Acute effects of short duration, maximal exercise on cardiac troponin I in healthy horses

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Abstract
This study evaluated the effects of exercise on cardiac troponin I (cTnI) concentrations in healthy, adult horses.
Fifteen fit, healthy horses determined to have a normal cardiovascular system completed a standardized exercise test on a high-speed treadmill. Heparinized blood was collected for plasma cTnI concentrations before maximal exercise, and 1, 3, 6, 9, 12 and 24 h post-exercise. The cTnI concentrations were measured with a commercial system (Stratus CS, Dade Behring, Inc.). Results were analysed by a multivariate ANOVA, where indicated post hoc analysis was done by Tukey–Kramer HSD and significance was placed at $p < 0.05$.

All horses had elevations in cTnI concentrations after maximal exercise. Values for cTnI trended higher at 3 h ($0.066 \pm 0.011$ ng ml$^{-1}$) and 6 h ($0.062 \pm 0.011$ ng ml$^{-1}$) post-exercise compared with pre-exercise ($0.039 \pm 0.007$ ng ml$^{-1}$), although this did not reach statistical significance. Mean cTnI concentrations were within our normal reference range at all time points, although four individuals were above our normal range after exercise.

These data show that short-term, high-intensity exercise induces a small rise in plasma cTnI in normal horses. This should be kept in mind when evaluating cTnI concentrations in horses that have recently completed intense exercise. In addition, these data suggest that 3–6 h after intense exercise may be the optimal time for measurement of cTnI concentrations in horses with suspected exercise-induced myocardial damage.

Keywords: myocardial damage; equine; treadmill exercise; cardiac enzymes

Introduction
Cardiac abnormalities are reported to be an important cause of poor performance in equine athletes$^{1,2}$. Exercise-induced dysrhythmias are the most common cardiac abnormality in athletic horses and are best diagnosed using exercising electrocardiograms. However, dysrhythmias during exercise can be secondary to diseases of other body systems and it may not be easy to determine that cardiac disease is the primary cause. In addition, myocardial dysfunction occurring during exercise is difficult to document. While echocardiography is an excellent diagnostic tool to assess ventricular function, its use is limited to the immediate post-exercise period to detect exercise-induced abnormalities$^{3,4}$, and requires sophisticated equipment and expertise that are not always available. It would be advantageous to have a diagnostic test that could supplement existing tests evaluating the effects of exercise on cardiac function in horses with suspected subclinical or exercise-induced myocardial disease.

The biochemical marker CK-MB (an isoenzyme of creatine kinase found in the heart) has been used to evaluate acute myocardial damage; however, its use has been hindered by a lack of both specificity and sensitivity$^{5-8}$. Recently, cardiac troponin I (cTnI) has been recognized as a sensitive and specific marker of myocardial damage in both people and dogs$^{9-13}$. cTnI is a myocardial protein modulating the interaction between actin and myosin, thus helping to regulate the contraction of cardiac muscle. In adult
persons, it is expressed almost exclusively in the heart\textsuperscript{6}. cTnI is found in the circulation after myocardial cell necrosis, and has been shown to be more sensitive and specific than CK-MB in the detection of myocardial damage\textsuperscript{9–11}. It has been highly correlated with the development of regional areas of dysfunction and myocardial infarction\textsuperscript{13,14}. In humans, cTnI concentrations are also elevated after severe congestive heart failure\textsuperscript{15}, myocarditis\textsuperscript{16,17}, sepsis, hypovolaemia and atrial fibrillation\textsuperscript{18}. Elevations have been correlated with reductions in cardiac function after chemotherapy\textsuperscript{19} and a poorer prognosis in patients with acute chest pain\textsuperscript{20}. In dogs, cTnI has been observed to be elevated in association with primary cardiac disease, pericardial effusion, chest trauma and gastric dilatation volvulus\textsuperscript{21–24}. While myocardial infarction is uncommon in horses, cTnI concentrations are influenced by a variety of other disease states that are more commonly seen in the horse. Elevated cTnI concentrations were seen in a horse with a ruptured aortic jet lesion and ventricular tachycardia\textsuperscript{25}, and in a horse with myocardial necrosis and ventricular tachycardia\textsuperscript{26}.

CK-MB has been examined in human athletes to assess exercise-induced cardiac damage; however its usefulness is limited because of cross-reactivity with skeletal muscle creatine kinase\textsuperscript{27–30}. Because cTnI is specific for cardiac muscle, it has been evaluated in endurance athletes to assess myocardial damage secondary to extreme exertion\textsuperscript{27,31–35}. Increased concentrations have been detected in a small subset of participants\textsuperscript{27,31,34}. Some of these athletes have shown transient echocardiographic evidence of functional changes after these events; however, no consistent correlations of functional and biochemical changes have been found and the significance of these biochemical changes is not known\textsuperscript{36–39}. Evidence of cardiac damage has also been evaluated in highly trained athletes after high- and moderate-intensity exercise of shorter duration. Neither cardiac dysfunction nor increases in troponin were seen in these athletes\textsuperscript{40}.

cTnI may be useful in the detection of myocardial damage in the exercising horse; however, the effect of high-intensity exercise on cTnI concentrations in healthy horses is not known. Normal ranges for cTnI concentrations have been described in healthy adult horses at rest\textsuperscript{41}; however, to the authors’ knowledge, the acute effect of intense exercise on cTnI concentrations in healthy horses has not been examined. We undertook this study to evaluate the effect of short-duration, intense exercise on cTnI concentrations in clinically normal and fit horses on a treadmill. Our hypothesis was that high-intensity exercise would induce increases in cTnI concentrations.

Materials and methods

Animals

In order to determine the effect of intense exercise on cTnI concentrations, 15 fit, clinically normal performance horses between the ages of 2 and 10 years and trained to run on a high-speed treadmill were evaluated. All horses were actively competing in their field, with the majority being racehorses in active race training. There were ten Thoroughbreds, four Standardbreds and one Warmblood; five were females and ten males. All horses were judged to be free of cardiac disease on the basis of normal physical examinations, complete two-dimensional, M-mode and colour Doppler echocardiographic examinations (Vingmed Sound, Horten, Norway)\textsuperscript{42,43}, and resting and exercising electrocardiograms (ECGs). This study was approved by the University Institutional Animal Care and Use Committee.

Experimental protocol

After a complete physical and cardiac examination, complete echocardiographic examination and resting ECG, horses were trained to the high-speed treadmill (Classic 4000 High Speed Equine Treadmill, Walmanik International Corp., Freedom, PA, USA). They then completed the exercise test, which consisted of a warm-up, followed by exercise to fatigue for 2000–2400 m, at speeds eliciting heart rates of at least 200 beats per min for the test (8.5–16 m s\textsuperscript{-1}, 0–0.8° incline, depending on the individual horse’s ability). All horses had continuous base-apex telemetric electrocardiography (Hewlett Packard, Model M1403A, Hewlett Packard Co, Andover, MA, USA) performed before, during and after exercise had been completed, pre- and post-exercise stress echocardiograms\textsuperscript{34}, and pre- and post-exercise M-mode measurements of the left ventricle (LV) performed. The pre- and post-exercise echocardiograms were recorded on VHS tapes and digitized for later evaluation. Resting examinations consisted of standard two-dimensional and M-mode images\textsuperscript{42–44}. In addition, four views for pre-exercise stress echocardiography were performed as previously described\textsuperscript{45} (two right parasternal long-axis and two right parasternal short-axis views of the LV) and saved to software designed to calculate wall motion indices in human patients (Echopac, GE Vingmed, Horton, Norway). Post-exercise images consisted of the same four views collected for pre-exercise stress echocardiography, and the conventional right parasternal short-axis M-mode images obtained for measurements of the LV, aortic root and calculation of percentage of fractional shortening. After completion of the exercise test, selected horses had 24-h ambulatory ECG (Holter monitors) placed. These horses were
chosen based on owner agreement to leave them hospitalized overnight.

**Sample collection**

Blood samples were collected pre-exercise and 1, 3, 6, 9, 12, 24 h post-exercise for measurement of cTnI concentrations. Three millilitres of whole blood were collected into heparinized syringes by venipuncture of the jugular vein. Plasma was separated immediately and frozen at −20°C until analysis (within 1 month). cTnI concentrations were measured with a commercially available enzyme immunoassay system (Stratus CS stat fluorometric analyser; Stratus CS, Dade Behring, Bear, DE, USA) with a range of detection of 0–50 ng ml⁻¹ and an analytical sensitivity of 0.03 ng ml⁻¹. With this analytical sensitivity, concentrations <0.03 ng ml⁻¹ can be measured; however, these values are less accurate and may not be significantly different from 0 ng ml⁻¹. This system uses antibodies against human cTnI that have been evaluated for cardiac tissue reactivity in the horse, and validation of measurement of cTnI concentrations has been performed in the horse.³⁴

Continuous base–apex exercising electrocardiography was printed, as well as digitized and recorded onto a computer for later analysis (DATAQ, Akron, OH, USA). Stress echocardiography³⁴, using a commercial software program (Echopac) and M-mode measurements of the LV were completed pre-exercise and within 2–3 min after exercise had ended for each test.

**Statistics**

Results of cTnI concentrations were analysed by repeated measures ANOVA with post hoc analysis done by Tukey–Kramer HSD. For these analyses, the statistical software package Jmp 4 was used (SAS Institute, Cary, NC, USA). Statistical significance was placed at p < 0.05.

**Results**

All horses had normal physical examinations, normal resting echocardiograms and an expected increase in fractional shortening, myocardial thickening and inward wall motion of the LV on the post-exercise echocardiograms. Resting and exercising electrocardiograms were normal, and similar maximal heart rates were obtained during exercise (200–220 BPM), with all horses exhibiting an expected heart rate recovery time (HR < 100 BPM within 2 min). Horses were eliminated from the study if they had dysrhythmias, excessively elevated exercising heart rates or prolonged heart rate recovery times. The horses with Holter monitors placed had a normal cardiac rhythm in a 24-h period.

Plasma cTnI concentrations were not significantly different between any of the time points (p = 0.48) (Fig. 1). Although no significant differences were seen in cTnI concentrations by sample time, all individual horses had a trend towards an increase in cTnI concentration at both the 3- and 6-h time point, and the overall mean cTnI concentration trended higher at both the 3- and 6-h time point. Power analysis indicated that 237 samples, rather than the 103 samples taken in this study, would be necessary to detect a significant difference. The mean values at all time points were within the normal range of 0.08 ng ml⁻¹ for horses observed in our clinic (unpublished data), and within the normal reported range for Thoroughbred horses using a similar immunoassay. However, intense exercise induced increases in cTnI concentrations that were slightly above our normal range in four horses, with individual values ranging from 0 to 0.18 ng ml⁻¹ 3–6 h post-exercise in these apparently normal horses (Table 1).

**Discussion**

This study evaluated the effect of short-term, high-intensity exercise on cTnI concentrations in actively competing, clinically normal horses. The aims of the present study were to determine whether

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**Table 1** Plasma cardiac troponin I (cTnI) in normal horses before and after high-intensity exercise

<table>
<thead>
<tr>
<th>Time post-ex</th>
<th>Sample size</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>15 (1)</td>
<td>0.039</td>
<td>0.027</td>
<td>0.03</td>
<td>0–0.10</td>
</tr>
<tr>
<td>1 h</td>
<td>15 (4)</td>
<td>0.055</td>
<td>0.033</td>
<td>0.05</td>
<td>0–0.11</td>
</tr>
<tr>
<td>3 h</td>
<td>15 (4)</td>
<td>0.066</td>
<td>0.044</td>
<td>0.06</td>
<td>0–0.18</td>
</tr>
<tr>
<td>6 h</td>
<td>15 (4)</td>
<td>0.062</td>
<td>0.044</td>
<td>0.05</td>
<td>0–0.17</td>
</tr>
<tr>
<td>9 h</td>
<td>15 (4)</td>
<td>0.053</td>
<td>0.037</td>
<td>0.05</td>
<td>0–0.11</td>
</tr>
<tr>
<td>12 h</td>
<td>15 (3)</td>
<td>0.048</td>
<td>0.035</td>
<td>0.04</td>
<td>0–0.12</td>
</tr>
<tr>
<td>24 h</td>
<td>15 (3)</td>
<td>0.047</td>
<td>0.031</td>
<td>0.04</td>
<td>0–0.10</td>
</tr>
</tbody>
</table>

Abbreviations as follows: pre, pre-exercise; 1 h, 1 h post-exercise; 3 h, 3 h post-exercise; 6 h, 6 h post-exercise; 9 h, 9 h post-exercise; 12 h, 12 h post-exercise; 24 h, 24 h post-exercise. Values within parentheses below the sample size indicate the number of observations with cTnI concentrations greater than our normal upper limit of 0.08 ng/ml.
high-intensity exercise elevates cTnI concentrations in fit, athletic, clinically normal horses and to determine at approximately what time after exercise cTnI concentrations peak, in order to determine when to sample horses with suspected exercise-induced cardiac disease. In addition, we wished to confirm our established normal values in resting horses.

The ranges we obtained in this group of horses at rest were similar to that of concentrations our lab has determined to be normal (≤0.08 ng ml⁻¹) using the Stratus CS (unpublished data). This is similar to the range reported by Smith et al., using a similar analyser⁴⁵. Although only a small number of studies have reported cTnI concentrations in healthy horses, the reported ranges for normal concentrations are wide⁴⁵,4¹,4⁶. These differences are likely due to differences in analyser systems. Different analysers have not been standardized and may use different target amino acids to make antibodies. This results in values that are not directly comparable from one analyser to another¹₂,4⁷–5². The antibodies may target either the N- or C-terminus, and even if the same terminus is targeted, differences in epitope selection, monoclonal versus polyclonal antibody type, specificity of the antibody for detection of free cTnI or complexed cTnI, and whether changes in cTnI structure such as degradation or phosphorylation have occurred, can all influence detection of cTnI. Recently, a study comparing three different analysers was conducted in dogs⁵³. The results suggested that, while the correlation between the three analysers was good, measurements of agreement were poor. The fold differences, variability and limits of agreement were too large to be clinically acceptable for comparisons between those analysers, and clinically significant differences in absolute concentrations were seen.

Plasma cTnI concentrations increased slightly, but insignificantly 3 and 6 h post-exercise but, by 24 h, they had returned to pre-exercise values. Although the magnitude of the elevations was insignificant, each horse had a predictable, consistent increase between 3 and 6 h, which declined to resting concentrations by 24 h. These increases appear to be attributable to exercise, as they were consistent, followed a similar time frame for all horses, and consistently returned to baseline by 24 h. In addition, some of these horses were part of two additional studies involving cardiac catheterizations to establish pressure indices during intense exercise, and cTnI concentrations were measured as a part of those studies⁴⁴,⁵⁴. The cTnI concentrations were of similar magnitude and increased similarly post-exercise in those studies⁵⁵. However, to our knowledge, intra-individual variability has not been determined in horses and could have contributed to the differences seen over time. Although the population of horses was heterogeneous, the majority were racehorses. All were either race- or performance horses in competition and were sufficiently fit for competition in their required events. In addition, all horses were subjected to a similar treadmill exercise test, reaching what would be considered a maximal heart rate (≥200 BPM). Therefore, each horse performed a test of similar intensity for their individual ability. Although the horses may have varied slightly in degree of fitness, Phillips et al. did not see an effect of fitness on the baseline cTnI concentrations in Thoroughbred horses⁴¹.

Although the increases in cTnI concentrations were consistent, they were very small, and the means were still within our normal range for horses. However, exercise-induced increases in cTnI that were slightly outside of our normal range in some individuals. At the 1, 3, 6 and 9 h time points, four horses had cTnI concentrations over our normal upper value of 0.08 ng ml⁻¹, and the values in three of these horses remained above the upper value at 12 and 24 h post-exercise. This mild elevation in fit horses immediately post-exercise should be kept in mind when evaluating horses with suspected myocardial damage that have just performed high-intensity exercise, as values slightly higher than the normal range obtained in resting horses might be encountered in normal horses. Alternatively, these four horses may not have been entirely free of cardiac disease and may have had exercise-induced myocardial damage that was undetectable by standard diagnostic methods.

Our results suggest that maximal cTnI concentrations occur 3–6 h after high-intensity exercise has been completed. Therefore, this may be the optimal time to measure cTnI concentrations to detect exercise-induced myocardial damage in horses. The immunoassay system used to measure cTnI concentrations is highly specific for cardiac troponin, with very little cross-reactivity to skeletal muscle troponin; therefore, increases in cTnI observed in these horses are unlikely to be due to cross-reactivity with skeletal muscle troponin.¹¹,⁴⁵ In support of this, horses with acute, exercise-induced myopathy have had similar plasma cTnI concentrations to those they had prior to the onset of myopathy (Durando, unpublished data).

In humans, extreme exertion, such as occurs in marathons, ultramarathons and triathlons has been shown to increase cTnI concentrations in a subset of athletes²⁷,³¹,³⁴,⁵⁶. Along with biochemical evidence of damage, some investigators have found a smaller subset with left ventricular dysfunction on echocardiography immediately post-exercise. Others have not found any correlation between biochemical changes and decreases in cardiac function after strenuous exercise, and the relationship between cardiac dysfunction and increases in troponins is not known³⁶–³⁹. Myocardial damage in these athletes is assumed to be
transient and exercise induced, because the athletes had normal baseline echocardiograms and troponin concentrations, and those followed over several days have had a return to baseline troponin concentrations. While the mechanism is not established, it is speculated to be due to myocardial cell membrane damage and leakage of free, cytosolic cTnI not from permanent necrosis and cell death–as occurs after vascular thrombosis. Structurally bound cardiac troponin released after major myocyte injury and necrosis often results in greater elevations in circulating cTnI concentrations than has been observed following exercise. In humans and dogs, a small percentage of cTnI is not bound to thin filaments and may be released with transient injury to the myocyte, resulting in early, modest increases in cTnI.

The effect of prolonged, aerobic endurance exercise on cTnI concentrations in humans is likely not a good comparison with high-intensity, short-duration treadmill exercise used in the horses in the present study. While endurance competition may be similar to long-distance equine endurance competition, they are both very different from modern-day Thoroughbred and Standardbred horse-racing. However, cTnI concentrations have also been evaluated in short-duration, intense exercise in humans, which is more appropriate to compare with high-intensity treadmill exercise in horses. Shave et al. evaluated cTnI and cTnT in highly trained healthy athletes after maximal treadmill exercise, and found no increases in troponins post-exercise. These findings differ somewhat from our findings, which may be related to either the protocol used or inherent differences between horses and humans. In the study in human athletes, samples were taken before, immediately following and 48 h after exercise, whereas in our study we took multiple samples over 24 h. Increases in cTnI in horses did not occur until 3–6 h post-exercise, while the 1-h post-exercise samples were similar to pre-exercise. However, horses performing a maximal exercise test, in which they become hypoxemic during exercise, may also sustain greater cardiovascular stress than people performing a maximal-ramp stress test and have a correspondingly mild increase in cTnI for this reason.

cTnI concentrations were also used to examine the effects of symptom-limited exercise in patients with moderate heart disease compared with those with mild or no cardiac disease. Exercise to fatigue did not increase cTnI concentrations in healthy human subjects, whereas it can in patients with moderate cardiac disease. Whether exercise induces increases in cTnI concentrations to a greater extent in horses with cardiac disease than in healthy horses remains to be determined. Additional studies measuring cTnI concentrations after exercise in fit, actively competing performance horses—as well as in horses with cardiac disease—should be performed.

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