

Variation of pre- and post-race cardiac troponin concentrations in Thoroughbred and Arabian racehorses

Nilgün Paksoy¹, Ali Haydar Kırmızıgül², Cemalettin Ayvazoğlu³, Hamza Yalçın⁴

¹University of Harran, Faculty of Veterinary Medicine, Department of Biochemistry, Şanlıurfa, Türkiye

²University of Kafkas, Faculty of Veterinary Medicine, Department of Internal Medicine, Kars, Türkiye

³University of Ardahan, Nihat Delibalta Göle Vocational School, Ardahan, Türkiye

⁴University of Harran, Faculty of Agriculture, Department of Biostatistics, Şanlıurfa, Türkiye

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Abstract

In horses, cardiac troponins are specific and sensitive biomarkers of myocardial injury. Increased cardiac troponin T (cTnT) and cardiac troponin I (cTnI) concentrations may indicate myocardial damage, but the physiological release is also possible post-exercise or race. In this study conducted to investigate the effects of racing on cTnT and cTnI concentrations in Thoroughbred and Arabian racehorses, blood samples were collected from seven Thoroughbred stallion and eight Arabian stallion racehorses, aged between 3 and 6, before and 30 min after a 1400-metre race. The mean cTnT concentration increased from 0.007 ± 0.001 ng/ml to 0.008 ± 0.001 ng/ml in Thoroughbred racehorses and from 0.007 ± 0.002 ng/ml to 0.008 ± 0.001 ng/ml in Arabian racehorses. The mean cTnI concentration increased from 0.008 ± 0.001 ng/ml to 0.142 ± 0.005 ng/ml in Thoroughbred racehorses and from 0.008 ± 0.002 ng/ml to 0.165 ± 0.054 ng/ml in Arabian racehorses. The increases in post-race cTnT ($P < 0.05$) and cTnI ($P < 0.001$) concentrations of both breeds were statistically significant. The results of the study suggested that racing in racehorses has an effect on the myocardium and this effect was reflected as an increase in cardiac troponin. However, it would be useful to make multiple measurements at certain time intervals after the race to accurately determine whether the increase is physiological or pathological.

Cardiac biomarker, cTnT, cTnI, horse, race

The effects of exercise or racing on the cardiovascular system are among the recent research topics in equine sports medicine (Fazio et al. 2023; Mehrazin et al. 2025). In horse racing, one of the most common organizations that use horses for sporting purposes, the race performance of horses can be considered the horse's yield. In order to increase racing performance, reliable biomarker research is carried out to evaluate the racehorse's general health status, early and accurate diagnosis of possible diseases, establish treatment strategies, and determine the prognosis. Although the field of cardiac biomarkers is constantly evolving, it has not fully taken its place in the general veterinary biochemical profile (Langhorn and Willesen 2016). It has been reported that in equine myocardial diseases, diagnosis is difficult due to a deficiency of specific cardiac signs and therefore may be overlooked (Nath et al. 2012). Poor performance and sudden death are increasingly associated with heart disease in horses (Hellings et al. 2020). Echocardiography and exercise electrocardiography are widely used in equine medicine, but these techniques are unreliable in detecting subclinical heart disease (Reef et al. 2014).

Among the reliable cardiac biomarkers, cardiac troponins are considered ideal biomarkers (Mehrzin et al. 2025). Troponins are regulatory proteins and play a key role in muscle contraction. Cardiac troponins are myocardial-specific proteins. In human medicine, both cTnT and cTnI have been reported to be useful markers in diagnosing myocardial injury (Hellings et al. 2020) and highly sensitive to myocardial necrosis and reported to have

Address for correspondence:

Nilgün Paksoy
Harran University, Faculty of Veterinary Medicine
Department of Biochemistry
63200 Eyyübiye, Şanlıurfa, Türkiye

Phone: 0-414-3183000/3913
E-mail: nilgunpaksoy@harran.edu.tr
<http://actavet.vfu.cz/>

prognostic significance even with minor changes in their concentrations (Langhorn and Willesen 2016; Carretón et al. 2017). However, studies reporting that cTnT increase is also associated with exercise suggest that cardiac troponin elevate is not only pathological but might be a reflection of exercise physiology (Vilela et al. 2014; Klinkenberg et al. 2016).

Different tests and reference values are used in equine clinical medicine for cTnI (Nostell and Haggstrom 2008; Slack et al. 2012; Van Der Vekens et al. 2015a) but there is limited information about cTnT in horses (Helling et al. 2020). In the literature review, studies investigating the cTnT and cTnI values of Thoroughbred and Arabian racehorses participating in the same type of race are scarce. Therefore, this study aimed to evaluate the changes in cTnT and cTnI concentrations of Thoroughbred and Arabian racehorses pre- and post-race.

Materials and Methods

Ethical statement

The investigation was conducted with approval from the Harran University Local Animal Experiments Ethics Committee (HRU-HADYEK), under the authority of its permit number 2022/009/01.

Study design and population

For this study, seven Thoroughbred stallion (3.28 ± 0.75) and eight Arabian stallion (3.87 ± 1.12) racehorses aged between 3 and 6 years were selected. These horses were clinically healthy and actively competing stallions, registered with the Jockey Club of Türkiye in Şanlıurfa, Türkiye. The horses selected in the study underwent routine clinical examination, including the cardiovascular system. All horses, whose body temperature (37.9 ± 0.1), pulse ($42 \pm 2/\text{min}$), and respiratory rate ($21 \pm 3/\text{min}$) were recorded, and who underwent cardiac and pulmonary auscultation, were clinically healthy. The pulse and respiratory rate measured before the race may be due to the horses' increased excitement as they realise they are preparing for the race (Mukai et al. 2007). Horses included in the study did not have any clinical signs of heart disease. The race for each horse was a 1400-metre race. The race times varied between 1 min 25 s and 1 min 30 s for Thoroughbred racehorses and 1 min 38 s and 1 min 48 s for Arabian racehorses.

Blood sampling and laboratory analyses

Blood samples were collected into serum tubes (BD Vacutainer, BD, Franklin Lakes, NJ) by venepuncture of v. jugularis within 30 min before the race and 30 min after the race. Following collection and coagulation of the blood samples, tubes were centrifuged at 3,000 g for 10 min, and separated serum samples. Troponin concentrations remain stable at -20°C for up to 3 months (Langhorn and Willesen 2016). Thus, serum samples were immediately frozen and stored at -20°C until analysis. Samples were transferred to the laboratory under cold chain conditions and analyzed within 10 days.

Cardiac cTn-T and cTn-I in serum were measured using commercial horse-specific enzyme-linked immunosorbent assay (ELISA) kits (Horse Cardiac Troponin T Kit-Cat No: ELK9455, ELK Biotechnology, Denver, Colorado, USA; Horse Cardiac Troponin I Kit-Cat No: ELK9456, ELK Biotechnology) by ELISA equipment (Thermo Scientific Multiscan GO, TYPE: 1510, Waltham, Massachusetts, USA).

Statistical analyses

All analyses and graphs pertaining to the data were conducted in the open source R program (de Mendiburu 2021; Wei and Simko 2021; Harrell 2023). Variables were subjected to Shapiro-Wilk test for normal distribution and Levene test for homogeneity of variance ($P < 0.05$). Repeated measures ANOVA was performed to observe the main effects and interaction effects together. Paired *t*-test was applied to examine the difference between pre- and post-race measurements for a single breed. Furthermore, Pearson correlation analysis was conducted to investigate the relationships in conjunction with pre- and post-race measurements for each breed.

Results

Alterations in the pre- and post-race cTnT and cTnI concentrations and their statistical significance in Thoroughbred and Arabian racehorses are presented in Table 1 and Fig. 1.

It was observed that the pre- and post-race cardiac troponin values of Thoroughbred and Arabian racehorses were similar and not affected by breed differences. In addition, it was determined that cTnT ($P < 0.05$) and cTnI ($P < 0.001$) values in each breed increased significantly post race compared to before race.

Table 1. Alterations in pre- and post-race cTnT and cTnI concentrations and statistical significance of Thoroughbred and Arabian racehorses.

Cardiac troponins	Thoroughbred (n = 7)			P	Arabian (n = 8)			P	Total (n = 15)			P
	Min-Max	Mean ± SD	Mean ± SD		Min-Max	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD	
cTnT (ng/ml)	Pre-race	0.0061–0.0088	0.007 ± 0.001 ^a	*	0.0034–0.0088	0.007 ± 0.002 ^a	0.007 ± 0.001 ^a	*	0.007 ± 0.001 ^a	0.007 ± 0.001 ^a	0.007 ± 0.001 ^a	*
	Post-race	0.0067–0.0095	0.008 ± 0.001 ^b		0.0047–0.0095	0.008 ± 0.001 ^b	0.008 ± 0.001 ^b		0.008 ± 0.001 ^b	0.008 ± 0.001 ^b	0.008 ± 0.001 ^b	
cTnI (ng/ml)	Pre-race	0.0063–0.0095	0.008 ± 0.001 ^a	***	0.0034–0.0106	0.008 ± 0.002 ^a	0.007 ± 0.002 ^a	***	0.007 ± 0.002 ^a	0.007 ± 0.002 ^a	0.007 ± 0.002 ^a	***
	Post-race	0.1285–0.1666	0.142 ± 0.005 ^b		0.0955–0.2771	0.165 ± 0.054 ^b	0.154 ± 0.041 ^b		0.154 ± 0.041 ^b	0.154 ± 0.041 ^b	0.154 ± 0.041 ^b	

^{a,b} Different superscripts in the same row indicate significant differences (*: $P < 0.05$, ***: $P < 0.001$).

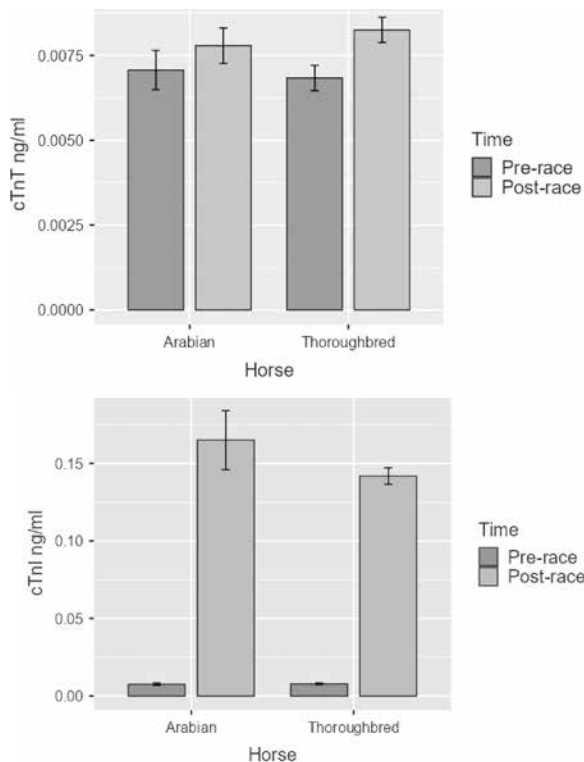


Fig. 1. Box plot of Thoroughbred and Arabian racehorses pre- and post-race cTnT and cTnI concentrations.

There was no significant correlation found between pre-race and post-race cardiac troponin concentrations in Thoroughbred racehorses. When examining the troponin concentrations of Arabian racehorses, significant correlations were found between post-race cTnT with pre-race cTnI ($r = 0.904$) and pre-race cTnT ($r = 0.929$), between pre-race cTnI with pre-race cTnT ($r = 0.897$), as well as between post-race cTnI with pre-race cTnT ($r = 0.709$) concentrations (Plate I, Fig. 2).

Discussion

The results of this study suggest that racing affects the myocardium in both Thoroughbred and Arabian racehorses. Troponins are intracellular proteins. When a cardiomyocyte is damaged or destroyed, troponins are released in circulation from the cardiomyocyte (Barison et al. 2011). Therefore, troponins circulating in the blood are biomarkers that evaluate the integrity of the myocardium, providing information about cardiac-specific injury (Carretón et al. 2017). Cardiac troponin T and cTnI have been verified to be reliable cardiac

biomarkers for the diagnosis of myocardial damage in human and equine medicine (Van Der Vekens et al. 2015b; Fazio et al. 2023). It is also stated that physical exercise can cause physiologically temporary myocardial damage and thus affect the concentration of cardiac troponins (Langhorn and Willesen 2016; Pourmohammad et al. 2020). After physical exercise, the cardiac muscle undergoes mild hypoxia which causes the sarcolemma permeability to change, leading to a loss of macromolecules. It has been reported that a short period of hypoxia can induce cTnI release without death of cell (Aengevaeren et al. 2021; Fazio et al. 2023).

Serum cTnT and cTnI concentrations in clinically healthy horses have been reported to be ≤ 0.01 ng/ml and ≤ 0.2 ng/ml, respectively (Van Der Vekens et al. 2013; Pourmohammad et al. 2020). Pre-race cardiac troponin concentrations of all horses in this study were agreed with those reported within the literature. There was a significant difference between the cardiac troponin levels in the serum pre- and post-race in both breeds. However, the statistical difference in cTnI levels ($P < 0.001$) was more noticeable and significant than cTnT levels ($P < 0.05$). The more significant increase in post-race cTnI concentrations was attributed to the slightly lower molecular weight of cTnI (24 kDa) than cTnT (37 kDa) (Missov and de Marco 1999) and the more tightly bound cTnT to the contractile apparatus (Shaw et al. 2004). This suggests that cTnI may be more sensitive and specific than cTnT in assessing the cardiac effects of exercise or race in racehorses.

Cardiac troponin T concentrations 30 min after the race were significantly higher than before the race in both Thoroughbred and Arabian racehorses ($P < 0.05$). This finding was consistent with a study by Ayvazoğlu et al. (2023) on Arabian horses. In studies conducted with various horse breeds, it has been reported that cTnT levels increase in samples taken at various time intervals after the race. In one of these studies, cTnT levels were investigated in Thoroughbred horses competing in the Chuckwagon race, and a significant difference was detected between the results obtained pre race and at the 2nd, 3rd, 4th, and 6th h post race ($P < 0.0001$) (Shields et al. 2017). In another study, it was reported that there was a significant difference between pre-race cTnT levels in Standardbred racehorses and samples taken 1–2 h after the race ($P < 0.0001$) (Hellings et al. 2020). Increased concentrations of cTnT after racing or exercise suggest that cTnT is released into the circulation. However, the significance of this increase varies between $P < 0.05$ – 0.0001 in studies where factors such as horse breeds, types of races or exercises, and sampling times differ. The existence of different rates of change in different horse populations suggests that variables such as age, sex, condition, type and duration of exercise, sampling times, and analytical factors encountered in human studies may also apply to horses (Shave et al. 2010; Rossi et al. 2014; Hellings et al. 2020).

In this study, pre-race cTnI concentrations ranged between 0.0061–0.0088 ng/ml in Thoroughbred racehorses, and between 0.0034–0.0088 ng/ml in Arabian racehorses. According to the results of the study conducted by Nostell and Haggstrom (2008), it was determined that the resting serum cTnI concentrations of 32 of 34 Thoroughbred horses were below 0.022 ng/ml. In the study carried out with Arabian racehorses, pre-race serum cTnI concentration was higher than the results of this study and was 0.130 ± 0.01 ng/ml (Ayvazoğlu et al. 2023). Post-race cTnI values were measured 30 min after the race and this mean value was determined as 0.142 ± 0.005 ng/ml for Thoroughbred racehorses and 0.165 ± 0.054 ng/ml for Arabian racehorses. A significant increase in cTnI concentrations was detected in both breeds ($P < 0.001$). Giers et al. (2023) who studied TnI concentrations in horses of various breeds, sexes, and age groups, reported that the increase in cTnI levels was non-significant in samples taken 30 min after the race. However, samples taken the next morning showed a significant increase. Another study conducted with seven Italian saddle horses in Sicily indicated that within the scope of the exercise protocol, the cTnI values of show jumping horses during rest, after exercise, in recovery periods 30 and 60 min

were 0.37 ± 0.10 , 0.55 ± 0.14 , 0.42 ± 0.10 , 0.49 ± 0.09 ng/ml, respectively. Significant difference was detected only in the after-exercise values (Fazio et al. 2023). In a study conducted with Arabian racehorses in Kars, Ardahan, and Iğdır provinces of Türkiye, measured pre- and post-exercise cTnI concentrations were 0.130 ± 0.01 and 0.169 ± 0.01 ng/ml, respectively, and the difference was reported to be significant (Ayzaoğlu et al. 2023). In another study in which cTnI levels were determined in Arabian racehorses, pre-exercise and post-exercise cTnI levels were measured in horses that were included in an exercise program that included warming up, walking, trotting, and galloping for 6 days a week. The average cTnI concentration, which was 0.139 ± 0.008 ng/ml before exercise, was recorded as 0.156 ± 0.008 ng/ml and 0.161 ± 0.008 ng/ml at the 5th and 18th h after exercise. The study reported a significant increase in cTnI values due to myocardial stress caused by exercise (Pourmohammad et al. 2020). The increase in cTnI may be due to the release of cTnI from cardiomyocytes affected by the race or an increase in cell membrane permeability caused by changes in local pH or oxygen tension during the race (Hickman et al. 2010; Baker et al. 2019; Giers et al. 2023). The fact that measurements were not made at various intervals after the race makes it difficult to determine whether this increase was due to myocardial necrosis or a physiological response. The mean $T_{1/2}$ of cTnI in horses has been reported to be 0.47 h. A half-life that does not decrease or is prolonged within ± 0.47 h after exercise or race may indicate ongoing myocardial necrosis, which may be evidence of myocardial pathology (Kraus et al. 2013). As a matter of fact, in human models, it has been reported that the increase after exercise reaches its peak within the first 4 h, but if there is myocardial necrosis, this peak occurs after approximately 8 h and the decrease occurs after a few days (Tjora et al. 2011; Baker et al. 2019). In the study conducted by Rossi et al. (2015) with five Standardbred racehorses, it was reported that cTnI concentrations reached the peak level at 2–6 h after exercise and decreased to the baseline level within 24 h. Results from this study show that horses generally have low cTnI concentrations at rest, but racing causes increases in cTnI concentrations in horses. However, the increased values pre- and post-race are lower than in other studies, likely because the horses are already running and in high condition. In this study, if blood samples had been collected more frequently after the race, it would have been possible to detect the algorithm of increases and decreases in cTnI concentrations. However, for practical reasons, this was not possible.

According to the results of this current study, the average concentrations of cardiac troponins pre- and post-races were similar in both Thoroughbred and Arabian racehorses. Moreover, both breeds had similar differences in their pre- and post-race cardiac troponin concentrations. The only variances due to racial differences were the correlations. The correlation between pre- and post-race cardiac troponin concentrations of racehorses was determined only between the data of Arabian racehorses. These findings suggest that racing may have an impact on the heart of racehorses, but this effect may not be directly related to the breed itself. Although the racehorses were of different breeds, they were of similar ages, and in good condition because they currently raced, and were competing in the same 1400-metre race.

In conclusion, it was determined that the cTnT and cTnI concentrations of Thoroughbred and Arabian racehorses that were clinically healthy and currently competing athletes in a similar age range, were affected by racing. However, to better and accurately understand whether cardiac troponin increases are pathological or physiological, comprehensive studies involving different competitions or exercises, different sexes, wider age ranges, and multiple post-race measurements of cardiac troponins and other cardiac parameters are needed.

Conflict of interest

The authors declare they have no conflicts of interest.

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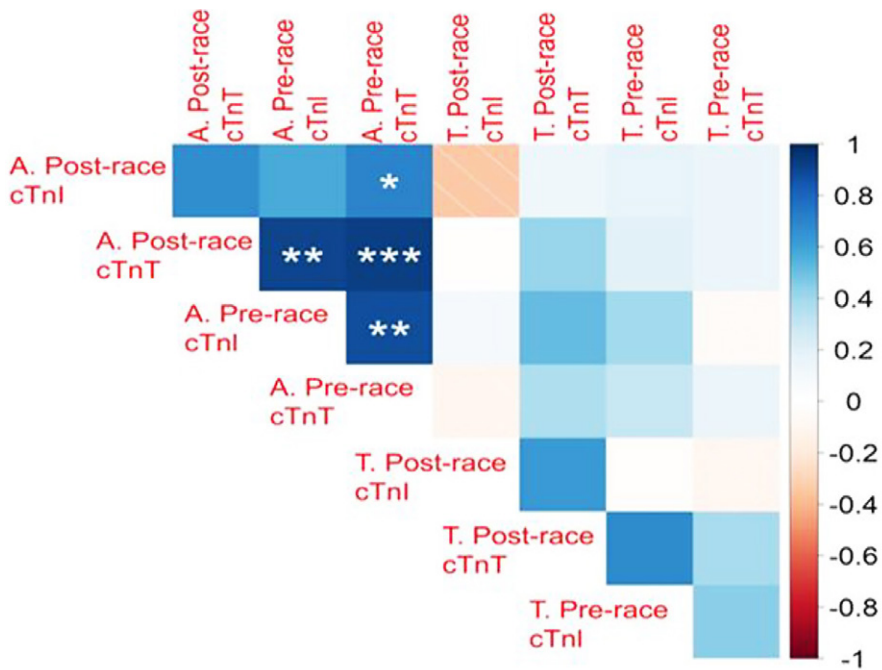


Fig. 2. Pearson correlation coefficient for the Thoroughbred (T) and Arabian (A) racehorses pre- and post-race cTnT and cTnI concentrations. *, **, *** represent $P < 0.05$, 0.01 , and 0.001 , respectively.