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Cardiac Troponin I in Racing Standardbreds

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Background: Upper reference limits for cTnI have not been established for healthy Standardbred racehorses.

Objectives: To establish cTnI upper reference limits for Standardbred racehorses and determine if increases in plasma cTnI concentration can be detected in 1–2 hours after a race.

Animals: Samples were obtained from 586 apparently healthy Standardbreds aged 2–14 years before racing and from the winners of 144 races 1–2 hours after the end of the race.

Methods: Prospective, observational study; convenience sampling; assay validation; and reference limits determinations were performed according to ASCVP guidelines. Plasma cardiac troponin I concentrations before racing were determined, potential outliers identified, and the 95th and 99th percentile upper reference limits calculated using nonparametric methods. The correlation between cTnI concentration and age, differences in median cTnI concentrations by subgroups and differences between cTnI concentrations before and after racing in winning horses were determined.

Results: The 95th and 99th percentile upper reference limits for all horses excluding outliers were < 0.04ng/mL and 0.06 ng/mL. There were no significant differences in cTnI concentrations based on age ($P = .06$), sex ($P = .35$), gait ($P = .55$), or race classification ($P = .65$) and a weak correlation of cTnI with age ($\rho = 0.09$, $P = .03$). There were no significant differences between cTnI concentrations before and after racing in winning horses ($P = .70$).

Conclusions and Clinical Importance: Because of lack of standardization across cTnI assays, the reference limits apply only to the Stratus CS immunoassay. Future studies looking at the effects of high intensity, short duration exercise on cTnI should consider sampling more than 2 hours after racing or using an ultrasensitive assay.

Key words: Heterophilic antibody; Horse-anti-mouse antibodies; Outlier detection; Precision.

There is increasing interest and concern in horse racing and veterinary communities regarding cardiac disease in racehorses and other equine athletes, especially as a potential cause of collapse or sudden death.[1] Sudden cardiac death in Thoroughbred racehorses is often a diagnosis of exclusion based on the absence of other lesions at post mortem, and might be suspected in cases of acute pulmonary hemorrhage and edema without definitive cardiac pathology.[1–3] In the absence of structural cardiac disease, arrhythmia is the presumed mechanism of acute cardiac failure and death. Several predisposing factors for cardiac arrhythmia in racehorses have been suggested, including subclinical myocardial injury. Cardiac troponin I is a sensitive and specific marker of myocardial injury in numerous mammalian species including horses[4,5] and concentrations can be readily determined in serum and plasma. This ease of determination makes cTnI analysis a potentially useful screening tool for the detection of myocardial injury. The gene sequence of equine cTnI has been shown to be highly homologous to human cTnI, suggesting that monoclonal antibody-based cTnI assays are likely to detect equine cTnI.[6] However, to the authors' knowledge, rigorous cTnI assay validation according to the American Society of Veterinary Clinical Pathology (ASVCP) quality control principles[7] has not been performed for equine samples and upper reference limit determinations have not been made for a large population of racing Standardbreds.

Abbreviations:

ALP	alkaline phosphatase
ASVCP	American Society of Veterinary Clinical Pathology
CLSI	Clinical and Laboratory Standards Institute
cTnI	cardiac troponin I
CV	coefficient of variation
LoB	limit of blank
LoD	limit of detection

Release of cTnI during and after exercise has been described in apparently healthy human athletes,[8–10] sled-dogs,[11] and a small number of equine athletes after racing or race training,[12–14] endurance rides,[15] or treadmill exercise.[16] Comparison of these studies is made difficult by the variation in sampling time-points after exercise, the marked differences in duration and intensity of exercise, and the relatively small number of individuals evaluated. It also remains unclear from these studies whether cTnI is released secondary to physiologic stress, pathologic mechanisms, or a combination of factors. At this time, the literature does not provide clear information as to the best sampling time-points or the subgroup of athletes most likely to have cTnI elevations.

The specific objectives of this study were to establish reference intervals for a large population of healthy racing Standardbreds according to ASVCP guidelines[17] using the Stratus CS immunoassay,[a] determine the effects of age, sex, gait, and race classification on cTnI concentrations, and to determine if increased plasma cTnI concentrations could be detected in winning horses 1–2 hours after racing.

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Materials and Methods

Assay Validation

The performance characteristics of the Stratus CS cTnI immunoassay[a] were determined for equine heparin plasma samples according to the ASVCP veterinary-specific quality control guidelines.[7] Quality control measures included daily system checks and calibration[b,c] of the equipment according to manufacturer's recommendations. Before specific method validation experiments, a stability study was performed to determine the effects of freezing on cTnI concentrations. Fresh plasma samples with different cTnI concentrations (0.03, 0.87, 1.01, and 4.98 ng/mL) were separated into 2 aliquots. The 1st aliquot was tested in duplicate, whereas the 2nd aliquot was frozen for 4 months at −70°C. The 2nd aliquot was then thawed at room temperature for 30 minutes and analyzed in duplicate. In addition, 3 plasma samples of known cTnI concentrations (0.87, 3.14, and 25.9 ng/mL) that had been frozen at −70°C for 3 years were thawed at room temperature for 30 minutes and analyzed in duplicate. Average percent differences between freeze-thawed and fresh samples were calculated.

Linearity was determined by analyzing a series of 7 samples of known cTnI concentrations (range 0–26 ng/mL) in triplicate and using simple linear regression.[18] Precision studies were performed using 4 plasma cTnI concentrations (0.02, 0.04, 0.10 and 1.00 ng/mL). Within-day precision was determined by analyzing each concentration 20 times in a single day. Between-day precision was determined by analyzing each concentration in duplicate once daily for 20 days. Imprecision was then calculated as the coefficient of variation (CV).[7] Functional sensitivity was defined as the concentration at which there was a 20% CV.[7] The limit of blank (LoB) was estimated by calculation of the mean and standard deviation of 20 replicates of a zero calibrator comprised of pooled cTnI-negative equine plasma.[7] The limit of detection (LoD) was estimated by analyzing 20 replicates of an equine plasma sample with low cTnI concentration (0.02 ng/mL).[7]

A recovery experiment was performed using cTnI-negative equine control plasma spiked with standard calibrator solutions (human serum origin).[b] Duplicate measurements of the control sample and each of 5 different spiked samples were made. Average percent recovery and proportional systematic error were then calculated.[19] Interference by hemolysis was determined by rapidly drawing cTnI-negative whole blood back and forth 10 times between sample tube and syringe through a 20 gauge needle,[20] creating plasma that was markedly hemolyzed based on visual assessment. The hemolyzed plasma was then added to 5 equine plasma samples of varying cTnI concentrations (0.03, 0.06, 0.27, and 0.57 ng/mL) to create samples that were either mildly or moderately hemolyzed based on visual assessment. The samples were measured in duplicate and the average difference between spiked and unspiked sample calculated.[19]

Sampling Methods

Reference individuals and race winners were selected from a population of Standardbreds racing at a single 5 eighth-mile race track over a 12 week period. During this time period, all horses entered to race underwent a soundness examination before racing, which included brief electrocardiographic examination[d] (median recording duration of 44 seconds; range 30–80 seconds) and lameness evaluation on the day of the race. All horses also underwent brief electrocardiographic examination (median duration of 48 seconds; range 30–85 seconds) between 10 and 30 minutes after racing. No other cardiac examination was performed and horses were presumed to be in general good health.

Sampling methods were convenience based. Blood collection for cTnI analysis was performed before racing in conjunction with venous blood gas sampling[e] already in place as part of the racing integrity program. The specific days of sampling were chosen for reasons of convenience, whereas individual horses in each race were selected for blood sampling by roll of the dice. These samples were obtained before clearing the horse to warm-up, approximately 2 hours before racing. Samples were obtained after racing from the winners of 144 consecutive races, 1–2 hours after completion of the race. Horses were excluded from analysis if they were scratched from racing, were detained after a race because of performance problems, did not complete their race, or had cardiac arrhythmias detected using ECG before or after racing.

All samples were obtained by jugular venipuncture (needle and syringe) and transferred to lithium heparin vacutainer tubes[f] for further processing. The heparinized blood samples were centrifuged at 2000 × *g* for 10 minutes, the plasma separated and split into at least 2 aliquots, then frozen within 60 minutes of collection and stored at −70°C until analyzed. Cardiac troponin analysis was performed within 4 months of blood collection using the Stratus CS 2-site fluorometric immunoassay.[a] The monoclonal and capture antibodies used in this assay have previously been shown to be highly sensitive and specific for equine cardiac troponin I.[5] Visibly hemolyzed or icteric samples were excluded from analysis. This study was approved by and performed in cooperation with the Pennsylvania Harness Racing Commission at Chester Downs and the Meadows Standardbred Horseman's Association. Information pertaining to age, sex, gait, and race classification was obtained from the United States Trotting Association website.

Statistical Methods

Outlier detection and upper reference limit determination were performed according to ASVCP guidelines,[17] which are based on CLSI guidelines.[21] Commercially available software was used for all calculations.[g,h] Distribution of the data was determined by visual inspection of the histogram. Normality of the data was rejected based on D'Agostino-Pearson testing ($P < .0001$). Transformation of the data could not be accomplished because of the large number of zeros in the data set. Visual inspection of the histogram and the authors' clinical experience were used to identify potential outliers. The records of the potential outliers were reviewed and plasma samples underwent recentrifugation and reanalysis, split sample analysis, and determination of alkaline phosphatase (ALP) concentrations.[i] The highest cTnI sample was screened for heterophilic antibodies using a commercial blocking system.[j]

After removal of outliers, nonparametric methods were used for determination of the 95th and 99th percentiles and 90% confidence intervals for all horses and by sex, gait, and race classification.[17,21] For statistical purposes all colts, stallions, and ridgelings were grouped as males and all fillies and mares were grouped as females. Open handicap races, stakes races, and elimination races were grouped into a single category and compared to claiming and condition races. The possible correlation between cTnI concentration and age was investigated using Spearman rank correlation. Median cTnI concentrations were compared among subgroups using the Kruskal-Wallis equality of populations rank test. The need for partitioning based on sex or gait was determined by visual inspection of each subgroup's upper reference limit, and 90% confidence intervals together with consideration of the assay's precision profile. A Wilcoxin signed-rank test was performed to determine if there was a statistically significant difference between cTnI concentrations before and after

racing in winning horses with paired samples. Significance was assigned to P values $\leq$ .05.

Results

Assay Validation

The stability study revealed the average percent difference between fresh samples and those frozen for 4 months to be 0.8% (range 0–1.4%) and between fresh and those frozen 3 years to be 1.4% (range 0.9–2.1%). The assay was linear over the range of tested concentrations [$y = 0.998 (\pm 0.001)x + 0.002(\pm 0.009)$]. The within-day repeatability study demonstrated imprecision as 6.4 % at a cTnI concentration of 1.00 ng/mL, 9.0% at 0.10 ng/mL, 20% at 0.04 ng/mL, and 63% at 0.02 ng/mL. The 20-day precision profile demonstrated a coefficient of variation in 10% at 0.10 ng/mL, and a coefficient of variation in 20% at 0.04 ng/mL (Fig 1) compared with the manufacturer's reported values of 0.06 ng/mL and 0.03 ng/mL, respectively.[22] The LoB was 0.01 ng/mL and the LoD was 0.02 ng/mL. The average percent recovery was 95% (proportional systematic error of 5%). Mild visible hemolysis resulted in an average interference of 0.01 ng/mL (±0.01) and moderate visible hemolysis resulted in an average interference of 0.03 ng/mL (±0.01).

Data Distribution, Outlier Detection, and Upper Reference Limit Determination

There were 754 prerace samples from 586 horses meeting inclusion criteria available for cTnI analysis. Only cTnI concentrations from the first time each horse was sampled were included in statistical analyses, leaving 586 individual horses with a median age of 5 years (range 2–14 years). The remaining demographic data are shown in Table 1.

Distribution of the cTnI data obtained before racing is shown in Figure 2. Five horses with cTnI concentrations between 0.08 and 0.17 ng/mL were identified as potential outliers. These potential outliers were 4 (n = 3), 6 (n = 1), and 8 (n = 1) years of age and comprised 4 pacers and 1 trotter, (3 geldings and 2 mares). Repeat analysis of the same sample and split samples from the 5 potential outliers showed no change in cTnI concentration. The mean ALP concentration for outliers was 115 U/L (range 88–137; reference range 109–315 U/L). Use of a heterophile blocking tube for the highest reference value (0.17 ng/mL) resulted in a cTnI concentration of 0.05 ng/mL.

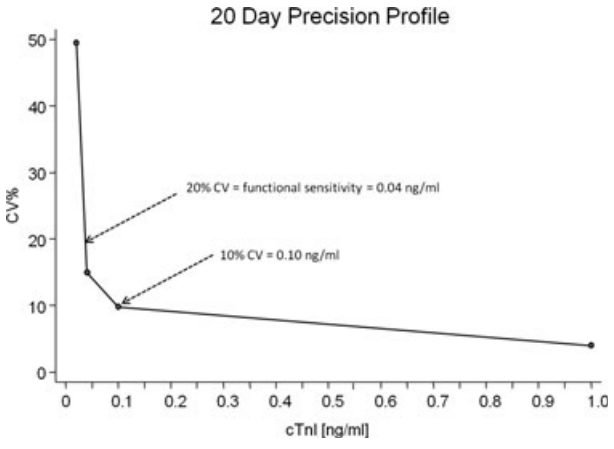

Fig 1. Stratus CS 20-day precision profile for equine heparin plasma samples. Note the marked increase in imprecision at low cTnI concentrations.

The upper limits of the 95th and 99th percentile for each cohort excluding the single confirmed outlier are shown in Table 1. There were no statistically significant differences in median cTnI concentrations based on age (P = .06), sex (P = .35), gait (P = .55) or race classification (P = .65). There was a weak, positive correlation between age and cTnI concentration (ρ = 0.09, P = .03).

cTnI Concentrations from Winning Horses after Racing

One hundred and forty-four samples after racing were available for cTnI analysis. Eleven horses were sampled twice each, leaving 133 individual horse samples. Forty-four of these were sampled both before and after racing. Median age was 4 years (range 2–14 years). Horses comprised 20 intact males, 45 females, and 68 geldings, and there were 107 pacers and 26 trotters. Samples were obtained from the winners of 38 claiming races, 63 condition races, 7 open handicap races, 5 elimination races, and 20 stakes races. All cTnI samples were below the analytical sensitivity of the assay and no significant differences were found between cTnI concentrations before and after racing (P = .70).

Discussion

This study establishes the 95th and 99th percentile upper reference limits for heparin plasma cTnI in a large population of apparently healthy, fit Standardbreds using the Stratus CS immunoassay. It is important to interpret these results in light of the convenience sampling methods and the assay's performance characteristics, particularly, the 20-day precision profile. The assay used in this study demonstrated a 10% CV at 0.10 ng/mL and a 20% CV at 0.04 ng/mL for equine plasma samples. This latter value is equal to the functional sensitivity of the assay and represents the lowest limit at which information is reliably quantifiable.[7] The authors, therefore, suggest using a single upper reference limit for all cohorts as this would prevent over interpretation of a lower cTnI threshold, where the value suffers from higher analytical imprecision. Whether the 95th percentile or the 99th percentile upper reference limit should be used will depend on future studies that examine cut-offs based on clinically relevant decisions. Because of different proprietary antibodies used in various cTnI assays, absolute concentrations of cTnI are not comparable between assays

Table 1. Heparin-plasma 95th and 99th percentile upper reference limits (URLs) by sex, gait, and race classification for the Stratus CS cTnI assay. Outliers (n = 1) removed from analysis.

Category	n	Median (ng/mL)	Range (ng/mL)	95th Percentile URL (90% CI)	99th Percentile URL (90% CI)
All horses	585	0	0–0.15	0.02 (0.01, 0.02)	0.06 (0.04, 0.12)
Male	94	0	0–0.05	0.01 (0.01, 0.04)	0.05 (*)
Female	198	0	0–0.08	0.01 (0.01, 0.03)	0.08 (*)
Gelding	293	0	0–0.15	0.02 (0.01, 0.03)	0.06 (*)
Pacer	457	0	0–0.15	0.01 (0.01, 0.02)	0.06 (0.04, 0.15)
Trotter	128	0	0–0.08	0.03 (0.01, 0.05)	0.07 (*)
Claiming	180	0	0–0.06	0.02 (0.01, 0.03)	0.04 (*)
Condition	316	0	0–0.15	0.02 (0.01, 0.03)	0.08 (0.03, 0.15)
Handicap/Stakes	89	0	0–0.03	0.01 (0, 0.03)	0.03 (*)

*unable to calculate confidence intervals

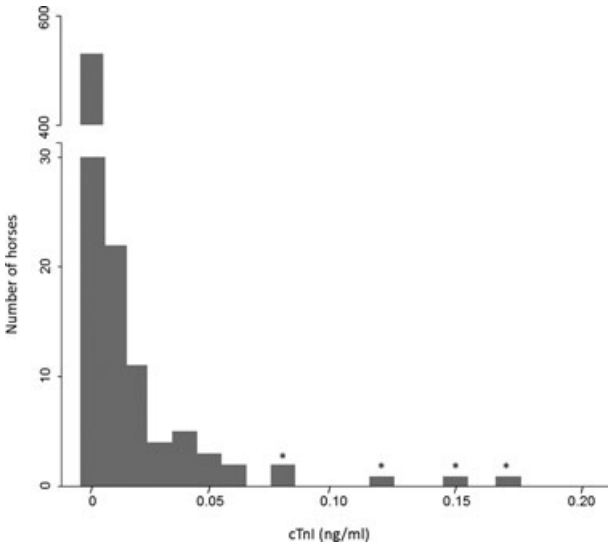

Fig 2. Distribution of heparin plasma cTnI concentrations from 586 apparently healthy, racing-fit Standardbreds. Note the non-Gaussian, right skewed data with the majority of reference values = 0.00 ng/mL. Potential outliers are marked with an * (2 with a value of 0.08 ng/mL and one each at 0.12, 0.15 and 0.17 ng/mL).

and the reference limits apply only to the Stratus CS cTnI assay used in this study.

Outlier detection is a critical part of determining whether a particular value should be excluded from the final calculation of a reference limit. Sources of outliers might include preanalytical factors, such as inclusion of unhealthy individuals, poor sample handling, and transcription errors, or analytical factors, such as fibrin clots, hemolysis, heterophile antibodies, horse-anti-mouse antibodies, and increased ALP.[23–27] Incomplete clotting of serum samples can lead to nonspecific binding of fibrin with the capture and labeling antibodies or fibrin interference with the assay's enzymatic reaction.[24] To avoid this, heparinized blood samples were obtained, carefully centrifuged, and separated. Both recentrifuging the outlier sample and split sample analysis produced results identical to the original suggesting that fibrin clots were unlikely sources of outlier data. Hemolysis was considered an unlikely interferent given the lack of visible hemolysis in the samples and the minimal interference caused by mild visible hemolysis during assay validation.

Circulating heterophilic antibodies and horse-anti-mouse antibodies have the potential to interfere with 2-site cTnI immunoassays by cross-linking the mouse monoclonal capture and labeling antibodies.[26,27] Heterophilic antibodies are produced against poorly defined antigens and are generally weak antibodies with multispecific activities. Anti-animal antibodies are produced against well-defined antigens and have strong avidities. They develop as a result of exposure to animal proteins either by iatrogenic mechanisms (vaccination or other therapeutic agents) or noniatrogenic mechanisms (transfer of dietary antigens across the gut wall). The prevalence of heterophilic or anti-mouse antibodies in the equine population remains unknown. The prevalence of human-anti-mouse antibodies in healthy individuals was 12% in one study, but estimates vary widely.[26,28] Several blocking agents capable of binding and inactivating heterophilic and human-anti-animal antibodies are commercially available. Use of a heterophilic blocking tube[f] on 1 sample in this study resulted in a decrease in cTnI concentration from 0.17 to 0.05 ng/mL, suggesting that these antibodies exist in horses and can interfere with a cTnI analysis.

Human serum ALP concentrations as little 129 U/L have been shown to cause falsely increased cTnI concentrations in an older generation cTnI assay.[25] Both the older generation assay and the current assay are fluorometric enzyme immunoassays that employ mouse monoclonal anti-cTnI antibody labeled with ALP. During the final wash phase of the assay, excess labeling antibody is washed off, substrate for the ALP is added, and fluorescence is

measured. It is possible that high endogenous ALP present in a sample may not completely wash off, leading to falsely elevated results. The plasma ALP concentrations of the 5 equine outliers were within normal limits, but 1 was within the range of ALP reported to cause interference in human samples. Given the normal ALP concentrations in healthy horses, it is likely that many of the horses in this study had ALP concentrations within the range reported to cause false elevations in human cTnI concentrations. Additional studies are needed to determine if ALP plays a role in falsely increased cTnI concentrations in equine plasma.

Given the large number of horses sampled and the racetrack setting, it was not possible to completely screen horses for cardiac disease and unhealthy individuals may have been unknowingly sampled. This is a limitation of convenience sampling. To address this, application of a mathematical outlier detection method would have been ideal. However, our data presented several problems for this approach. The distribution of the data was non-Gaussian and straightforward analysis using the most commonly applied outlier detection techniques would have been inappropriate, because these tests assume a normal distribution.[29,30] Transformation of the data was hindered by the large number of zeros in the data set, which prevent log or Box Cox transformation. Therefore, analysis of the histogram and previous clinical experience with the assay were the subjective methods used to identify potential outliers. This information was then used as the basis for the repeated sample analyses and heterophilic antibody testing. With this approach, only 1 reference value out of 5 potential values could be confirmed as an outlier. Following the guidelines of the ASVCP and CLSI, the remaining 4 potential outliers were retained in the final analyses. The fact that not all potential outliers were tested for heterophilic antibodies should be considered a limitation of the study.

Subgroup analysis was performed to determine if median cTnI concentrations were significantly different based on sex, gait, or race classification, and correlation between age and cTnI was investigated. There was a weak, positive correlation between age and cTnI concentration in our population of horses. This might reflect the relatively young age of the majority of horses in the study, and a study involving a true geriatric equine population might better elucidate differences based on age, similar to what has been reported in healthy humans and dogs.[31,32] A comparison of median cTnI concentrations by gait was performed with the consideration that trotting Standardbreds might be genetically distinct from pacing Standardbreds and therefore have different cTnI concentrations. Comparison by race classification was a crude estimate of athletic ability with the more elite or talented horses racing in open handicap, stakes, and elimination races. No differences in median cTnI concentrations were identified in either of these cohorts nor when analyzed by sex.

In addition to a comparison of medians, partitioning criteria were considered for sex and gait subgroups to determine if separate reference limits should be reported for each subgroup. Several methods have been proposed for determining the need for partitioning, most of which rely on Gaussian distribution of the data.[17] A method for partitioning of non-Gaussian data with unequal subgroup size has been proposed by Lahti et al and application of this procedure was considered.[33] However, for all horses and subgroups, the upper reference limits fall either below the functional sensitivity of the assay or within a range where precision is >10%, thus diminishing the ability to reliably distinguish different subgroups. Therefore, the authors recommend using a single upper reference limit for all cohorts.

All 133 winning horses sampled after racing had undetectable plasma cTnI concentrations 1–2 hours after racing, regardless of race classification. This might represent a true lack of myocardial cell injury in association with exercise, the inability to detect small increases in cTnI because of assay sensitivity limitations or sample collection that is too early when considering the kinetics of cTnI release from the myocardial cell. In a study of healthy Thoroughbreds, plasma cTnI elevations either above baseline or above the normal reference range were shown to occur between 3 and 6 hours after high-speed treadmill evaluation.[9] In a separate study of healthy Standardbreds, 5/22 developed small increases in plasma cTnI either 1–2 hours or 10–14 hours after racing.[11] For the latter study, the functional sensitivity was not reported for the assay, making interpretation of small changes in plasma cTnI difficult. Future studies that include sampling more than 2 hours after exercise or the use of higher sensitivity assays or both might elucidate the effects of exercise on plasma cTnI values. Later sampling was not possible in this study because of lack of housing at the racetrack, resulting in most horses leaving the premises shortly after being released from the detention area.

On the basis of a few studies in human athletes, it is probable that duration and intensity of exercise affect the timing and magnitude of cTnI release.[8–10] Racehorses represent a unique class of athlete, where exercise is performed at maximal intensity for a very short duration. Even studies describing high intensity, short duration running exercise in human athletes refer to exercise performed over 30 minutes[10] as compared with just over 2 minutes or less for Standardbred racehorses. Comparison of cTnI release in racehorses to endurance horses, sled dogs, and human marathon runners or cyclists is likely to result in conflicting information if these factors are not considered. For convenience purposes, only winning racehorses were sampled in our study. It is possible that sampling a population of nonwinners could produce evidence of myocardial injury. This hypothesis was not tested and our results show only that apparently healthy, winning Standardbred racehorses, when sampled 1–2 hours after racing, do not

have detectable increases in cTnI concentrations using the Stratus CS assay system. This information can be used for designing future studies.

Similar to previous reports in a small number of fit Thoroughbreds and Standardbreds, most Standardbreds in this study had undetectable plasma cTnI concentrations at rest.[12,13] This is most likely attributable to the limited sensitivity of the cTnI assays rather than a true absence of circulating cTnI. Studies have shown that when using a research-based ultrasensitive assay system with a lower limit of detection of 0.0002 ng/mL, plasma cTnI concentrations in healthy humans, dogs, and other laboratory species are in the range of 0.001–0.020 ng/mL.[34,35] In a recent study, using a commercially available ultrasensitive cTnI assay with a detection limit of 0.001 ng/mL, 7 healthy Standardbreds had cTnI concentrations between 0.008 and 0.028 ng/mL.[36] As ultrasensitive assays become more readily available, there will be a need to establish reference intervals and determine biological variability so that small changes in cTnI can be interpreted. Once done, these assays might help elucidate the effects of exercise on the equine myocardium.

In summary, this study establishes the 95th and 99th percentile upper reference limits for heparin plasma cTnI concentrations in a large population of apparently healthy, racing-fit Standardbreds using the Stratus CS immunoassay. We could identify no differences in cTnI concentration based on sex, gait, or race classification and cTnI concentration only weakly correlated with age. An increase in cTnI concentration could not be detected in winning horses 1–2 hours after completion of a race. Additional studies are needed to determine if marked hemolysis, heterophilic antibodies, horse-anti-mouse antibodies, increased ALP or fibrin clots cause false elevations in cTnI concentrations in this assay system. Studies looking at the effects of high intensity, short duration exercise on cTnI concentrations should consider sampling more than 2 hours after racing, consider the use of an ultrasensitive cTnI assay or both.

Footnotes

[a] Stratus CS, Siemens Diagnostic Healthcare, Newark, DE
[b] Stratus CS Acute Care cTnI Cal Pak, Siemens Diagnostic Healthcare
[c] Dimension clinical chemistry system, cardiac troponin I calibrators, Siemens Diagnostic Healthcare
[d] Vet Biolog II, QRS Diagnostic, LLC, North Plymouth, MN
[e] ABL 815M Flex, Radiometer, West Sussex, UK
[f] BD Vacutainer lithium heparin 158units/10 mL tube, BD, Franklin Lakes, NJ
[g] Medcalc version 12.0, MedCalc Software, Mariakerke, Belgium
[h] Stata 11.1, Statacorp LP, College Station, TX
[i] Vitros 350 dry slide chemistry, Ortho-Clinical Diagnostics, Rochester, NY
[j] Heterophilic Blocking Tube, Scantibodies Laboratory Inc, Santee, CA

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