Background: A “high-sensitivity” cardiac troponin-T (hscTnT) assay recently has been validated for use in horses and is a specific biomarker of myocardial damage. Postexercise release kinetics of cTnT utilizing the hscTnT assay have yet to be established in horses.

Objectives: To determine: (1) cTnT release kinetics in racing Thoroughbreds after a high-intensity 5/8th mile Chuckwagon race; (2) the effects of age on pre- and postrace cTnT concentrations; and (3) sampling guidelines for clinicians evaluating horses presenting after exercise.

Animals: Samples were obtained from 38 Thoroughbred geldings aged 5–16 years before racing and immediately, 2, 3, 4, 6, 12, and 24 hour postrace.

Methods: Prospective, observational study with convenience sampling. A fifth-generation hscTnT assay was used for plasma sample analysis, and concentrations were compared at all time-points. Correlations were determined between cTnT concentrations and age. Biochemistry analysis was performed to assess rhabdomyolysis, renal failure, and exercise-induced dehydration.

Results: All horses with measureable cTnT concentrations had significant postexercise increases in cTnT with a median peak (8.0 ng/L) at 3-hour postrace. All horses had peak postexercise cTnT concentrations 2- to 6-hour postrace ≤ the 99th percentile upper reference limit of 23.2 ng/L, after which all cTnT concentrations decreased until returning to baseline by 12–24 hours. There was no correlation over time between cTnT concentrations and age.

Conclusions and Clinical Importance: In racing Thoroughbreds completing short-duration, high-intensity Chuckwagon races, cTnT concentrations are expected to be increased 2- to 6-hour postrace and to decrease by 12-24 hours while remaining ≤3.2 ng/L throughout. This study contributes to establishing guidelines for clinical use of the hscTnT assay in exercising horses.

Key words: Cardiomyopathy; Clinical chemistry; Horse; Physiology-exercise.

Cardiomyopathies of equine athletes can be diagnosed with biochemical markers. Cardiac troponins (cTn) are highly specific biomarkers of myocardial cell damage, have been utilized widely in human and veterinary medicine, and play a role in helping to identify cardiac pathology in equids. They are central to the diagnoses of acute myocardial infarctions (AMI in humans), but increases in cTn also are apparent in conditions that result in cardiac stress in the absence of obstructive coronary disease.1–4 Meeting the stringent recommendations of the Clinical and Laboratory Standards Institute (CLSI), the National Academy of Clinical Biochemistry (NACB), and the American Society of Veterinary Clinical Pathology (ASVCP), the “high-sensitivity” TnT (hscTnT) assay recently has been validated for use in horses with total imprecision ≤10% at the 99th percentile and measurable cTn analyte concentrations obtained in >50% of the populations studied.5 Upper reference limits (URL) for the 95th and 99th population distribution percentiles have been defined for apparently healthy horses and Thoroughbred Chuckwagon racing geldings5 and plasma concentrations have been described in 2 populations of horses with cardiac damage.6 In numerous studies of healthy humans, in laboratory rodents, in sled-dogs, as well as a few studies of horses, increases in cTn concentrations have been identified after exercise.7–15 After myocardial injury in humans, loss of cell membrane integrity results in 2 phases of cTn release: a
mild-to-moderate increase within 1- to 2-hour postinjury, and a more substantial increase 4- to 6-hour postinjury which may persist for 7-14 days. We are unaware of similar postmyocardial injury cTn release kinetic studies in horses. Similarly, although available in human athletes for hscTnT and hscTnl assays, no postexercise cTnT kinetics studies have been reported in horses (although 1 previous study utilized an older generation cTnI assay). The mechanisms whereby troponins, for any reason, are liberated into the bloodstream remain incompletely characterized. Even minor increases in cTnT above threshold concentrations, however, confer worse prognosis in human patients across a wide spectrum of acute and chronic cardiac and noncardiac disease processes. Increased cTnT concentrations after exercise, therefore, can generate clinical concern, and differentiation among normal cTnT reference population concentrations, expected concentrations after a variety of exercise types, and possible pathologic concentrations warrant defining. Interpretation of troponin assay results with respect to sample timing postexercise, for example, must be also judiciously performed. The effects of age on baseline and postexercise troponin concentrations have been evaluated in humans. Another report in horses examined the effects of age on baseline and postexercise troponin-I concentrations. In horses, no such correlations among age, baseline cTnT concentrations, and cTnT release after exercise have been explored. Also, postexercise correlations between markers of skeletal muscle injury or kidney function and cTnT concentrations have not been evaluated in horses. In both the human and veterinary medical literature, there is no consensus regarding the prevalence, physiology, or clinical relevance and management of exercise-associated cTn release.

Our specific objectives were to: study the effects of short-duration, maximal-intensity Chuckwagon racing exercise on cTnT plasma concentrations in a population of healthy racing Thoroughbreds by defining postexercise cTnT release kinetics and determine the effects of age on cTnT concentrations pre- and postrace, using a high-sensitivity cTnT assay. We aimed to provide sampling guidelines for clinicians evaluating horses presenting after exercise to aid in distinguishing normal responses of the equine myocardium to high-intensity, short-duration racing exercise from potentially abnormal pathological cardiac processes.

**Materials and Methods**

**Sampling Methods**

**Population Sampled**

Sampling methods were convenience-based at the Calgary Stampede Chuckwagon races over 7 consecutive days. Racing times were recorded, and average speeds calculated over the Chuckwagon racing pattern. Plasma samples were taken from 38 healthy, fit, actively competing Thoroughbred Chuckwagon racing geldings. Horses were competing at this highest level competition for the sport and were deemed by all competitors and trainers to be in good general health, excellent physical condition, and had no known history of poor performance, or congenital or acquired cardiac abnormalities. A complete physical examination with heart auscultation was performed, and Telemetric electrocardiography (ECG) was performed stall-side and recorded for 2 minutes on all horses. Horses with abnormal physical examination findings, cardiac murmur grade ≥3/6, or any arrhythmia other than second-degree atrioventricular block were excluded from the study. All horses included in the study population had no arrhythmia of sinus origin noted. No echocardiogram was performed. In addition, all horses entered to race passed a veterinary lameness examination before competing. Any horse with baseline plasma cTnT concentrations that exceeded the 99th percentile URL using the hscTnT assay was excluded from data analysis because pre-existing myocardial damage could not be definitively ruled out.

**Blood Sampling Timing**

Plasma samples were taken when the horses were at rest in the morning before races (baseline), immediately after a 5/8th mile Chuckwagon race, then at 2, 3, 4, 6, 12, and 24 hour postrace.

**Blood Sample Handling**

Plasma samples were collected by jugular venipuncture in 5-mL lithium-heparinized tubes. Specimens were immediately centrifuged at 2000 × g for 10 minutes, separated, frozen within 90 minutes, and stored at −80°C until batch analyzed. The hscTnT assay, a fifth-generation electrochemiluminescence immunoassay, was used on the Cobas-e601 Analyzer for analysis of plasma samples, complying with manufacturer’s instructions and Calgary Laboratory Services (CLS) internal quality controls systems, and recently validated for use on equine plasma. Degree of hemolysis in all samples was assessed using the serum indices instrument application on the Roche Cobas-e601 platform and no specimen reached the hemolysis threshold for exclusion. Plasma activities of creatine kinase (CK), and aspartate aminotransferase (AST), and concentrations of creatinine, and total proteins (TP) were analyzed at a commercial laboratory. The study was approved by the University of Calgary Veterinary Sciences Animal Care Committee.

**Postexercise Release Kinetics for High-Sensitivity Cardiac Troponin-T Analyte Concentrations**

Postexercise release kinetics for cTnT were determined by assessment of the absolute values of cTnT plasma concentrations at all time-points (prerace, immediately postrace, as well as 2, 3, 4, 6, 12, and 24 hour postrace) using the hscTnT assay.

**Pre-Exercise and Selected Postexercise Measurements of CK, AST, Creatinine, and TP**

Plasma activities of CK and AST, and concentrations of creatinine and TP were measured using prerace (baseline), and 2-hour postrace blood samples. These were used to determine whether horses had pre-existing evidence of rhabdomyolysis or kidney dysfunction, and as indicators of plasma volume change after exercise.

**Determination of Effects of Age on Pre- and Postrace hscTnT Concentrations**

The possible correlation between cTnT concentration and age was determined. Prerace cTnT analyte concentrations, as well as all postrace cTnT analyte concentrations, were compared to the individuals’ ages.
Statistical Analyses

Commercially available software was used for all calculations (Microsoft Excel® and GraphPad Prism 6.0). Distribution of the data was determined by visual inspection of histograms. Normality of the cTnT concentration data was rejected based on D’Agostino-Pearson testing (P < 0.0001). The limit of detection of the hscTnT assay was 3.0 ng/L, and all horses with concentrations reported by the laboratory as <3.0 ng/L were assigned a concentration of 2.9 ng/L. Transformation of the data could not be accomplished because of the number of results below the limit of detection for the assay. Because of the severity of the right-skewed population in this instance, normalization of the data is unreliable with transformation techniques such as Box-Cox transformation. Consequently, visual inspection of the histogram was used to identify potential outliers in the populations tested. The Shapiro–Wilks test confirmed the normality of the CK, AST, creatinine, and TP distributions.

The Friedman Test was applied to the absolute value time course cTnT analyte concentration data to determine any differences in cTnT concentrations at various collection time-points. When a significant main effect was detected it was followed by Dunn’s multiple comparisons test. Paired-t tests were used to compare CK, AST, creatinine, and TP prerace to 2-hour postrace samples, and results were reported as mean ± SD. Possible correlation between baseline and postrace maximum cTnT analyte concentrations and age was investigated using Spearman rank correlation. Significance was assigned to P values ≤0.05.

Results

Average racing time over the Chuckwagon pattern was 72.7 seconds with average speeds of 13.8 m/s and peak-speeds of 18.6 m/s.

Thirty-seven apparently healthy, actively competing Thoroughbred Chuckwagon racing geldings (ages 5–16 years; median, 9 years) were included in the study. Another horse (1/37) had cTnT plasma concentrations that exceeded the 99th percentile URL previously published for the hscTnT assay5 at all but one time-point, and was removed from the study population before data analysis.

The absolute plasma cTnT analyte concentrations indicated a significant change with time (P < 0.0001; Fig 1). There were no significant differences in cTnT concentrations among prerace, immediately postrace, 12-hour postrace and 24-hour postrace samples (Fig 1). A significant difference was detected for the absolute plasma cTnT concentrations between the prerace, immediately postrace samples, 12, and 24 hour samples versus the 2, 3, 4, and 6 hour postrace concentrations, respectively (P < 0.0001 for all comparisons; Fig 1). Ten to 14 of 37 horses had a ≥ 2-fold increase in cTnT analyte concentrations between baseline and 2- to 6-hour postracing time-points (Table 1 and Fig 1). The plasma cTnT analyte median concentration (8.0 ng/L) was the highest at the 3-hour postrace time-point. The median cTnT concentration then decreased back to concentrations not different from baseline at the 12- and 24-hour postrace time-points. Only 2 horses had AST activities above the normal laboratory range for both pre- and 2-hour postrace samples. Only 1 horse had CK activities above the normal laboratory range, measured at the 2-hour postrace time-point. The maximal AST and CK activities recorded were 816 IU/L and 1112 IU/L, respectively. Plasma activity for AST and CK increased mildly (both P < 0.001) between pre- and 2-hour postrace time-points from 296 ± 130 IU/L (prerace AST) to 317 ± 137 IU/L (postrace AST) and from 133 ± 28 IU/L (prerace CK) to 277 ± 181 IU/L (postrace CK).

All horses had creatinine concentrations within the normal laboratory range with the exception of 2 horses at 2-hour postrace plasma (creatinine concentrations: 205 and 198 μmol/L, respectively). One horse had an increased plasma TP concentration prerace (72.0 g/L) and 1 horse had an increased TP concentration 2-hour postrace (71.0 g/L). Whereas plasma TP did not change (TP = 60.9 ± 5.8 g/L, prerace; TP = 60.7 ± 5.0 g/L, 2-hour postrace; P = 0.85), plasma creatinine concentration increased from 133 ± 20 μmol/L (prerace) to 153 ± 21 μmol/L (2-hour postrace; P < 0.001). No correlations were found between prerace baseline or any postrace maximal cTnT analyte concentrations and age (Fig 2).

Discussion

The goal of our study was to determine if high-intensity short-duration Chuckwagon racing exercise induces a significant increase in cTnT concentration in fit, athletic, clinically normal horses.

Postexercise cTnT analyte release kinetics were characterized by a significant increase from baseline, reaching a median peak at 3-hour postracing, and returning to baseline at 12- and 24-hour postracing. Data enabled comparison of plasma cTnT concentrations to age of the horse, and as no such correlations were found, cTnT results do not need to be interpreted with respect...
choosing a statistical test that is appropriate for your data.

To illustrate, consider a simple study where you are comparing the average heights of two groups of people, Group A and Group B. You perform a t-test to determine if there is a statistically significant difference in the average heights between the two groups.

Here are the steps you would take:

1. **State the null and alternative hypotheses**: The null hypothesis (H0) is that there is no difference in average heights between Group A and Group B. The alternative hypothesis (H1) is that there is a difference.

2. **Choose a significance level (α)**: Common choices are 0.05 or 0.01. This is the probability of rejecting the null hypothesis when it is true (Type I error).

3. **Select an appropriate statistical test**: For comparing two groups, a t-test is suitable if the data are normally distributed. If the data are not normally distributed, a non-parametric test like the Mann-Whitney U test might be more appropriate.

4. **Collect data and calculate the test statistic**: This involves measuring the heights of all individuals in Group A and Group B, then using the formula for the t-test to calculate the t-statistic.

5. **Determine the critical value or p-value**: Compare the calculated t-statistic to the critical value from the t-distribution table (for a given α and degrees of freedom) or calculate the p-value using statistical software.

6. **Make a decision**: If the calculated t-statistic is greater than the critical value (or the p-value is less than α), reject the null hypothesis. Otherwise, fail to reject the null hypothesis.

7. **Report the results**: Include the test statistic, degrees of freedom, and p-value in your report. For example, "A t-test was performed, and the calculated t-statistic was 2.53 with 18 degrees of freedom. The p-value was 0.02, which is less than the significance level of 0.05, suggesting a statistically significant difference in average heights between Group A and Group B."

By following these steps, you can ensure that your statistical analysis is rigorous and robust, leading to valid conclusions from your data.
Troponin and exercise physiology studies in humans are mostly available for endurance exercise, but more recently an increased focus on nonendurance, higher-intensity exercise can be noted. Collective research in humans examining nonendurance exercise has demonstrated significant increases in cTn concentrations after standardized treadmill running (30 minute at 85–90% VO2max), repetitive sprints (12 × 30-second sprints with set recovery periods in between), and rowing (30-minute high-intensity). In research on horses, limited work has been completed for endurance exercise, and only 1 report examined high-intensity short-duration exercise. The exact duration or intensity of exercise necessary to invoke significant changes in cTn concentrations has not been definitively determined in horses or humans. Seven research studies in horses have evaluated cTn concentrations in normal horses after nonendurance exercise. None of these studies identified significant increases in cTn concentrations partly as a consequence of the study population sizes (15, 28, 6, 24, 26, and 15 horses, respectively) but also as a result of the use of older generation, conventional sensitivity assays that have a higher LoD. We identified significant increases in cTn, using the hscTnT assay, in horses after high-intensity, short-duration racing exercise.

Although unlikely, the increased cTnT concentrations observed here may not have been solely a result of cardiac muscle release of cTn, but also possibly a result of skeletal muscle damage, impaired renal function or exercise-induced dehydration. Our study did not directly investigate the effects of rhabdomyolysis or decreased renal function on cTnT concentrations after racing exercise in horses, but it was confirmed that the study population was healthy and unaffected by rhabdomyolysis or clinically relevant renal impairment as documented by prerace and 2-hour postrace blood biochemistry analyses. Additional sampling time-points for blood biochemistry analyses may have further confirmed these findings, but the CK and AST activities obtained suggest that clinically relevant skeletal muscle damage was unlikely. Excellence cardiac tissue specificity furthermore was confirmed in a previous validation study in which the hscTnT assay reactivity to cardiac muscle was 256.2 higher than that of skeletal muscle (2.49 × 10^7 and 9.75 × 10^4 ng/g wet weight respectively). Horses with acute, exercise-induced myopathy have been reported to have similar plasma cTnI concentrations to those the subjects had prior to the onset of myopathy. Collectively, this observation suggests that skeletal muscle breakdown after Chuckwagon racing was unlikely to have caused significant increases in cTnT concentrations in our study and did not limit interpretation of the results. Similarly, the increases in cTnT detected were unlikely to have been affected by any changes in free water. Indeed, TP (as an approximation of plasma water content) did not change significantly in our study between baseline and 2-hour postrace. This was the first time-point at which a significant increase in cTnT concentration (compared to baseline) was noted. Similar conclusions recently were made regarding the increases in cTnI concentrations identified using a high-sensitivity cTnI assay in horses after endurance racing whereby horses with the highest cTnI concentrations did not appear to have more marked hematologic or biochemical changes compared with the remaining horses.

All horses with detectable concentrations (35/37) in our study had an increase in cTnT induced by the racing exercise with an average delta absolute change from prerace baseline to peak 3-hour postrace cTnT concentration of 4.3 ng/L (median, 3.6 ng/L; range, 0–17 ng/L). Not only do the results indicate significant increases in cTnT, but the magnitude of the increases also suggests a relatively uniform response to the same amount of exercise, which is consistent with findings in exercise physiology of humans whereby very myo exercise protocols similar in intensity and duration are correlated with cTn release kinetics and associated peaks.

The upper 99th percentile resting cTnT plasma analyte concentration reported for Thoroughbred Chuckwagon Racing horses, using the hscTnT assay, has been previously established to be <23.2 ng/L. In our study,
all cTnT concentrations measured were <2.32 ng/L, but 2/37 horses had peak cTnT plasma concentrations (23.0 ng/L for both horses) that closely approached the 99th percentile URL (see Table 1, Fig 1), and then decreased to baseline concentrations by 24-hour postexercise in accordance with the remainder of the study population. Departure from the normal expected cTnT kinetics after exercise may be a practical way to differentiate “normal” horses from those that: (1) have pre-existing subclinical (i.e. occult) myocardial damage, resulting in an unexpected response of the myocardium to exercise, or (2) the exercise dose itself induces myocardial damage in that particular individual. Data on cTnT kinetics in horses with myocardial damage, however, are not available, and any persistence in cTnT concentration increases postracing in horses with myocardial damage, as reported in humans after AMI, must be verified. In humans after AMI, loss of cell membrane integrity results in multiple phases of cTn release. There is indeed a relatively long half-life of cTn in serum of ≥2 hours because of persistent leakage after AMI. In contrast, because of rapid renal elimination the true half-life of both cTnT and cTnI in circulation is approximately 2 hour in humans, and only cTnI has been determined in horses to have a half-life of 0.47 hour. If ischemia or other insults to cellular homeostasis fail to induce necrosis (such as has been proposed to be the case after exercise), this short true half-life would make the cTnI kinetics of myocardial disease that is seen after AMI. In our study, cTnT concentrations returned to baseline after 12 to 24-hour thereby suggesting that racing exercise did not induce myocardial necrosis in these Chuckwagon horses.

The kinetics described above provided the basis for sampling protocol recommendations. In practical situations in which a clinician may be measuring cTnT as part of examination of a horse that has undergone high-intensity, short-duration exercise in the recent past, and pre-exercise (baseline) cTnT concentrations are unavailable, our results suggest that because postracing exercise plasma cTnT concentrations are expected to peak 3-hour postexercise and thus be measured anytime between 2- and 6-hour postexercise because no significant differences were noted among these collection times in our study. Although no horse, at any time, in the our study exceeded the URL for cTnT concentrations (i.e. 23.2 ng/L with the hscTnT assay), similar to results from exercise studies in humans, it may be normal for healthy horses to actually meet or possibly exceed the URL for cTnT concentrations at these peak time-points. Also, the cTnT concentrations decreased at, and after, 12-hour postrace for all horses that had concentrations above the LOD of the hscTnT assay. This and horses with a cTnT concentration at 12 or 24-hours postrace, that is, equal to or higher than a concentration observed between 2- and 6-hour postrace may warrant further evaluation for possible cardiac damage. Based on the above, we suggest the following repeat measure plasma cTnT ratio using the hscTnT assay (with values ≤1 representing normal horses):

\[
\frac{\text{Plasma cTnT sample taken between 12- to 24 - h postrace (ng/L)}}{\text{Plasma cTnT sample taken between 2 - to 6 - h postrace (ng/L) }} \leq 1
\]

The implication of the increased resting cTn concentrations measured in males vs. females and with increasing age, as confirmed in a study of 1540 people, is currently unknown. Individual factors such as sex and age are important to address for clinical relevance with respect to clinical decision limits (i.e. application has been corroborated in 1 study of 586 Standardbreds at rest. Age correlations also have been explored in subjects undergoing exercise challenges, and in a recent cTnT postexercise study of humans using a high-sensitivity assay, no age correlation was found between age and peak postexercise cTnT concentrations. Finally, in our study, no correlations were found between age and baseline or peak postexercise cTnT concentrations.

Limitations of our study included the lack of echocardiography, exercise or postexercise ECG, and possible inclusion of horses with nonphysiological murmurs of grade <3/6, and as such it is possible that horses with mild cardiac impairments were inadvertently included. Fitness levels of horses were not individually evaluated, and therefore, the intensity of racing exercise (e.g. as a percentage of VO2max) may have differed among individuals thereby impacting postexercise cTnT concentration kinetics. In addition, conclusions and clinician sampling guideline recommendations are based on a population of horses that underwent a specific racing dose of exercise (short-term, high-intensity), and thus extrapolation to other forms of exercise or equine activities must be made with caution. Finally, the results of our study define the response of myocardium to exercise in healthy horses, but the cTnT kinetics and associated peak concentrations in horses with myocardial disease may be different and warrant independent evaluation in future studies.

Conclusions

The adoption of cTn assays for use in veterinary medicine, coupled with concern about the possible association of underlying or exercise-induced myocardial injury and pathological exercise-associated arrhythmias, has led to numerous studies in horses exploring increases in cTn in exercising populations and after exercise. The development and validation of high-sensitivity cTn assays have allowed further assessment of the response of the equine myocardium to short-duration, high-intensity racing exercise. Our study demonstrates that (1) this exercise induces a significant increase in plasma cTnT from baseline (or 12- to 24-hour postexercise) concentrations between 2-hour and baseline; (2) cTnT
concentration peaks at 3-hour post-exercise; (3) cTnT concentration at 12 or 24 hour postexercise was lower than at 2- to 6-hour postexercise; (4) cTnT concentrations at 12 or 24 hour postexercise were not different than pre-exercise concentrations in any horse. The combination of postrace cTnT measurements using a hscTnT assay taken any time between 2- and 6-hour postrace, paired with a second plasma sample taken at 12 or 24 hours postrace to calculate the repeat measure ratio, and absolute cTnT plasma concentrations that fall under the 99th percentile URL of 23.2 ng/L by 12- to 24-hour postrace, are appropriate clinical guidelines to differentiate normal responses of the equine myocardium to high-intensity short-duration racing exercise from potentially pathological cardiac processes that may warrant further evaluation and monitoring. Future studies to determine absolute cTn concentrations during and after exercise above which myocardial damage is indicated, as well as to verify that a cTnT repeat measure ratio ≥23.2 ng/L 12 or 24 hours after racing, may be positively correlated with myocardial damage in a horse are needed. In clinical settings, to prevent misdiagnosis of cardiac pathology in otherwise healthy horses, results of cTn analyte measurements must be analyzed in light of sampling timing in relationship to exercise, and ideally interpreted in conjunction with detailed clinical history and other objective cardiac measurements such as ECG and echocardiographic findings. Recognition that exercise stimulates release of cTn will help veterinarians make informed clinical decisions about how to use cTn testing appropriately in horses after exercise.

Footnotes

a Televet-100, Engel Engineering, Offenbach, Germany
b Troponin-T hs, Roche Diagnostics, Indianapolis, IN
c Cobas-e601, Roche Diagnostics, Indianapolis, IN
d Idexx Laboratories, Beckman AU680 Chemistry Analyzer, Calgary, AB
e Microsoft Excel, Microsoft Corporation, Redmond, WA
f GraphPad Prism 6.0, GraphPad Prism Software, Inc., La Jolla, CA

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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