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A REVIEW ON BIOMARKERS OF BONE METABOLISM IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

Bone metabolism biomarkers of bone formation and bone resorption are released during the bone remodeling processes. In humans as well as in veterinary medicine, these bone biomarkers have attracted much attention in the last decade. However, there are few reports found in the application of bone metabolism biomarkers in camel medicine. In camels, the most commonly used bone formation biomarkers include osteocalcin (OC) and bone-specific alkaline phosphatase (b-ALP). Concerning the bone resorption biomarkers, the most commonly used one is pyridinoline cross-links (PYD). OC is synthesised mostly from by mature osteoblasts. It is believed that OC is associated with mineralisation of newly formed osteoid. Therefore it is considered as a putative biomarker of bone formation and mineralisation. b-ALP is an isoform of alkaline phosphatase and plays an important role in bone formation. With normal liver function in adults, about 50% of total b-ALP is produced from bone in serum. The PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. It is not only found in mature type I collagen, which is the major type of collagen in bone tissues, but also in collagen types II and III (Eyre *et al,* 1984). Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption. This review article was written to emphasise the importance of commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camels. It is believed that research on bone formation and bone resorption biomarkers in camels will be increasingly used in the future in the diagnosis and prognosis of bone diseases.

Key words: Biomarkers, bone formation, bone resorption, bone, camels

Early diagnosis of bone diseases constitutes a great challenge for the clinician. Although radiography is the most widely imaging technique used for the diagnosis of bone diseases, it fails to diagnose early stages of bone disorders (Al-Sobayil, 2010). It has been reported that approximately 30–40% changes in bone mineral density are required before bone changes are detected in radiography (Greenfield, 1986). Therefore, more specific, sensitive and applicable methods are needed to identify early changes in bone, assess the progression of bone damage and to monitor therapeutic response and the healing process.

Bone is a dynamic tissue, characterised by a continuously renewed through processes of bone removal parallel to bone formation and replacement, which occur in the so-called basic multicellular units. Main cells in the basic multicellular units are osteoblasts, deputed to bone formation, and osteoclasts, to bone resorption. Markers of bone metabolism are biochemical by-products that provide insight into the activity of bone cells. These biochemical markers are produced from the bone remodeling process included bone formation biomarkers and bone resorption biomarkers (Allen, 2003).

Biochemical markers of bone turnover are widely used in human clinical practice, mainly for non-invasive monitoring of bone metabolism and response to therapy of certain musculoskeletal and bone disorders (Swaminathan, 2001; Watts *et al,* 2001; Kanakis *et al,* 2004; Sabour *et al,* 2014). In animals, bone biomarkers are mostly used in preclinical and clinical studies as a rapid and sensitive method for assessment of bone response to medical treatment and surgical interventions and for the detection of musculoskeletal injuries (Allen, 2003; DeLaurier *et al,* 2004; Frisbie *et al,* 2008; Frisbie *et al,* 2010; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018 a,b).

The common biomarkers of bone formation include osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and amino and carboxy propeptides of collagen type I. The non-collagenous

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protein OC, a product of the osteoblasts, is regarded as a sensitive indicator of bone formation (Pullig *et al,* 2000). The b-ALP, a glycoprotein found on the surface of osteoblasts, has also been shown to be a sensitive and reliable indicator of bone metabolism. Although b-ALP and OC are considered bone formation biomarkers, their correlation in the camel blood was reported to be weak (Al-Sobayil, 2010). The lack of a strong correlation between the two biomarkers has been attributed to the fact that each of them reflects different stages of osteoblast function (Delmas *et al,* 1990). Moreover, b-ALP represents an early osteoblast biomarker because it presents in pre-osteoblasts and osteoblasts, whereas OC is considered a later biomarker of osteoblast differentiation and bone mineralisation (Naylor and Eastell, 1999). The most common biomarkers of bone resorption include pyridinoline cross-links (PYD), deoxypyridinoline enzyme tartrate resistant acid phosphatase and amino and carboxy telopeptides of collagen type I.

This review article emphasises the importance of commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camels. It is believed that the current review on bone formation and bone resorption biomarkers in camels will help researchers and clinicians in the future in the diagnosis and prognosis of bone diseases and their interpretation.

Bone formation biomarkers

Osteocalcin

Osteocalcin, also known as bone gamma-carboxyglutamic acid-containing protein, is a small vitamin K-dependent and calcium binding protein that contains 49 amino acids. It composes 1–20% of the noncollagenous protein of the organic matrix of bone depending on animal species, age, and site (Price, 1983; Conn and Termine, 1985). OC is synthesised by mature osteoblasts, odontoblasts and hypertrophic chondrocytes. Moreover, OC is the most abundant non-collagenous protein in bone comprised about 2% of total protein in the human body. It is believed that OC is associated with mineralisation of newly formed osteoid (Billinghurst *et al,* 2003). Therefore, it is considered as a putative biomarker of bone formation and mineralisation (Billinghurst *et al,* 2004).

Bone-specific Alkaline Phosphatase

Alkaline phosphatase is a membrane-bound enzyme that hydrolyses phosphate esters. Although total alkaline phosphatase (TAP) is not specific to bone, its levels in serum have shown correlation with bone formation rate as assessed by calcium kinetics in normal humans (Weaver *et al,* 1997). TAP levels in serum consist of several enzyme isoforms produced by bone, liver, intestine, kidney, spleen and placenta (Moss, 1987). The majority of TAP in serum is the liver and bone isoforms. Bone-specific alkaline phosphatase (BAP) is an isoform of alkaline phosphatase and plays an important role in bone formation (McIlwraith, 2005). With normal liver function in adults, about 50% of total ALP is produced from bone in serum.

In synovial fluid of active equine OA joints, the b-ALP concentrations were increased compared with normal joints (Fuller *et al,* 2001). The positive correlation between synovial fluid BAP and articular cartilage damage demonstrated a link between changes in bone and articular cartilage in OA. A correlation between cartilage damage and marker levels validates the use of synovial fluid BAP in OA assessment. In serum of horses with OC, BAP concentrations were significantly lower than in serum of normal horses. On the other hand, synovial fluid levels of BAP were significantly higher in horses with OC injury than in healthy horses (McIlwraith, 2005).

Bone resorption biomarkers

Pyridinoline cross-links

The PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. It is not only found in mature type I collagen, which is the major type of collagen in bone tissues (Von Der Mark, 1999), but also in collagen types II and III (Eyre *et al,* 1984). Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption (Thompson *et al,* 1992).

Application of cardiac biomarkers in camel medicine

Similar to published results in mares (Filipovic *et al,* 2010), the serum concentrations of the bone formation biomarkers OC and b-ALP in female camels did not change significantly during the periparturient period (3wk before to 3wk after parturition). In contrast, the serum concentrations of the bone resorption biomarker PYD decreased significantly at parturition compared to 3wk before parturition and then increased significantly at 3wk after parturition (Fig 1) (Tharwat and Al-Sobayil, 2015). In mares, the concentrations of PYD in the blood plasma significantly increased around day 20 after foaling, indicating an increased rate of bone

resorption (Filipovic *et al,* 2010). An increase in the markers of bone resorption was also observed in the serum of ewes and goats in the last month of pregnancy (Liesegang *et al,* 2006, 2007). Liesegang *et al* (2006) indicated that the activity of the osteoblasts is lowered in ewes during late pregnancy.

The decreased oestrogen in camels at 3 wk after parturition (Tharwat and Al-Sobayil, 2015), could contribute to an elevated bone resorption rate. The decreased oestrogen levels post-partum may enhance osteoclast activity that, in turn, would increase bone resorption. It has been reported that a cyclical variation in bone turnover occurs over the course of the oestrous cycle in post-partum dairy cows, with decreases in plasma oestrogen below a critical threshold correlating with enhanced bone resorption (Devkota *et al,* 2012).

In a study conducted recently by our group on 20 sexually mature, healthy male dromedary camels with 8 controls, the serum concentration of OC increased significantly immediately after (electroejaculation) EEJ compared to baseline values (Tharwat and Al-Sobayil, 2018a). However, the serum concentration of b-ALP and PYD differed significantly (Fig 2). Although OC and b-ALP are considered bone formation biomarkers, their correlation in the serum of camels was reported to be weak (Al-Sobayil, 2010). The lack of a strong correlation between the two biomarkers has been attributed to the fact that each of them reflects different stages of osteoblast function (Delmas *et al,* 1990).

The non-significant changes in the serum concentration of the bone resorption biomarker PYD after EEJ may indicate that increased physical activity may have the potential to decrease the collagen resorption in male camels. This result may be influenced, in part, by a systemic suppression of collagen resorption through the systemic actions of calciotropic hormones, with emphasis on testosterones. It has been shown that increased levels of testosterone significantly reduce bone loss (Steffens *et al,* 2012; Wiren *et al,* 2012), decrease collagen and glycosaminoglycan loss in the articular tissues (Ganesan *et al,* 2008) and increase the repair strength of the ligaments and tendons (Tipton *et al,* 1975).

In a study conducted recently by our group on 23 female racing camels that participated in a 5 km race, the serum concentration of serum OC and b-ALP increased but not significantly after race. On the contrary, the serum concentration of the bone

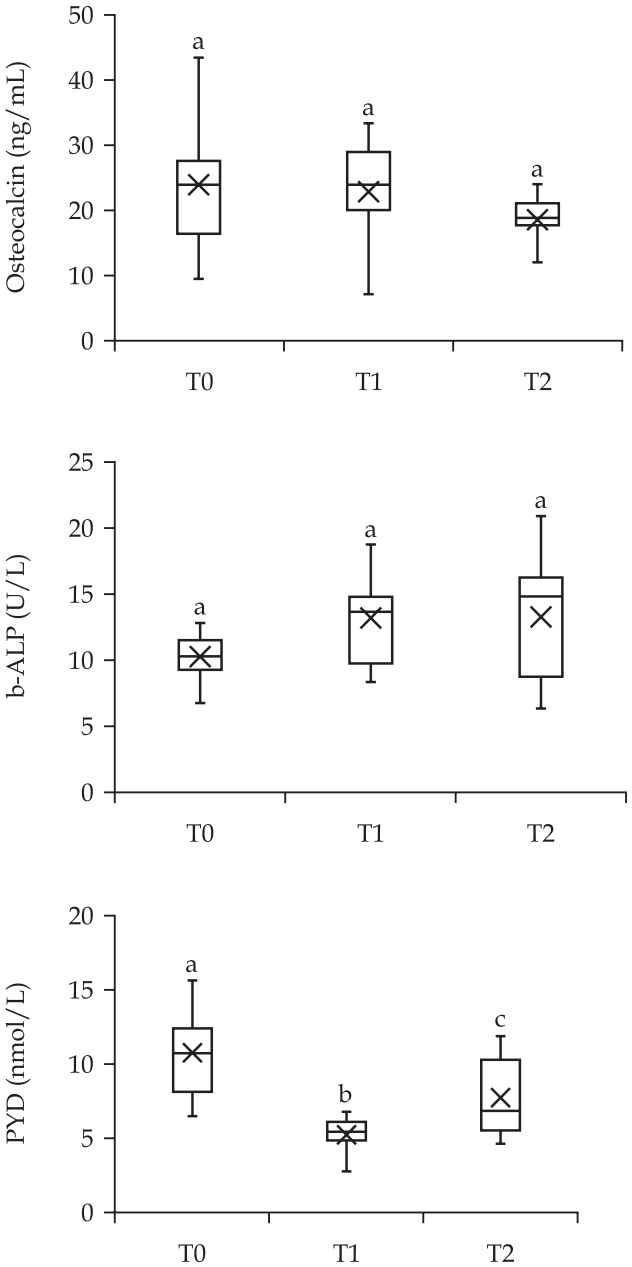

Fig 1. Box and whiskers plots of serum osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Values different letters differ significantly (P>0.5) (Tharwat and Al-Sobayil, 2015).

resorption biomarker PYD increased significantly after racing (Fig 3) (Tharwat and Al-Sobayil, 2018b). The non-significant elevations in the bone formation biomarker OC are consistent with findings of another exercise study involving highly conditioned Arabian horses (Porr *et al,* 1998). Similarly, in racing camels, both moderate and full-speed exercise had no effect

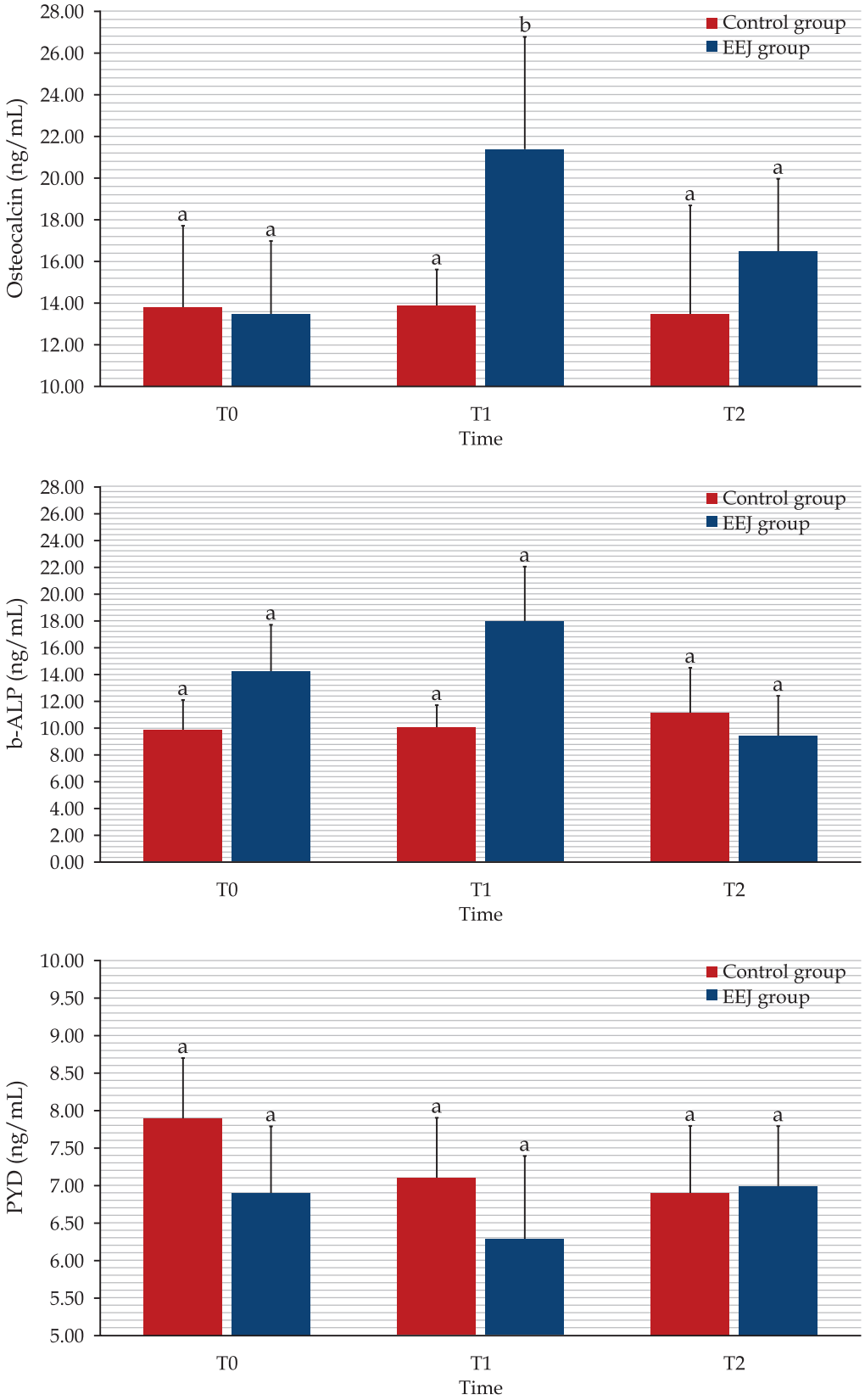

Fig 2. Effect of stimulation by electroejaculation (EEJ) on concentrations of serum osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in male dromedary camels (mean ± SD, n=20) compared to control group (n=10). T0: just before EEJ; T1: directly after EEJ; T2: 24h after EEJ. Values different letters differ significantly (Tharwat and Al-Sobayil, 2018a).

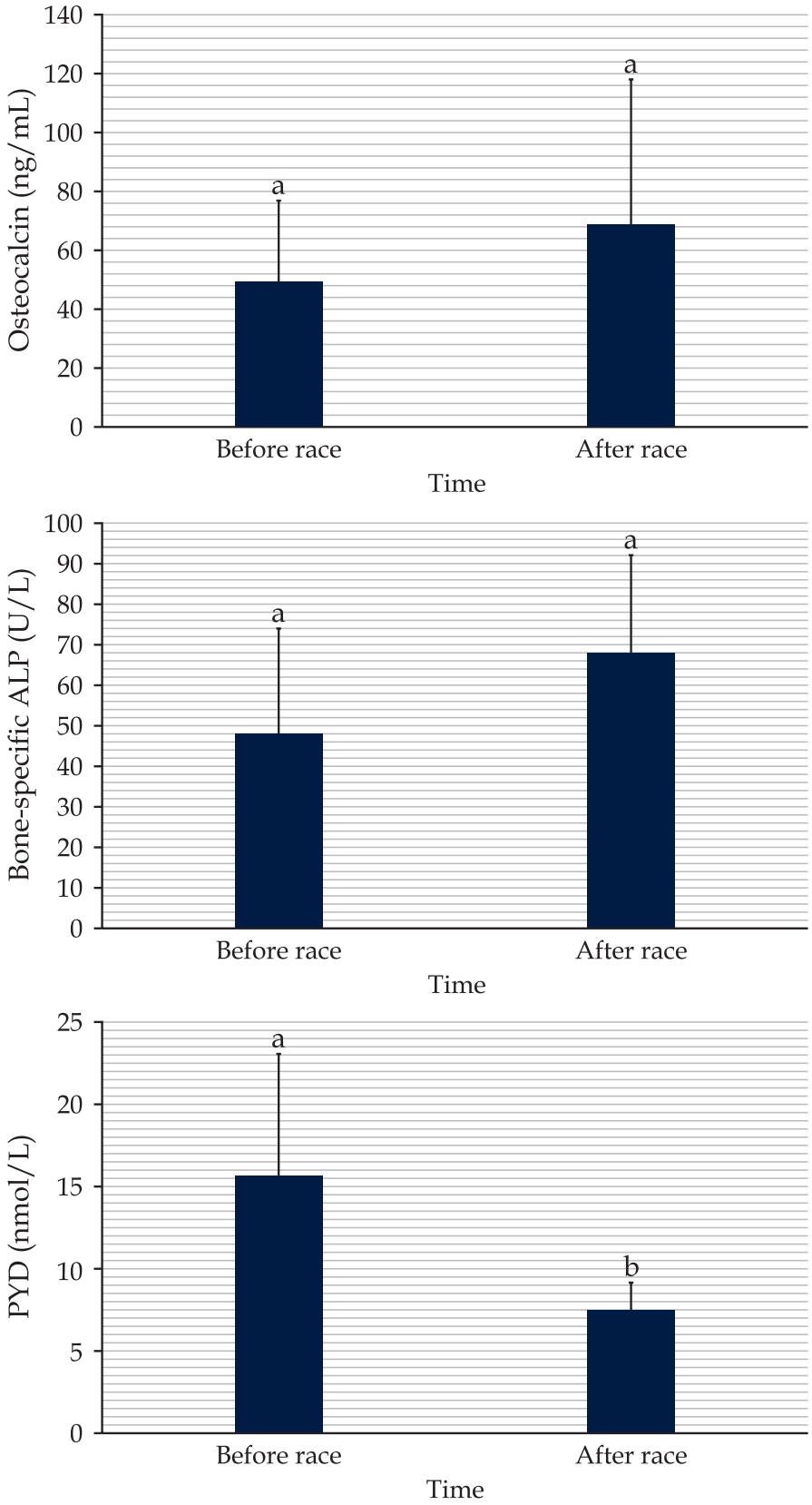

Fig 3. Serum concentration of osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in racing dromedary camels before and after race (Tharwat and Al-Sobayil, 2018b).

on the concentration of OC (Al-Sobayil, 2008). Rudberg *et al* (2000) reported short-lasting increases in b-ALP after 4.7 Km of jogging, a distance nearly similar ours (7 Km). It has been suggested that the increased content of b-ALP could be released from the osteoblast membranes under local factors such as changes in the pH, which triggers the release of b-ALP from the osteoblasts (Anh *et al,* 1998). The significant increases in PYD post-race in camels disagree with those of the study in horses assigned to 48-week race training (Caron *et al,* 2002), where no significant changes in serum PYD concentrations were detected post-race. It is clear from this study that the bone formation biomarkers are not influenced by the 5-km race. However, the bone resorption biomarker increased significantly. The influence of long-distance racing on these biomarkers is therefore warranted. Understanding the effect of racing on stimulation of the bone remodelling is important for the development of strategies to increase and maintain bone mass.

Conclusions

This mini review sheds light on the commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camel medicine. These biomarkers were changed in camel serum following parturition, stimulation by electroejaculation and race. It is expected that bone metabolism biomarkers in camels would be increasingly used in the clinical studies for the diagnosis, prognosis and assessment the response of the skeleton to medical and surgical interventions.

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