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SERUM CONCENTRATIONS OF ACUTE PHASE PROTEINS AND BONE BIOMARKERS IN FEMALE DROMEDARY CAMELS DURING THE PERIPARTURIENT PERIOD

Mohamed Tharwat[1,2] and Fahd Al-Sobayil[1]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia
[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt

ABSTRACT

The objective of the present study was to evaluate the influence of the periparturient period in dromedary camels on the acute phase proteins (APPs) haptoglobin (Hp) and serum amyloid A (SAA), and on the bone biomarkers osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and pyridinoline cross-links (PYD). For this purpose, blood samples were collected from 10 female dromedary camels during the periparturient period at 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2). Compared to values at T0, the serum concentrations of cortisol and estrogen increased significantly at T1 then decreased significantly at T2. On the contrary, the serum concentration of progesterone decreased significantly at T1 and T2. Concerning the APPs, the serum concentrations of Hp and SAA increased significantly at T1 and then decreased at T2. The serum concentrations of the bone formation biomarkers OC and b-ALP did not differ significantly among T0, T1 and T2 values. On the other hand, the serum concentration of the bone resorption biomarker PYD decreased significantly at T1 then increased at T2. The increased serum concentrations of Hp and SAA proved that an acute-phase response had occurred in the camels at parturition. The bone formation biomarkers did not alter significantly during the periparturient period, while the bone resorption biomarker decreased significantly at parturition.

Key words: Acute phase proteins, biomarkers, bone, camel, periparturient period

The periparturient or transition period, 3 wk before to 3 wk after parturition, is characterised greatly by the increased risk of disease (Lean *et al,* 2013; Tharwat *et al,* 2012a,b; Tharwat *et al,* 2015a). During this period, the animal experiences a series of nutritional, physiological, and social changes, and is more vulnerable to infectious and metabolic diseases (Ospina *et al,* 2013). Metabolically, the animal is in a state of nutrient reserve mobilisation, primarily that of adipose and labile protein but also that of bone (Lean *et al,* 2013). Successful transition of the animal from the fetal to the neonatal state involves tremendous physiological adaptations on the part of the neonate and the dam. The success or failure of this transition process equally dictates the survival of the offspring and the subsequent recovery of the dam (Lean *et al,* 2013; Ospina *et al,* 2013).

Acute-phase proteins (APPs) are a class of proteins whose blood concentrations increase (positive APPs) or decrease (negative APPs) in response to infection, inflammation or trauma (Murata *et al,* 2004). This response is called the acute-phase reaction or acute-phase response (APR). In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream. The liver responds by producing a large number of APPs (Petersen *et al,* 2004). The negative APPs include albumin, the most abundant constitutive plasma protein, and transferrin. The positive APPs include Haptoglobin (Hp), C-reactive protein, serum amyloid A (SAA), ceruloplasmin, fibrinogen and alpha 1-acid glycoprotein (Eckersall and Bell, 2010). In ruminants, the major APPs are Hp and SAA (Murata *et al,* 2004).

The serum concentrations of APPs were determined during the periparturient period in cattle (Rezamand *et al,* 2007; Huzzey *et al,* 2011; Trevisi *et al,* 2012; Schneider *et al,* 2013; Krause *et al,* 2014), and results indicated that disease cases in periparturient, high-yielding dairy cows correlated with signs of accentuated markers of inflammatory phenomena. The use of bone biomarkers appears to be valuable for evaluating the bone remodeling status in cows and

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mares during the periparturient period (Moreira *et al,* 2009; Filipovic *et al,* 2010; Sato *et al,* 2011, 2013). To the authors' knowledge, the effect of the periparturient period on the concentrations of the APPs and bone biomarkers in female dromedary camels has not been reported; thus, this study was designed to evaluate this influence. We believe that studying the APPs and bone metabolism biomarkers during the transition period in camels is expected to increase our understanding to the biology of reproduction in this species.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. Ten multiparous pregnant female dromedary camels reared at Qassim University Farm were enrolled in this study. Their mean body weight was 450 kg (range: 380-560 kg) and their mean age was 8.9 years (range: 7.5-11.0 years). The camels were considered healthy on the basis of physical examination (auscultation of the heart, lungs, rumen and intestine and measurement of heart rate, respiratory rate and rectal temperature) and laboratory evaluation (normal complete blood cell counts and biochemistry panel). All camels were maintained in a free-stall barn and kept under the Laboratory Animal Control Guidelines of Qassim University, which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publications No. 86 to 23, revised 1996).

Blood sampling

To minimise any circadian fluctuations, a fixed-time sampling at 11:00 AM was adopted. Two jugular blood samples were collected 3 wk before expected parturition (T1), within 12 h of parturition (T2) and 3 wk after parturition (T3). The first blood sample was collected in EDTA tubes for white blood cell count. The second blood sample was collected in plain tubes and centrifuged at 1200 × g for 10 min and the serum samples obtained were aliquotted in tubes and immediately stored at -20 °C pending the clinical chemistry analyses.

White blood cell, cortisol, estrogen and progesterone assays

White blood cell counts were carried out using an automated analyser (VetScan HM5, Abaxis, CA, USA). Estrogen, progesterone and cortisol were determined in the serum samples using electrochemiluminescent immunoassay kits (Roche Diagnostics, Indianapolis, IN, USA), with measuring ranges of 5.00-4300 pg/mL, 0.030-60.00 ng/mL, and 0.018-63.4 µg/dL, respectively. The intra- and inter-assay coefficients of variance for estrogen, progesterone and cortisol were 3.7 and 3.8%, 2.2 and 5.0% and 1.22 and 1.54%, respectively.

Acute-phase proteins assays

The serum concentrations of Hp and SAA were determined accordingly as recently reported in the literature for camels (Tharwat *et al*, 2015b). Haptoglobin was measured according to the prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp (Tridelta Development Ltd., Ireland).The analytical sensitivity of the assay was 0.0005 mg/mL, and the intra- and inter-assay CVs were 5-6% and 4-6%, respectively. The SAA was determined by a solidphase sandwich ELISA (Tridelta Development Ltd., Ireland). The analytical sensitivity of the assay was 0.15 µg/mL and the intra- and inter-assay CVs were 4.5% and 6%, respectively.

Bone metabolism biomarkers assays

The bone biomarkers OC, b-ALP and PYD serum concentrations were determined in the serum using commercial immunoassay kits (Quidel Corp., CA, USA). The serum concentrations of the bone biomarkers in the camels were determined as recently reported in the work of Al-Sobayil (2010). The limit of quantification of OC ranged from 2 to 32 ng/mL, and precision CVs within runs and between runs were 5-10%. The dynamic range of b-ALP was from 2 to 140 U/L, and the precision CVs within and between runs were 4-6% and 5-8%, respectively. The dynamic range of PYD was from 15 to 750 nM/L, and the precision CVs within and between runs were 6-10% and 3-11%, respectively.

Statistical analysis

Data are presented as medians ± standard deviations. Statistical analysis was done with the SPSS statistics package (version 18.0; SPSS, Chicago, IL, USA). Data normality was tested using the Kolmogorov–Smirnov test. Because data were not distributed normally, a nonparametric method (Kruskal–Wallis) was used to test the significance. Post hoc multiple comparisons among the time points T0, T1 and T2 were then carried out using the Dunnett test. The significance value was set at $P<0.05$.

Results

The serum concentrations of cortisol 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2) are summarised in Fig 1. Compared to a value of 1.15 ± 0.31 µg/dL at T0, the serum concentration of cortisol increased significantly at T1 to 7.40 ± 1.78 µg/dL (P<0.0001) then decreased significantly at T2 to 0.71 ± 0.19 µg/dL (P = 0.0006).

The serum concentrations of estrogen 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2) are summarised in Fig 2. Compared to a value of 216 ± 151 pg/mL at T0, the serum concentration of estrogen increased significantly at T1 to 739 ± 190 pg/mL (P<0.0001) then decreased significantly at T2 to 30 ± 10 pg/mL (P = 0.001). Fig 3 summarises the serum concentrations of progesterone 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2). Compared to a value of 2.0 ± 0.6 ng/mL at T0, the serum concentration of progesterone decreased significantly at T1 to 0.6 ± 0.4 ng/mL (P = 0.0001) and measured 0.1 ± 0.07 ng/mL at T2 (P<0.0001).

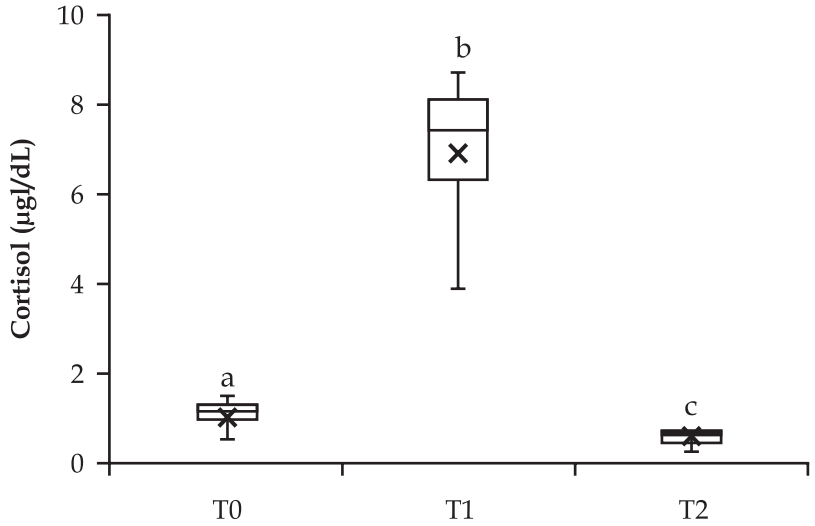

Fig 1. Box and whiskers plots of serum cortisol in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles.**T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

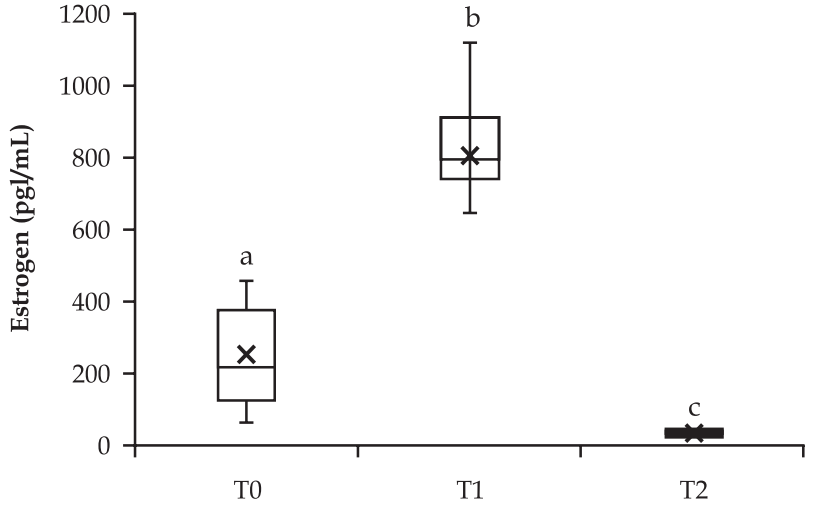

Fig 2. Box and whiskers plots of serum estrogen in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

Fig 4 summarises the serum concentrations of Hp at T0, T1 and T2 time points. Compared to a value of 0.58 ± 0.22 mg/dL at T0, the serum concentration of Hp increased significantly at T1 to 14.25 ± 4.31 mg/dL (P<0.0001). At T2, the Hp serum concentration measured 2.60 ± 0.84 mg/dL, which differed significantly from T0 and T1 values (P<0.0001). Fig 5 summarises the serum concentrations of SAA at T0, T1 and T2. Compared to a value of 0.73 ± 0.27 ng/mL at T0, the serum concentration of SAA increased significantly at T1 to a value of 81.00 ± 22.83 ng/mL (P<0.0001). At T2, the SAA serum concentration measured 4.59 ± 1.42 ng/mL, a significant decrease from T0 and T1 values (P<0.0001).

The serum concentrations of OC at T0, T1 and T2 are shown in Fig 6. Compared to a value of 23.80 ± 11.29 ng/mL at T0, the serum concentrations of OC measured 23.79 ± 8.30 ng/mL at T1 and 18.76± 3.58 ng/mL at T2. A comparison of OC values at T1, T2 and T3 did not reveal any significant difference (P>0.05). Fig 7 summarises the serum concentrations of b-ALP at T0, T1 and T2. Compared to a value of 10.25 ± 1.94 U/L at T0, the serum concentration of b-ALP measured 13.7 ± 3.73 U/L at T1 and 14.79 ± 4.94 U/L at T2. A comparison of b-ALP values at T1, T2 and T3 did not reveal any significant difference (P>0.05). The serum concentrations of PYD at T0, T1

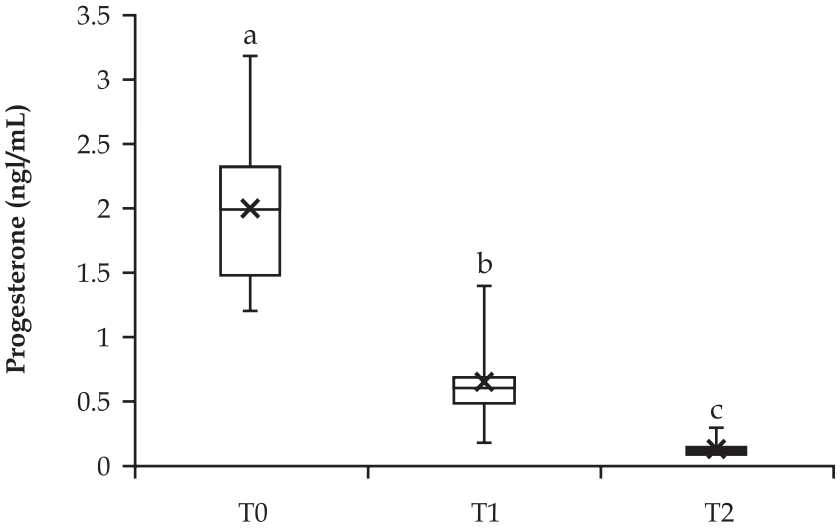

Fig 3. Box and whiskers plots of serum progesterone in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

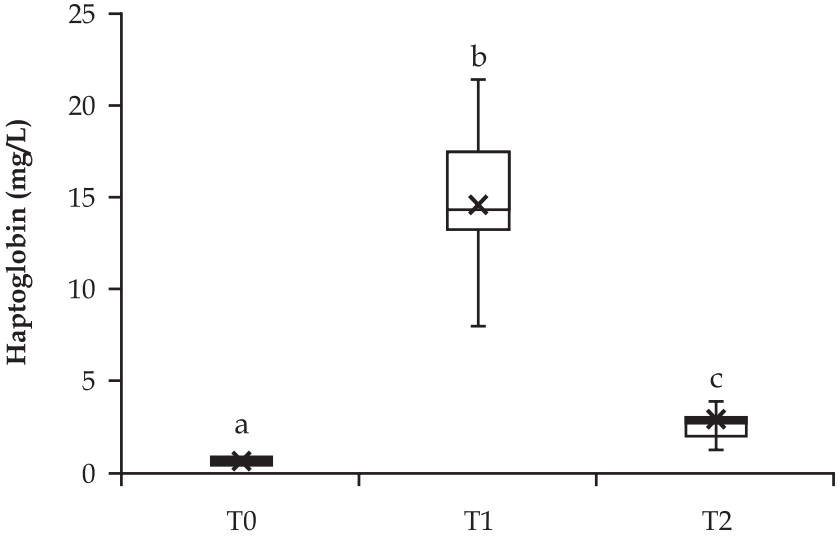

Fig 4. Box and whiskers plots of serum haptoglobin in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

and T2 are summarised in Fig 8. Compared to a value of 10.65 ± 3.14 nmol/L at T0, the serum concentration of PYD decreased significantly at T1 to a value of 5.3 ± 1.22 nmol/L (P<0.0001). At T2, the PYD serum concentration measured 6.75 ± 2.77 nmol/L, which differed significantly from T0 and T1 values (P<0.05).

Discussion

To the authors' knowledge, this is the first study to evaluate the influence of the periparturient period on the serum concentrations of the APPs and bone biomarkers in camels. The results of this study showed that APR had occurred in the female dromedary camels at parturition, manifested by significant increases in Hp and SAA as compared to 3 wk before and 3 wk after parturition. The APR is a rapid, nonspecific systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism. Acute-phase response is induced by the pro-inflammatory cytokines IL-1, TNF-α and especially IL-6 (Tizard, 2009). These cytokines activate receptors on various target cells and promote hormonal and metabolic changes leading to local and systemic effects, including APP synthesis in the liver (Petersen *et al,* 2004; Tizard, 2009). In veterinary medicine, the APPs can be used in diagnosis, prognosis and in monitoring response to therapy, as well as in general health screening (Eckersall and Bell, 2010).

Generally, APPs are secreted during the inflammatory response (Eckersall and Bell, 2010). However, in this study, the significant increases of Hp and SAA at parturition cannot be associated with pathological conditions, as the WBCs did not change significantly among T0, T1 and T2 values, thus confirming the absence of pathological conditions. These elevations could be due to cortisol and hormone release and to stress resulting in numerous changes (Huzzey *et al,* 2011). Parturition, often considered as a physical stress, represents a variety of physical and psychological stimuli that alter homeostasis and metabolism (Trevisi *et al,* 2012). The mechanism behind the stress-induced APPs release at parturition is not known, but a hypothesis based on a neuroendocrine-immune network concept has recently been put forward, indicating that non-inflammatory and psychophysical stressors activate the combined action of the sympatho-adrenal axis and the hypothalamic–pituitary–adrenal axis. This

would affect both the immunity-related cells and the release of glucocorticoids, and would ultimately lead to the production and release of APPs. In the current study, the elevations in APPs or inflammation biomarkers clearly differentiate the physiological response to parturition versus the inflammation related to pathologies during the periparturient period. Another study is therefore required to verify the influence of the periparturient period in camels with reproductive and metabolic disturbances.

Pregnancy and lactation are periods of significant influence on metabolism. Due to changes in the endocrine status and an increased need for minerals during these two periods, significant changes in mineral metabolism occur (Filipovic *et al,* 2010). In women and rodents, late pregnancy and lactation significantly influence the extent of bone remodeling (Kovacs, 2005). The rate of bone remodeling could be estimated indirectly, by measuring the concentrations/ activities of the bone remodeling markers in the blood or urine, which are indicators of the activity of bone cells (Delmas, 1995).

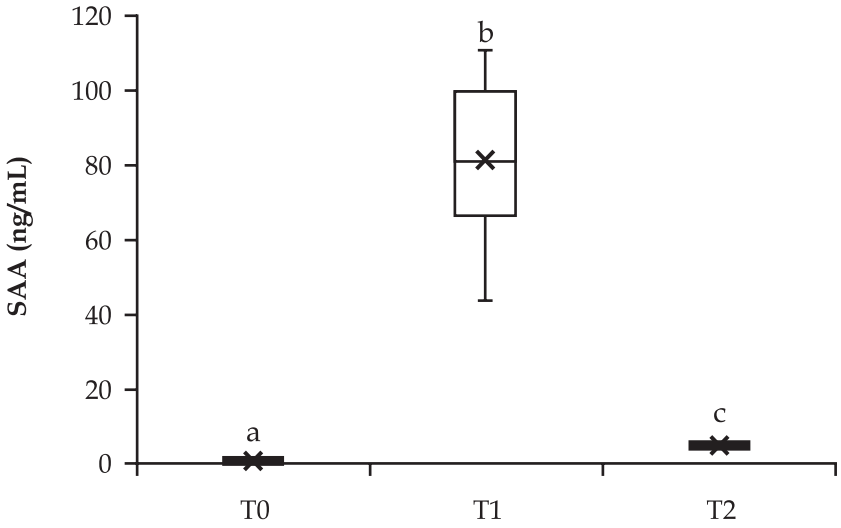

Fig 5. Box and whiskers plots of serum amyloid A (SAA) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

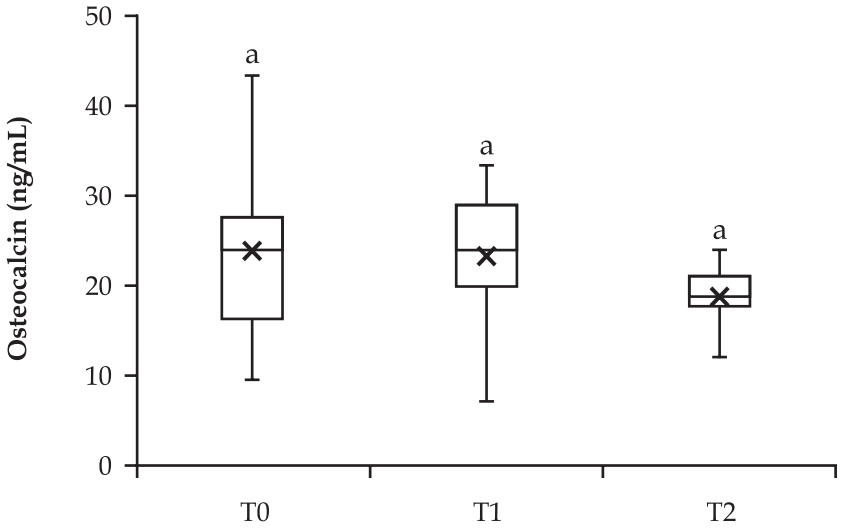

Fig 6. Box and whiskers plots of serum osteocalcin in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with same letters did not differ significantly (P>0.5).

The present investigations found that the bone formation biomarkers did not change significantly during the periparturient period of the camels. This is in agreement with data in dairy cows, where significant changes in the activity of b-ALP in the blood serum were not established during late pregnancy and early lactation (Filipovic *et al,* 2008). On the other hand, the activity of bone formation b-ALP in the serum of mares was lower in late pregnancy than in early lactation (Filipovic *et al,* 2010). In addition, a significant decrease in the b-ALP activity, an indicator of bone synthesis, was observed in the serum of ewes and goats from the first month of pregnancy up to the lambing (Liesegang *et al,* 2006, 2007). Liesegang *et al* (2006) indicated that the activity of the osteoblasts is lowered in ewes during late pregnancy.

The data in this study indicated that the bone resorption biomarker PYD decreased significantly at parturition compared to 3 wk before parturition. However, at 3 wk after parturition, the PYD serum values increased significantly compared to values at parturition. 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

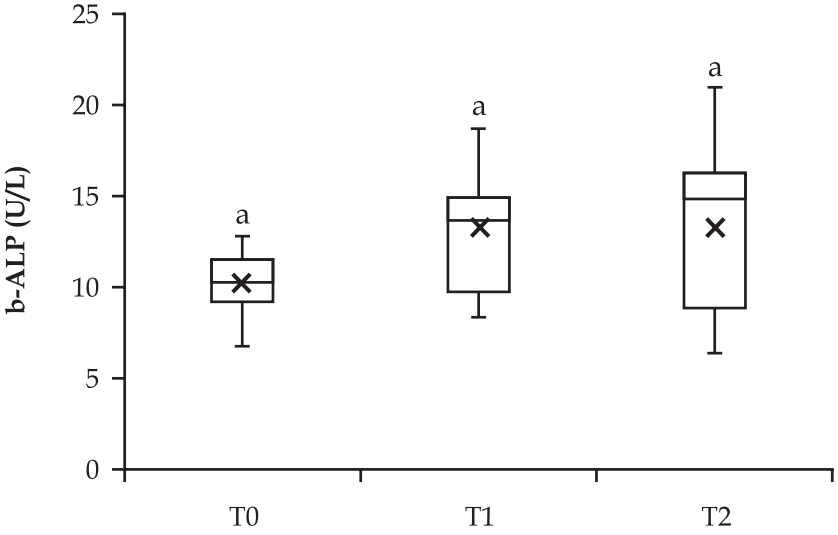

Fig 7. Box and whiskers plots of serum bone-specific alkaline phosphatase (b-ALP) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with same letters did not differ significantly (P>0.5).

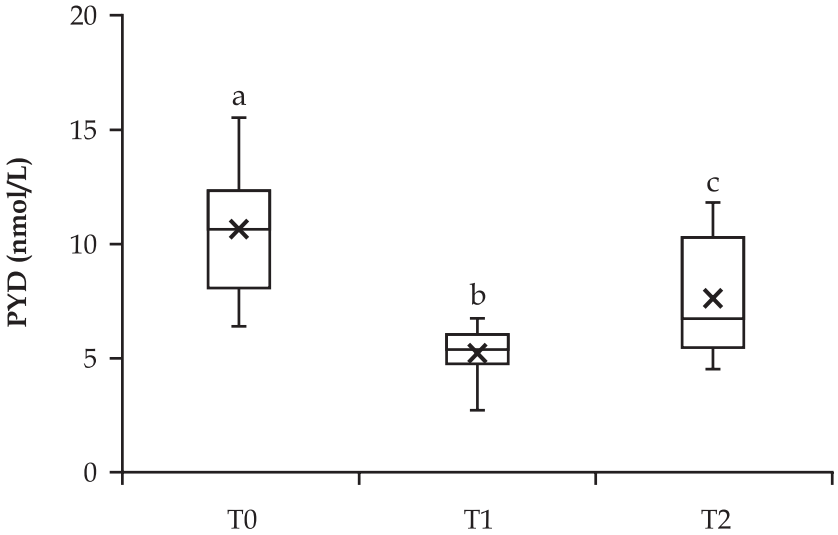

Fig 8. Box and whiskers plots of serum pyridinoline cross-links (PYD) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. **T0**, 3 wk before expected parturition; **T1**, within 12h of parturition; **T2**, 3 wk after parturition. Values with different letters differ significantly (P<0.5).

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). In the camels, the observed increase in the PYD concentration at 3 wk before and after parturition compared to values at parturition is in agreement with the data on the increase in bone resorption markers that was established at the beginning of lactation in dairy cows, ewes and goats (Liesegang *et al,* 2000, 2006, 2007; Holtenius and Ekelund, 2005; Filipovic *et al,* 2008).

This study showed a significant decrease of estrogen in the camels at 3 wk after parturition. These results are therefore consistent with a report in lactating women and another in mares, where low levels of estradiol were reported to influence the rate of bone turnover (Kovacs, 2005; Filipovic *et al,* 2010). Filipovic *et al* (2010) suggested that high concentrations of estradiol in the blood during late pregnancy could cause a decrease in the number of osteoblasts because estrogens suppress the self-renewal of osteoblast progenitors, while their anti-osteoclastogenic effects are thought to be secondary to impaired osteoblast formation (Manolagas *et al,* 2002). Hence, the lower concentrations of estradiol in the blood after parturition could contribute to an elevated bone resorption rate.

The present findings suggest that decreasing estrogen 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 estrous cycle in post-partum dairy cows, with decreases in plasma estrogen below a critical threshold correlating with enhanced bone resorption (Devkota *et al,* 2012). The same author also reported that alterations in the concentration of the bone resorption marker TRAP5b negatively correlated with estrogen levels; enhanced TRAP5b activity correlated with decreased estrogen concentrations below a defined level. This finding is consistent with the results of this study, where the estrogen level was increased at parturition while bone resorption was decreased. In mares, there was a fluctuation in the bone turnover rate during the estrous cycle; serum concentrations of the markers of bone formation and bone resorption were increased during the luteal phase compared with mares at other stages of the estrous cycle (Jackson *et al,* 2006).

The combined results of increased bone resorption at parturition in this study together with the increased estrogen concentration somewhat mirror the finding of increased serum activity of the bone resorption marker TRAP5b in post-menopausal women and the subsequent decreases in its activity in women given estrogen replacement therapy (Hallen *et al*, 2000).

This study showed that APR, manifested by significant increases of serum Hp and SAA, occurred at parturition compared to 3 wk before and after parturition. The increased Hp and SAA levels in the present study cannot be associated with pathological conditions, as evidenced by unchanged WBCs among T0, T1 and T2 time points. Furthermore, this investigation showed that the bone formation biomarkers did not change significantly during the periparturient period, while the bone resorption biomarker decreased significantly at parturition compared to 3 wk pre- and post-parturition. The significantly increased serum estrogen around parturition may have had a role in the increased bone resorption at this time.

References

Al-Sobayil FA (2010). Circadian rhythm of bone formation biomarkers in serum of dromedary camels. Research in Veterinary Science 89:455-459.

Delmas PD (1995). Biochemical markers of bone turnover. Acta Orthop. Scand. 66:(Suppl. 266):176-182.

Devkota B, Itagaki K, Kim D, Sasaki K, Osawa T, Furuhama K and Yamagishi N (2012). Relationship between sex hormone fluctuations and biomarkers of bone resorption in bovine plasma during the oestrous cycle. Veterinary Journal 194:256-258.

Eckersall PD and Bell R (2010). Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal 185:23-27.

Filipovic N, Stojevic Z, Zdelar-Tuk M and Kusec V (2008). Plasma parathyroid hormone-related peptide and bone metabolism in periparturient dairy cows. Acta Veterinaria Hungarica 56:235-244.

Filipovic N, Stojević Z, Prvanović N and Tucek Z (2010). The influence of late pregnancy and lactation on bone metabolism in mares. Research in Veterinary Science 88:405-410.

Halleen JM, Alatalo SL, Souminen H, Cheng S, Janckila AJ and Vaananen HK (2000). Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. Journal of Bone and Mineral Research 15:1337-1345.

Holtenius K and Ekelund A (2005). Biochemical markers of bone turnover in the dairy cow during lactation and the dry period. Research in Veterinary Science 78:17-19.

Huzzey JM, Nydam DV, Grant RJ and Overton TR (2011). Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows Journal of Dairy Science 94:5878-5889.

Jackson BF, Dyson PK, Hattersley RD, Kelly HR, Pfeiffer DU and Price JS (2006). Relationship between stages of the estrous cycle and bone cell activity in Thoroughbreds. American Journal of Veterinary Research 67:1527-1532.

Kovacs CS (2005). Calcium and bone metabolism during pregnancy and lactation. Journal of Mammary Gland Biology and Neoplasia 10:105-118.

Krause AR, Pfeifer LF, Montagner P, Weschenfelder MM, Schwegler E, Lima ME, Xavier EG, Brauner CC, Schmitt E, Del Pino FA, Martins CF,Corrêa MN and Schneider A (2014). Associations between resumption of postpartum ovarian activity, uterine health and concentrations of metabolites and acute phase proteins during the transition period in Holstein cows. Animal Reproduction Science 145:8-14.

Lean IJ, Van Saun R and Degaris PJ (2013). Energy and protein nutrition management of transition dairy cows. Veterinary Clinics of North America - Food Animal Practice 29:337-366.

Liesegang A, Eicher R, Sassi ML, Risteli J, Kraenzlin M, Riond JL and Wanner M (2000). Biochemical markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low standard milk yields. Journal of Dairy Science 83:1773-1781.

Liesegang A, Risteli J and Wanner M (2007). Bone metabolism of milk goats and sheep during second pregnancy and lactation in comparison to first lactation. Journal of Animal Physiology and Animal Nutrition 91:217-225.

Liesegang A, Risteli J and Wanner M (2006). The effects of first gestation and lactation on bone metabolism in dairy goats and milk sheep. Bone 38:794-802.

Manolagas SC, Kousteni S and Jilka RL (2002). Sex steroids and bone. Recent Progress in Hormone Research 57:385-409.

Moreira VR, Zeringue LK, Williams CC, Leonardi C and McCormick ME (2009). Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. Journal of Dairy Science 92:5189-5198.

Murata H, Shimada N and Yoshioka M (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. Veterinary Journal 168:28-40.

Ospina PA, McArt JA, Overton TR, Stokol T and Nydam DV (2013). Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. Veterinary Clinics of North America - Food Animal Practice 29:387-412.

Petersen HH, Nielsen JP and Heegaard PMH (2004). Application of acute phase protein measurement in veterinary clinical chemistry. Veterinary Research 35:163-187.

Rezamand P, Hoagland TA, Moyes KM, Silbart LK and Andrew SM (2007). Energy Status, Lipid-Soluble Vitamins, and Acute Phase Proteins in Periparturient Holstein and Jersey Dairy Cows With or Without Subclinical Mastitis. Journal of Dairy Science 90:5097-5107.

Sato R, Onda K, Kato H, Ochiai H, Kawai K, Iriki T, Kaneko K, Yamazaki Y and Wada Y (2013). An evaluation of the effect of age and the peri-parturient period on bone metabolism in dairy cows as measured by serum bone-specific alkaline phosphatase activity and urinary deoxypyridinoline concentration. Veterinary Journal 197:358-362.

Sato R, Onda K, Ochiai H, Iriki T, Yamazaki Y and Wada Y (2011). Serum osteocalcin in dairy cows: Age-related changes and periparturient variation. Research in Veterinary Science 91:196-198.

Schneider A, Corrêa MN and Butler WR (2013). Acute phase proteins in Holstein cows diagnosed with uterine infection. Research in Veterinary Science 95:269-271.

SPSS (2009) Statistical Package for Social Sciences. Chicago, IL, USA: SPSS, Inc. Copyright for Windows, version 18.0.

Tharwat M, Ali A, Al-Sobayil F (2015a). Hematological and biochemical profiles in goats during the transition period. Comparative Clinical Pathology 24:1-7.

Tharwat M, Ali A and Al-Sobayil F (2015b). The impact of racing on serum concentrations of acute phase proteins in racing dromedary camels. Comparative Clinical Pathology 24:575-579.

Tharwat M, Oikawa S and Buczinski S (2012b). Ultrasonographic prediction of hepatic fat content in dairy cows during the transition period. Journal of Veterinary Science & Technology 3:1.

Tharwat M, Takamizawa A, Hosaka YZ, Endoh D and Oikawa S (2012a). Hepatocyte apoptosis in dairy cattle during the transition period. Canadian Journal of Veterinary Research 76:241-247.

Tizard IR (2009). Veterinary Immunology an Introduction, 8th ed. Saunders Elsevier, St. Louis, Missouri.

Trevisi E, Amadori M, Cogrossi S, Razzuoli E and Bertoni G (2012). Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Research in Veterinary Science 93:695-704.