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Effect of Long Road Transport Journey on Serum Biomarkers of Bone Formation and Resorption in Athletic Horses

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ABSTRACT

We carried out this study to investigate the effects of a 300km road transport journey on the serum levels of bone formation and resorption biomarkers in athletic horses. The bone formation biomarkers included osteocalcin (OC), bone alkaline phosphatase (β-ALP), and the bone resorption biomarker included pyridinoline cross-links (PYD). Ten athletic horses were transported for 300 km on a paved road at 100 km/h. Sera were harvested before transport (T0), immediately after (T1), and 24 h of offloading (T2) and stored at -30ºC. The results showed significantly higher levels in the serum concentration of OC at T1 (17.05±2.12ng/mL) compared to its levels at T0 (12.95±1.92ng/mL), and its value became non-significant at T2 (15.33±3.99ng/mL). The serum concentrations of β-ALP did not differ among the tested three-time points (T0=28.79±10.39U/L, T1=33.28±12.76U/L, T2=29.12±10.59U/L). The concentration of PYD significantly increased at T1 (10.32±4.39nmol/L) compared to its levels at T0 (6.81±1.59nmol/L) and T2 (6.46±1.03nmol/L). In conclusion, the transportation of horses for 300km at a speed of 100km/h significantly increased the biomarkers of bone turnover. This might indicate a significant alteration that occurred in bone metabolism during transportation.

Key words: Bon; Biomarkers; β-ALP; Horses; Osteocalcin; Pyridinoline.

INTRODUCTION

In many countries around the world, appropriate handing of animals during transport is given more attention by those interested in this field (Sporer et al. 2008). In large animals, it is well known that transport has many stressors, including but not limited to dealing, loading, unloading, harsh weather, bad ventilation, and shortage of food and water (Padalino 2015). Transportation by trucks is a recognized stressor that has reverse effects on the production and health of livestock (Tharwat et al. 2013). Regrettably, the dealing, loading and unloading of animals constitute the most stressor during transportation (Minka et al. 2009).

Bone biomarkers are broadly used in human medicine, mainly for observation of bone response to treatment of special musculoskeletal diseases (Sabour et al. 2014). In veterinary field, the biomarkers of bone are commonly used in animals as a quick and sensible mean for evaluation of bone reaction to medical therapy and invasive interference, and for the diagnosis of musculoskeletal problems (Al-Sobayil and Tharwat 2021). The commonly used bone formation markers are osteocalcin (OC) and bone alkaline phosphatase (β-ALP). However, the most commonly used bone resorption markers are pyridinoline cross-links (PYD) and deoxypyridinoline (Al-Sobayil and Tharwat 2021).

As other animals, horses are transported by different means such as shipping, train, road and also by air. During such event, significant changes occur such as variability in heart rate, cardiac biomarkers, cortisol, lactate, and hemato-biochemical variables. It has been therefore suggested that transportation is stressful for horses on the basis of high cortisol levels and changes in the heart rate (Tharwat and Al-Sobayil 2014). This investigation was designed to highlight the influence of a 300km road transport on the serum levels of bone turnover biomarkers in athletic horses.

MATERIALS AND METHODS

Ethical Approval and Transportation of Horses

Animal Ethical Committee, Scientific Research Deanship in the University of Qassim, Saudi Arabia

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approved this study. The study design has been reported previously (Tharwat and Al-Sobayil 2014). Briefly, 10 athletic Arabian horses aged 9.8±2.3 years and weighed 345±21kg were used in the study. On a paved road, transport of the horses has been performed between 15:00 to 20:00 hours with an ambient temperature of 25ºC and a relative humidity of 60%. Two trucks were used, 5 horses each at a density of 1.2×3.0m for a journey of 300km. No tranquilizers were used throughout the transportation.

Blood Sampling and Assays of Bone Biomarkers

From each horse, 3 serum samples were collected and stored at -30ºC (before transport, T0; immediately after transport, T1; 24 h of transport, T2). The serum concentrations of OC, β-ALP and PYD were evaluated using immunoassay kits as reported (Tharwat et al. 2014; Tharwat and Al-Sobayil 2015, 2018a,b; Tharwat 2020a,b; Al-Sobayil and Tharwat 2021).

Statistical Analysis

Assessment of data normality was carried out using D'Agostino Pearson test. Data were presented as mean±SD as there was no significant deviation from normality. Statistical analysis was performed using the SPSS-program version 25.0 (SPSS 2017). Multiple comparisons among the 3 time points (T0, T1 and T2) were carried using the Dunnett's test and the significance value was set at P≤0.05.

RESULTS

After unloading of the horses, none of them developed any abnormality such as depression or loss of appetite. In Fig. 1, levels of bone formation (OC, β-ALP) and bone resorption (PYD) biomarkers in serum were displayed. Compared to a mean value of 12.95±1.92ng/mL before transport (T0), the OC value increased immediately after unloading (T1) where it was measured 17.05±2.12ng/mL with a statistically significant difference between them (P=0.001). Twenty-four hours after transport (T2), the OC serum values were tested 15.33±3.99ng/mL with a non-significant difference when compared to values pre- or immediately after transport (P=0.15, 0.30, respectively). The serum concentrations of β-ALP did not change significantly at the 3 time points. At T0, T1 and T2 it measured 28.79±10.39U/L, 33.28±12.76U/L, 29.12±10.59 U/L, respectively (P>0.05). Compared to a mean value of 6.81±1.59nmol/L before loading (T0), the PYD levels increased significantly after transport where it measured 10.32±4.39nmol/L (P=0.05). At T2, the PYD serum values were measured 6.46±1.03nmol/L, with a significant change when compared to T1 (P=0.03), but with a non-significant level when compared to T0 (P=0.61).

DISCUSSION

In the horse, a 12h journey resulted in significantly increased levels in the stress biomarker malondialdehyde compared to pre-transport levels (Onmaz et al. 2011). Similar, an eight-hour road transport in the horse elevated the plasma total antioxidant values just after transport end (Niedźwiedź et al. 2013). This clearly indicates that transportation is stressful for horses. A question of what the effects of horse transportation on the bone biomarkers will be was raised. Therefore, this study was carried out trying to answer this question. It is believed that ongoing research on bone biomarkers will be increasingly used in the future in the diagnosis and prognosis of musculoskeletal and bone injuries in horses. These bone formation and bone resorption biomarkers are biochemical by-products that provide insight into the activity of bone cells and are created from the bone remodeling process (Allen 2003).

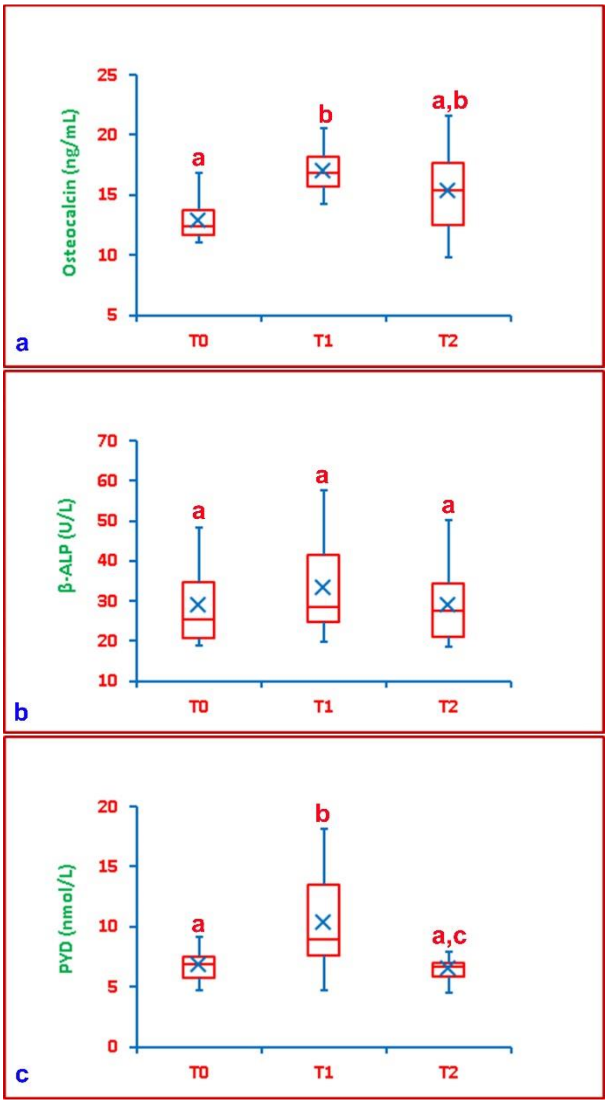

Fig. 1: Box and whiskers plot of the bone biomarkers osteocalcin (a), β-alkaline phosphatase (β-ALP; b) and pyridinoline cross-links (PYD; c) in 10 horses enrolled in a 300 km transport journey. T0, immediately before transport; T1, just before transport; T2, 24 hours after transport. [a,b,c] different letters indicate a significant difference (P≤0.05).

Osteocalcin is the most plentiful protein in bone that is particularly expressed in osteoblasts (Komori 2020). A recent unexpected development of bone biology reported that OC is not only a bone remodeling marker but also an active hormone contributing to the organization of a number of physiological processes. Of these functions regulated by OC is glucose homeostasis (Wei and Karsenty 2015). Knowledge about the hormone OC has therefore significantly expanded the area of bone biology because of the physiologic operation adjusted by this hormone such as metabolism of energy and growth of the brain (Karsenty 2017). Clinically, OC is a biomarker of bone formation, and an exercise increase this process and also improves glucose metabolism, making a link between OC and glucose metabolism (Al-Sobayil and Tharwat 2021).

In this study, transportation of horses for 300 km increased the serum OC to significant levels immediately after unloading compared to pre-transport levels. The OC values remained high 24h after transport compared to preloading measurements but didn't reach the significant levels. Increases in serum OC levels after transport in this study may be clinically important. Results from human studies demonstrated that exercise training induces significant increases in serum levels of OC; possibly due to some influences such as increasing the insulin sensitivity, beside its direct effect on bone osteoblastic activity (Rahimi et al. 2021). Elevation in insulin sensitivity may be caused by muscle contraction during road transport, similar to exercise (Rahimi et al. 2021).

The enzyme alkaline phosphatase has 2 types; the first is tissue-specific and the second is tissue non-specific). The intestine, placenta, and germinal tissue contain the first type. However, the second type is principally expressed in bone, liver and kidney, and it plays a core role in bone calcification (Vimalraj 2020). On the surface of osteoblasts, the β-ALP is found, and has been shown to be a sensible index of bone formation. Approximately, half of the ALP is produced from bone in animals with healthy liver. The correlation between β-ALP and OC in the blood was reported to be weak although both are bone formation markers (Al-Sobayil and Tharwat 2021). This was evident in the results of this study as the serum values of OC increased significantly after transport, nevertheless β-ALP did not differ significantly among values measured immediately before and after transport, and 24h of the end of transport.

The bone resorption biomarker PYD is not only presented in mature type I collagen, which is the main type of collagen in bone tissues, but also in collagen types II and III (Al-Sobayil and Tharwat 2021). The PYD is manufactured during ripeness of collagens and freed into the blood from degeneration of this grown collagens (Kuo and Chen 2017). Increased levels of PYD in the blood or urine are mostly believed as an indicator of bone resorption (Thompson et al. 1992). In this study, the serum levels of PYD increased significantly after transport compared to values before loading but it declined 24h later to values close to that before transport. The increased PYD in this study suggests excessive bone resorption as a result of road transportation in the horses. The clear product of bone formation and resorption is called bone mass, which are firmly adjusted by the balance between endogenous/ and exogenous factors (Lombardi et al. 2012). These bone turnover markers are very reactive to exercise varying according to the type of exercise (Gombos et al. 2016). Forces produced by muscle contractility make significant functions in motivating the response of skeletal system to the mechanical process of loading (Kohrt et al. 2009).

Conclusion

It is concluded that transportation of horses for 300km round trip journey did not change significantly the bone formation biomarker OC and β-ALP after transport. However, the bone resorption biomarker PYD increased significantly after transport compared to values before loading. This might be an indication to a significant alteration occurred in bone metabolism during transportation. What will be the potential effects on bone metabolism biomarkers if horses are transported for longer distances and were exposed to more stressful conditions? A future study is therefore warranted to answer this question.

Author's Contribution

Both authors planned the study and carried out the practical work. M. Tharwat wrote the original draft and made the figure. F. Al-Sobayil read and revised the manuscript. Both authors have read and approved the manuscript.

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