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# Changes in cardiac troponin I (cTnI), T (cTnT), and some biochemical parameters in Arabian racehorses after training

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## Abstract

The aim of this study was to compare the changes in the content of cTnI, cTnT and some biochemical parameters (CK–MB, LDH, AST, ALT) in Arabian racehorses before and after training. Diagnosis of myocardial disease in horses is very difficult due to the lack of specific cardiac signs. Cardiac diseases are seen as a cause of sudden death or decreased performance in horses. It has also been reported that excessive exercise may cause transient myocardial damage. In our study, 20 healthy stallion Arabian racehorses aged 4–10 y (6.00 ± 0.52 y) were used. Five millilitres of blood was collected from the *V. jugularis* of clinically healthy horses into serum tubes before and after training. Training time was determined as 30 min for each horse. Pre-training cTnI, cTnT, CK–MB, LDH, AST, and ALT contents were determined to be 0.130 ± 0.01 ng/mL, 0.007 ± 0.00 ng/mL, 231.15 ± 8.96 U/L, 692.45 ± 34.12 U/L, 309.92 ± 18.48 U/L, and 11.83 ± 0.92 U/L, respectively. After training, cTnI, cTnT, CK–MB, LDH, AST, and ALT contents were determined to be 0.169 ± 0.01 ng/mL, 0.008 ± 0.00 ng/mL, 289.80 ± 10.96 U/L, 704.25 ± 22.03 U/L, 328.47 ± 19.58 U/L, and 15.24 ± 1.03 U/L, respectively. As a result, it was determined that exercise triggered myocardial damage to some extent in Arabian horses. Exercise stimulates troponin release and the differences that may occur in troponin tests in horses after exercise should be taken into consideration.

**Key Words:** Arabian horse, cardiac disease, exercise, training, troponin
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## Introduction

Horses, which have been raised for agricultural, transportation, military, and sporting purposes throughout history, are important elements of the animal husbandry industry. Horse breeds are examined in two different groups, the warm-blooded and cold-blooded breeds. The most common warm-blooded breeds are Arabian horses (Kocaman & Fidancı, 2016). The Arabian horse is the oldest of the existing horse breeds on Earth and has played a major role in the creation of many horse breeds and has become more prominent after 1950 due to its racing abilities (Köseman & Özbeyaz, 2009; Kocaman & Fidancı, 2016).

Diagnosis of myocardial disease in horses is very difficult due to the lack of specific cardiac signs (Nath *et al.,* 2012). Cardiac diseases are seen as a cause of sudden death or decreased performance in horses (Hellings *et al.,* 2020). In addition, arrhythmias have been reported to occur in healthy horses at rest or after training in different studies (Lorello *et al.,* 2019; Massie *et al.,* 2021). Clinically, equine myocardial disease can present with non-specific symptoms such as fever,

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tachycardia, myalgia, and a reluctance to move. The conditions associated with cardiac dysfunction are mainly assessed by auscultation and echocardiography (ECG) (Nath *et al.,* 2012). However, the benefit of applying cardiac biomarkers in the clinical examination of critically-ill patients is being investigated and is promising, with a rapid increase in their use in veterinary medicine recently (Langhorn and Willesen, 2016). Various biomarkers [including cardiac troponin I (cTn-I), cardiac troponin T (cTn-T), creatine kinase–myocardial band (CK–MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST)] are used to detect cardiac dysfunction. The serum activities of these biomarkers have been reported to increase during myocardial injury (Aydoğdu *et al.,* 2016; Aygün & Yıldız, 2018; Beydilli & Gökçe, 2020; Ayvazoğlu *et al.,* 2022). In addition, cTn-I and cTn-T, which are specific for the heart, have been found to be highly sensitive to myocardial necrosis (Kırbaş *et al.,* 2021) and even small changes in their contents have been reported to have prognostic significance (Langhorn & Willesen, 2016; Carretón *et al.,* 2017).

It has been reported that excessive exercise may cause transient myocardial damage (Langhorn & Willesen, 2016). The aim of this study was to compare the changes in the contents of cTnI, cTnT, and some biochemical parameters (CK–MB, LDH, AST, ALT) in Arabian racehorses before and after training.

## Materials and Methods

Ethical approval for this study was granted by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: KAÜ-HAYDEK/2022-169).

The material of the study consisted of 20 healthy Arabian stallion racehorses between the ages of 4 and 10 years, registered to the TJK (Turkish Jockey Club) in public hands in the Kars, Ardahan, and Iğdır provinces of Turkey. The horses used in the study were subjected to a clinical examination with a particular focus on the cardiovascular system. Body temperature, pulse, and respiratory rate were recorded and cardiac and pulmonary auscultation was performed. All horses used in the study had normal clinical examinations.

Five millilitres of blood was collected from the *V. jugularis* of clinically healthy horses into serum tubes (BD Vacutainer, BD, Franklin Lakes, NJ) before and after training. Training time was determined as 30 minutes for each horse. Laboratory work was carried out in Ardahan University Central Laboratory. The blood samples were centrifuged at 3000 × *g* for 10 min and the serum was separated. Sera were stored at -20 ºC until cTnI, cTnT, CK–MB, LDH, AST, and ALT contents were measured. Since troponin concentrations are reported to remain stable at -20 ºC for a maximum of 3 months (Langhorn & Willesen, 2016), serum samples used in this study were stored for a maximum of 10 days.

Content of serum cTn-I, cTn-T, and CK–MB were determined using ELISA equipment (Thermo Scientific Multiscan GO, TYPE: 1510) and calculated using commercial test kits (respectively; ng/ml, ng/mL, U/L) as instructed by the manufacturer (Horse Cardiac Troponin I BT-LAB Kit-Cat No: E0028Ho, BT Lab, Korea; Horse Cardiac Troponin T BT-LAB Kit-Cat No: E0200Ho, BT Lab, Korea; Horse Creatine Kinase MB isoenzyme Elisa Kit-Cat No: SL0032Ho, SunLong Biotech Co., China). LDH was determined using a commercial spectrophotometric kit (TML, Turkey). Values of serum AST and ALT were measured using a Cobas C501 autoanalyzer (Roche-Cobes, Switzerland).

Statistical comparisons of data were performed using the SPSS® software program (SPSS 22.0, Chicago, IL, USA). Paired *t*-tests were used to compare the changes in the content of cTnI, cTnT, and some biochemical parameters (CK–MB, LDH, AST, ALT) in Arabian racehorses before and after training. Data are expressed as mean ± standard error (SE), and the statistical significance was set at $P < 0.05$.

## Results and Discussion

In the current study, 20 healthy, male Arabian racehorses aged 6.00 ± 0.52 y (min: 4 y, max: 10 y) were used. The content of cardiac biomarkers in the serum of Arabian racehorses before and after training is given in Table 1. There was a statistically significant increase in cTnT, cTnI, CK–MB, and ALT contents after 30 min of training. In the blood serum, the cTnT parameter was measured as 0.008 ± 0.00 ng/mL after training, and 0.007 ± 0.00 ng/mL before training. The cTnT value was found to be numerically higher in the post-training group ($P < 0.05$). In the post-training group, cTnI was 0.169

± 0.01 ng/mL and CK–MB was 289.80 ± 10.96 U/L. In the pre-training group, cTnI was 0.130 ± 0.01 ng/mL and CK–MB was 231.15 ± 8.96 U/L ($P$ <0.01). Pre-training ALT (11.83 ± 0.92 U/L) was numerically lower than post-training ALT (15.24 ± 1.03 U/L) ($P$ <0.05). LDH (692.45 ± 34.12 U/L) and AST (309.92 ± 18.48 U/L) measured before training were numerically lower than LDH (704.25 ± 22.03 U/L) and AST (328.47 ± 19.58 U/L) measured after training, but no statistically significant difference was found between the groups ($P$ >0.05).

**Table 1** Changes in biomarkers in Arabian racehorses before and after training (n = 20)

| Parameters | Group | Mean ± SE | *P*-value |
|---|---|---|---|
| cTnT (ng/mL) | Pre workout | 0.007 ± 0.00 | 0.017 |
| | Post workout | 0.008 ± 0.00 | |
| cTnI (ng/mL) | Pre workout | 0.130 ± 0.01 | 0.007 |
| | Post workout | 0.169 ± 0.01 | |
| CK–MB (U/L) | Pre workout | 231.15 ± 8.96 | <0.001 |
| | Post workout | 289.80 ± 10.96 | |
| LDH (U/L) | Pre workout | 692.45 ± 34.12 | 0.773 |
| | Post workout | 704.25 ± 22.03 | |
| AST (U/L) | Pre workout | 309.92 ± 18.48 | 0.495 |
| | Post workout | 328.47 ± 19.58 | |
| ALT (U/L) | Pre workout | 11.83 ± 0.92 | 0.019 |
| | Post workout | 15.24 ± 1.03 | |

cTn-I: cardiac troponin I; ANP: atrial natriuretic peptide; NT-proBNP: N-terminal pro-brain natriuretic peptide; CRP: C-reactive protein; CK–MB: creatine kinase–myocardial band; LDH: lactate dehydrogenase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; $P$ <0.05 indicates statistical significance

Although cardiac diseases in horses are considered to be a cause of sudden death or poor performance, it is very difficult to diagnose these diseases due to a lack of specific symptoms (Nath *et al.,* 2012; Hellings *et al.,* 2020). Although cardiac diseases in veterinary medicine are usually evaluated using auscultation and echocardiography (Nath *et al.,* 2012), recent studies have determined that cardiac biomarkers provide more specific results (Langhorn & Willesen, 2016; Carretón *et al.,* 2017; Kırbaş *et al.,* 2021).

It has been reported that gender affects the adaptation of antioxidant capacity change and male and female horses respond differently to exercise-induced reactive oxygen species (ROS) production (Pourmohammad *et al.,* 2020). A different study reported that oestrogen protects the heart (Kim *et al.,* 1996). For these reasons, female animals were not included in the study.

Troponins, which play a role in the contraction/relaxation of myofibrils and are considered to be the gold standard due to their high sensitivity/specificity for myocardial damage, enter the circulation when cardiac myocytes are destroyed (Langhorn & Willesen, 2016; AlSaad, 2020). Troponins consist of three subunits (I, T, and C). However, troponins specific to the heart are cTnI and cTnT (Kırbaş *et al.,* 2021). Moreover, cTnI is considered to be a more sensitive and more specific marker than cTnT due to its lower molecular weight (Langhorn & Willesen, 2016; Carretón *et al.,* 2017). When heart damage occurs, cTnI and cTnT contents in the blood peak within 6–12 h and 2–5 h, respectively (Gresslien & Agewall, 2016; Chow *et al.,* 2017; Hellings *et al.,* 2020). Clinically healthy horses have been reported to have a cTnI content ≤ 0.2 ng/mL (Pourmohammad *et al.,* 2020) and a cTnT content ≤ 0.01 ng/mL (Van Der Vekens *et al.,* 2013). It has also been reported that even small changes in cTnI and cTnT content have prognostic significance (Langhorn & Willesen, 2016; Carretón *et al.,* 2017). In the current study, cTnI and cTnT contents in horses before training were determined as 0.130 ± 0.01 ng/mL and 0.007 ± 0.00 ng/mL, respectively. These results indicate that none of the horses had any existing heart damage before training. The horses used in the study were unlikely to have heart disease as no disease was reported in clinical and laboratory examinations in the past. Therefore, the results of the study were consistent with both the literature and the clinical examination.

It has been reported that exercise may cause transient myocardial damage or stress (Langhorn & Willesen, 2016; Pourmohammad *et al.,* 2020). In other studies, it has been reported that cTnI and cTnT increase a few hours after exercise (Hellings *et al.,* 2020; Pourmohammad *et al.,* 2020). However, in other studies, no significant change in cTnI and cTnT contents was reported before and immediately after the race (Trachsel *et al.,* 2013; Shields *et al.,* 2018). In the current study, cTnI and cTnT contents

were 0.169 ± 0.01 ng/mL and 0.008 ± 0.00 ng/mL, respectively, in samples taken after 30 minutes of training. When the post-training troponin contents were compared with the pre-training contents, there was a statistically significant increase in cTnI ($P$ <0.01) and cTnT ($P$ <0.01) contents. Our findings are consistent with the literature that cTnI and cTnT may cause myocardial damage or stress (Langhorn & Willesen, 2016; Pourmohammad *et al.,* 2020). In another study, although the values measured a few hours after exercise were significant (Pourmohammad *et al.,* 2020; Hellings *et al.,* 2020), a statistical difference was found in the findings measured immediately after training in our study.

Creatine kinase–myocardial band is a cardiac biomarker that has been reported to increase with exercise and chest pain, although elevated contents are not always indicative of acute myocardial infarction (Tharwat & Al-Sobayil, 2014). Furthermore, CK–MB is a less sensitive biomarker than troponins for assessing myocardial injury (Sim *et al.,* 2008). CK–MB content in healthy horses is reported to be 235.70 ± 35.59 U/L (Ahmadpour *et al.,* 2020), which concurs with our study. In a study on Arabian horses, it was reported that endurance exercise did not cause significant increases in CK–MB content (Michima *et al.,* 2010). Similarly, in a study conducted on greyhounds, although there were slight increases in CK–MB concentrations after racing, these increases were not statistically significant (Tharwat & Al-Sobayil, 2014). Exercise caused a substantial increase in CK–MB contents in horses (Di Filippo *et al.,* 2017). In the current study, CK–MB content increased by approximately 25% after training compared to before training ($P$ <0.01). CK–MB alone does not provide clear information about cardiac damage, but will provide more detailed information about cardiac damage when evaluated together with cTnI and cTnT.

Serum activities of some enzymes, such as LDH and AST, have been reported to increase during cardiac injury (Aydoğdu *et al.,* 2016). In a study conducted on Thoroughbred racehorses, it was reported that LDH, AST, and ALT contents increased ($P$ <0.05) after exercise (Allaam *et al.,* 2014). In another study, LDH and AST contents in the same horses exercised in May and September were measured and it was reported that LDH and AST contents increased after exercise (Kedzierski *et al.,* 2009). In addition, it was found that LDH and AST contents increased after exercise in horses with piroplasmosis (Bravo-Barriga *et al.,* 2022). In a two-race study on endurance horses, it was reported that AST and ALT contents increased substantially in both races compared to the pre-race period (Larsson *et al.,* 2013). In the current study, similar to the literature, an increase in LDH, AST, and LDH contents was detected after training. The increase in LDH after exercise is thought to be due to oxidative peroxidation (Jović *et al.,* 2013) and damage to musculoskeletal cells and hepatocytes caused by hypoxia due to exercise intensity. Muscle injuries are indicated by elevated AST and CK contents (Bravo-Barriga *et al.,* 2022). In addition, AST and ALT are parameters that clearly indicate hepatocellular damage and ALT and AST increase together in the event of damage to the hepatocytes (Yoldaş *et al.,* 2012).

## Conclusions

In conclusion, it was determined that cardiac biomarkers were increased after exercise in Arabian racehorses that did not show any clinical symptoms, but more studies should be done to check cardiac biomarkers at different time intervals after training to detect permanent myocardial damage. In addition, it was concluded that exercise stimulates the release of troponin and the differences that may occur in the troponin test in horses after exercise should be taken into consideration.

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**Authors' Contributions**

CA (ORCID: 0000-0003-2064-0657) conceptualization, data collection, methodology, analysis, writing, and original draft preparation; ŞK (ORCID: 0000-0003-3727-8893) data collection, methodology, and review; ÜY (ORCID: 0000-0002-2599-8589) Laboratory analysis, writing, and review; ZGY (ORCID: 0000-0001-6660-2643) Laboratory analysis, writing, and review; PAD (ORCID: 0000-0002-7010-0475) analysis, writing, review, and editing; ACT (ORCID: 0000-0002-6296-6762) Laboratory analysis, writing, and editing.

**Conflict of Interest Declaration**

The authors declare that they have no conflicts of interest relative to the content of this paper.

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