg, L.J., Luyten, P., van der Linden, N., Urgel, K., Snijders, D.P., Knackstedt, C., Dennert, R., Kietselaer, B.L., Mingels, A.M., Cardinaels, E.P., et al., 2016. Cardiac troponin T and I release after a 30-km run. American Journal of Cardiology 118, 281–287.
Kong, Z., Nie, J., Lin, H., George, K., Zhao, G., Zhang, H., Tong, T.K., Shi, Q., 2017. Sex differences in release of cardiac troponin T after endurance exercise. Biomarkers: Biochemical Indicators of Exposure, Response, and Susceptibility to Chemicals 22, 345–350.
Lyle, C.H., Uzal, F.A., McGorum, B.C., Aida, H., Blissitt, K.J., Case, J.T., Charles, J.T., Gardner, I., Horadagoda, N., Kusano, K., et al., 2011. Sudden death in racing Thoroughbred horses: an international multicentre study of post mortem findings. Equine Veterinary Journal 43, 324–331.
Nostell, K., Haggstrom, J., 2008. Resting concentrations of cardiac troponin I in fit horses and effect of racing. Journal of Veterinary Cardiology 10, 105–109.
Ozarda, Y., 2016. Reference intervals: current status, recent developments and future considerations. Biochemia Medica 26, 5–16.
Phillips, W., Giguere, S., Franklin, R.P., Hernandez, J., Adin, D., Peloso, J.G., 2003. Cardiac troponin I in pastured and race-training Thoroughbred horses. Journal of Veterinary Internal Medicine 17, 597–599.
Reef, V.B., Bonagura, J., Buhl, R., McGurrin, M.K., Schwarzwald, C.C., van Loon, G., Young, L. E., 2014. Recommendations for management of equine athletes with cardiovascular abnormalities. Journal of Veterinary Internal Medicine 28, 749–761.
Rossi, T.M., Pyle, W.G., Maxie, M.G., Pearl, D.L., Physick-Sheard, P.W., 2014. Troponin assays in the assessment of the equine myocardium. Equine Veterinary Journal 46, 270–275.
Rossi, T.M., Kavsak, P.A., Maxie, M.G., Pearl, D.L., Pyle, W.G., Physick-Sheard, P.W., 2019. Post-exercise cardiac troponin I release and clearance in normal Standardbred racehorses. Equine Veterinary Journal 51, 97–101.
Sabatine, M.S., Morrow, D.A., de Lemos, J.A., Jarolim, P., Braunwald, E., 2009. Detection of acute changes in circulating troponin in the setting of transient stress test-induced myocardial ischaemia using an ultrasensitive assay: results from TIMI 35. European Heart Journal 30, 162–169.
Sharma, S., Jackson, P.G., Makan, J., 2004. Cardiac troponins. Journal of Clinical Pathology 57, 1025–1026.
Shave, R., George, K., Gaze, D., 2007. The influence of exercise upon cardiac biomarkers: a practical guide for clinicians and scientists. Current Medicinal Chemistry. 14, 1427–1436.
Shave, R., Baggish, A., George, K., Wood, M., Scharhag, J., Whyte, G., Gaze, D., Thompson, P.D., 2010. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. Journal of the American College of Cardiology 56, 169–176.
Shields, E., Seiden-Long, I., Massie, S., Passante, S., Leguillette, R., 2016. Analytical validation and establishment of reference intervals for a' high-sensitivity' cardiac troponin-T assay in horses. BMC Veterinary Research 12, 104.
Shields, E., Seiden-Long, I., Massie, S., Leguillette, R., 2017. 24-hour kinetics of cardiac troponin-T using a "high-sensitivity" assay in Thoroughbred chuckwagon racing geldings after race and associated clinical sampling guidelines. Journal of Veterinary Internal Medicine 32, 433–440.
Slack, J., Boston, R.C., Soma, L., Reef, V.B., 2012. Cardiac troponin I in racing standardbreds. Journal of Veterinary Internal Medicine. 26, 1202–1208.
Sou, S.M., Puelacher, C., Twerenbold, R., Wagener, M., Honegger, U., Reichlin, T., Schaerli, N., Pretre, G., Abacherli, R., Jaeger, C., et al., 2016. Direct comparison of cardiac troponin I and cardiac troponin T in the detection of exercise-induced myocardial ischemia. Clinical Biochemistry 49, 421–432.

Thygesen, K., Mair, J., Katus, H., Plebani, M., Venge, P., Collinson, P., Lindahl, B., Giannitsis, E., Hasin, Y., Galvani, M., et al., 2010. Recommendations for the use of cardiac troponin measurement in acute cardiac care. European Heart Journal 31, 2197–2204.

Thygesen, K., Alpert, J.S., Jaffe, A.S., Chaitman, B.R., Bax, J.J., Morrow, D.A., White, H. D., 2018. Fourth universal definition of myocardial infarction (2018). Circulation 138, 618–651.

Tian, Y., Nie, J., Huang, C., George, K.P., 2012. The kinetics of highly sensitive cardiac troponin T release after prolonged treadmill exercise in adolescent and adult athletes. Journal of Applied Physiology 113, 418–425.

Van Der Vekens, N., Decloedt, A., Sys, S., Ven, S., De Clercq, D., van Loon, G., 2015a. Evaluation of assays for troponin I in healthy horses and horses with cardiac disease. Veterinary Journal 203, 97–102.

Van Der Vekens, N., Decloedt, A., Ven, S., De Clercq, D., van Loon, G., 2015b. Cardiac troponin I as compared to troponin T for the detection of myocardial damage in horses. Journal of Veterinary Internal Medicine 29, 348–354.

Vilela, E.M., Bastos, J.C., Rodrigues, R.P., Nunes, J.P., 2014. High-sensitivity troponin after running–a systematic review. Netherlands Journal of Medicine 72, 5–9.

Weippert, M., Divchev, D., Schmidt, P., Gettel, H., Neugebauer, A., Behrens, K., Wolfarth, B., Braumann, K.M., Nienaber, C.A., 2016. Cardiac troponin T and echocardiographic dimensions after repeated sprint vs. moderate intensity continuous exercise in healthy young males. Scientific Reports 6, 24614.

Wu, A.H.B., 2017. Release of cardiac troponin from healthy and damaged myocardium. Frontiers in Laboratory Medicine 1, 144–150.