DOI : 10.5958/2277-8934.2020.00040.5 *Vol 27 No 3, p 295-300*

EFFECT OF GENERAL ANAESTHESIA WITH HALOTHANE OR ISOFLURANE ON SERUM CONCENTRATIONS OF INFLAMMATION AND BONE BIOMARKERS IN DROMEDARY CAMELS

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

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

This study was carried out to determine the effect of halothane or isoflurane general anaesthesia on the serum concentrations of the inflammation biomarkers C-reactive protein (CRP) and haptoglobin (Hp), and on the bone biomarkers osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and pyridinoline cross-links (PYD) biomarkers. Six healthy female camels were premedicated with xylazine and anaesthesia was induced with ketamine and maintained with either isoflurane (isoflurane group, *n*=6) or halothane (halothane group, *n*=6). A washout period of 2 weeks was allowed between the two anaesthetic protocols. Three blood samples were obtained from each camel; immediately before anaesthesia (T0), 80 min of recovery (T1), and 48 h after recovery from anaesthesia (T2). In the halothane group, the CRP values decreased significantly 48 h after anaesthesia compared to preanaesthetic values (P=0.04). In the isoflurane group, the serum concentration of CRP has increased significantly 80 min after recovery compared to preanaesthetic values (P=0.0009), but decreased significantly 48 h after anaesthesia compared to 80 min of recovery (P=0.0005). The most important finding in the halothane and isoflurane groups in the current study was the sharp increase in the serum concentration of Hp where it dramatically increased from 0.2±0.04 mg/L preanaesthetic (T0) in both groups to 43.4 mg/L and 20.8±4.6 mg/L (T1), respectively. The bone formation (OC, b-ALP) and bone resorption (PYD) biomarkers serum levels in this investigation did not show any significant changes following either halothane or isoflurane general anaesthesia compared to preanaesthetic values at any test point. In conclusion, isoflurane is superior to halothane as an inhalation anaesthetic in dromedary camels as acute phase reaction occurred sharply in halothane compared to isoflurane anesthetised camels.

Key words: Anaesthesia, bone biomarkers, camels, halothane, inflammation biomarkers, isoflurane

The inflammation biomarkers or acute phase proteins (APPs) are group proteins whose concentrations decrease or increase in sera in animals subjected to external or internal challenges (Eckersall and Bell, 2010). The acute-phase response (APR) is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism during inflammatory events (Tharwat, 2020a).

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 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 (Tharwat and Al-Sobayil, 2020).

The use of inhalation anaesthetics such as halothane, isoflurane, and sevoflurane in large animals is increasing, especially for prolonged procedures (Ahmed *et al,* 2015; Al-Sobayil *et al,* 2016). The aim of the present study was to determine the effect of halothane or isoflurane general anaesthesia after xylazine and ketamine administration on the serum concentrations of the inflammation biomarkers CRP and Hp, and bone biomarkers OC, b-ALP and PYD.

Materials and Methods

Camels, anaesthesia and blood sampling

Six adult female dromedary camels (*Camelus dromedarius*) weighing 321-503 kg and aged 5-12 y

SEND REPRINT REQUEST TO MOHAMED THARWAT email: mohamedtharwat129@gmail.com

were used. The experiment was divided into two parts, two weeks apart, each using six camels. The camels were premedicated with xylazine HCl (0.2 mg/kg, IV, Bomazine 10%, BOMAC Laboratories Ltd., New Zealand). Anaesthesia was induced 20 min later with Ketamine (2 mg/kg, IV, Ketamine 10%, Alfasan, Woerden, Holland) and was maintained with either isoflurane (Floran, HIKMA Pharmaceuticals, Amman, Jordan) or halothane (Anestane®, HIKMA Pharmaceuticals, Amman, Jordan) using 100% oxygen at a flow rate of 6 L/min. The animals were first subjected to isoflurane and then to halothane anaesthesia, with a washout period of 2 weeks between the two anaesthetic protocols. The anesthetised camel was moved onto a padded operating table, positioned in right lateral recumbency and was connected *via* the endotracheal tube with a semiclosed-circle rebreathing anaesthetic machine (SurgiVet Foal Circuit Set, Smith Medical North America, Waukesha, WI, USA). Anaesthesia was discontinued after 1 h and the camels received supplemental oxygen (6 L/min) through the endotracheal tube. After tracheal extubation, oxygen was insufflated through a nasal tube until sternal recumbency was achieved. Three blood samples were obtained from each camel for serum analyses of inflammation and bone metabolism biomarkers. The first blood sample was collected immediately before anaesthesia (T0), the second (T1) was collected 80 min of recovery, and the third was collected 48 h after recovery from anaesthesia (T2).

Assays of inflammation and bone metabolism biomarkers

The serum concentration of CRP was determined using a commercially available human kit (Minineph Hunan CRP kit, Binding Site Group Ltd.), according to the manufacturer's instructions. The determination of soluble antigen concentration by nephelometric method involves a reaction with the antibody bound to a latex particle to form insoluble complexes. When light is passed through the suspension, a portion of the light is scattered by a photodiode. The amount of light scattered is directly proportional to the CRP concentration in the serum samples. The analytical sensitivity of the assay was 0.44 mg/L, and intra- and inter-assay CVs were 3.6-6.6% and 3.6-6.0%, respectively. The serum concentration of Hp was determined in the sera using a colorimetric assay (Haptoglobin kit, second generation, Tridelta Ltd., Ireland) as reported recently (Tharwat and Al-Sobayil, 2015a; Tharwat and Al-Sobayil, 2015b; Tharwat and Al-Sobayil, 2018a; Tharwat, 2020a; Tharwat, 2020b). The analytical sensitivity of the assay was 0.0005 mg/mL, and intra- and inter-assay CVs were 5-6% and 4-6%, respectively.

The serum concentrations of the bone metabolism biomarkers OC, b-ALP and PYD were determined using commercial human immunoassay kits (Metra Biosystems Inc., a division of Quidel Corp.) as reported recently (Tharwat and Al-Sobayil, 2015b; Tharwat and Al-Sobayil, 2018a; Tharwat and Al-Sobayil, 2018b; Tharwat and Al-Sobayil, 2020). The limit of quantification of OC ranged from 2 to 32 ng/mL, and precision CVs within and between runs were 5-10%. The dynamic range of BAP was 2-140 U/L, and precision CVs within and between runs were 4-6% and 5-8%, respectively. The dynamic range of PYD was 15-750 nmol/L, and precision CVs within and between runs were 6-10% and 3-11%, respectively.

Statistical method

Data are presented as means ± SD, and were analysed statistically using the SPSS statistical package (SPSS, Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA, Copyright© for Windows, version 18.0, 2009). A repeated measures analysis of variance was employed as the statistical model to evaluate the differences over time in the isoflurane and halothane anesthetised camels. The Duncan test was used to calculate multiple comparisons. Student t test was used to evaluate differences between isoflurane and halothane anesthetised animals. Results were considered significant at $P<0.05$.

Results and Discussion

The serum concentrations of CRP in 130 and isoflurane anaesthetic camels are illustrated in Fig 1A. In the halothane group, although the serum concentration of CRP decreased 80 min after recovery (T1) (1.7±0.2 mg/L compared to 2.7±1.8 mg/L preanaesthesia); this decrease was insignificant (P=0.1). Forty eight hours after anaesthesia (T2), the CRP values (1.2±0.2 mg/L) decreased significantly compared to preanaesthetic values (T0) (P=0.04) and compared to 80 min of recovery (P=0.0002). In the isoflurane group, the serum concentration of CRP was increased significantly 80 min after recovery (5.1±2.0 mg/L) compared to preanaesthetic values (1.8±0.4 mg/L) (P=0.0009). At 48 h after anaesthesia, the CRP value (1.6±0.3 mg/L) were decreased significantly compared to 80 min of recovery (P=0.0005), but not significantly when compared to preanaesthetic values (P=0.2).

The serum concentrations of Hp in halothane or isoflurane anaesthetic camels are illustrated in Fig 1B. In the halothane group, the serum concentration of Hp has increased sharply 80 min after recovery (T1) (43.4 mg/L vs 0.2±0.04 mg/L preanaesthetic; p=0.0005). Forty eight hours after anaesthesia (T2), the Hp value decreased to 14.3±2.7 mg/L; a significant difference when compared to preanaesthetic value (T0) (P=0.0001) and when compared to 80 min of recovery (P=0.01). In a similar pattern, in the isoflurane group, the serum concentration of Hp has increased dramatically 80 min after recovery (20.8±4.6 mg/L vs 0.2±0.05 mg/L preanaesthetic; p=0.0001). Forty eight hours after anaesthesia, the Hp value decreased to 3.7±2.6 mg/L; a significant difference when compared to preanaesthetic value (P=0.002) and when compared to 80 min of recovery (P=0.0001).

Fig 2A illustrates the serum concentrations of OC in halothane or isoflurane anaesthetic camels. In the halothane group, the serum OC values were 26.6±18.4 ng/mL preanaesthesia (T0), 23.1±5.1 ng/mL 80 min of recovery (T1) and 28.3±20.1 ng/mL 48 h after anaesthesia (T2) with no significant difference among the 3 values (P>0.05). In the isoflurane group, the serum OC values were 25.3±9.2 ng/mL preanaesthesia, 24.2±20.5 ng/mL 80 min of recovery and 26.8±23.8 ng/mL 48 h after anaesthesia with no significant difference among the 3 values (P>0.05).

The serum concentrations of b-ALP in halothane or isoflurane anaesthetic camels are illustrated in Fig 2B. In the halothane group, the serum OC values were 14.8±4.0 U/L preanaesthesia (T0), 16.1±5.4 U/L 80 min of recovery (T1) and 16.9±6.71 U/L 48 h after anaesthesia (T2) with no significant difference among the 3 values (P>0.05). In the isoflurane group, the serum OC values were 12.3±3.3 U/L preanaesthesia, 12.0±4.7 U/L 80 min of recovery and 13.0±6.2 U/L 48 h after anaesthesia with no significant difference among the 3 values (P>0.05).

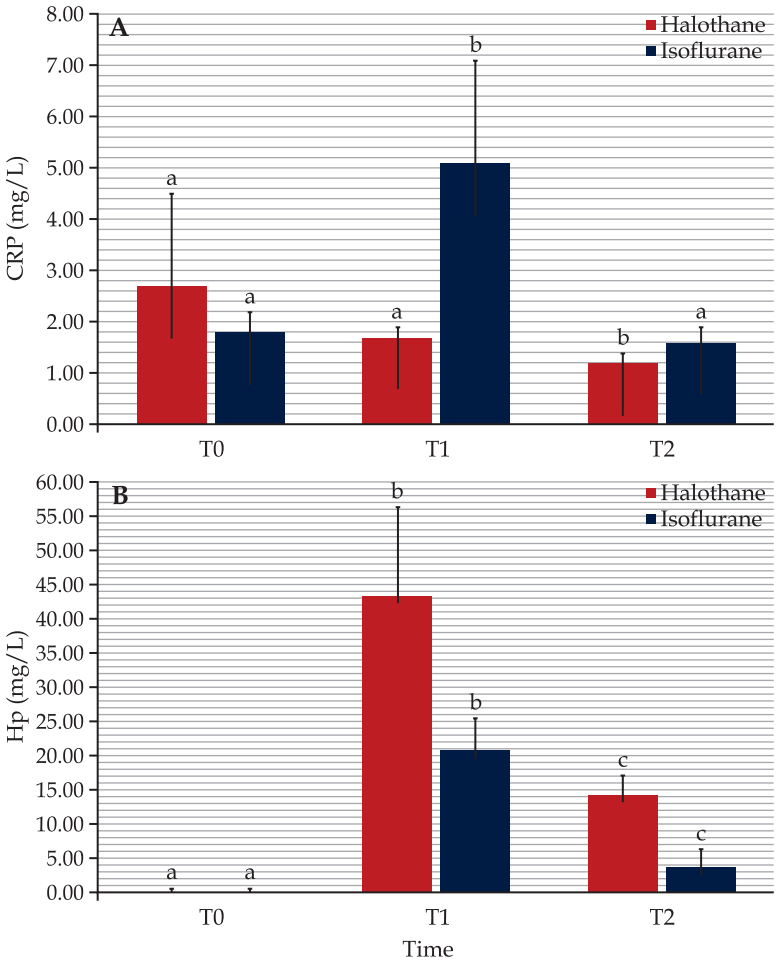

Fig 1. Serum concentrations of acute phase protein C-reactive protein (CRP) (**A**) and haptoglobin (Hp) (**B**) in camels (n = 6) undergoing isoflurane and halothane anaesthesia. T0, immediately before anaesthesia; T1, 80 min of recovery; T2, 48 h after anaesthesia. Different letters differ significantly at P<0.05.

Fig 2C illustrates the serum concentrations of PYD in halothane or isoflurane anaesthetic camels. In the halothane group, the serum OC values were 7.6±1.7 nmol/L preanaesthesia (T0), 5.8±0.4 nmol/L 80 min of recovery (T1) and 8.9±3.8 nmol/L 48 h after anaesthesia (T2) with no significant difference among the 3 values (P>0.05). In the isoflurane group, the serum OC values were 8.9±4.5 nmol/L preanaesthesia, 8.0±2.8 nmol/L 80 min of recovery and 6.8±3.5 nmol/L 48 h after anaesthesia with no significant difference among the 3 values (P>0.05).

To the authors' knowledge, this is the first study in dromedary camels evaluating the effect of general anaesthesia by either isoflurane or halothane on the serum concentrations of inflammation (CRP and Hp) and bone metabolism (OC, b-ALP and PYD) biomarkers.

The APR is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism (Yazwinski *et al*, 2013). This response is induced by the pro-inflammatory cytokines IL-1, TNF-α and especially IL-6. 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). The APPs can be used in diagnosis, prognosis and in monitoring response to therapy, as well as in general health screening and animal welfare (Eckersall, 2000; Eckersall and Bell, 2010). The APPs have received attention as biomarkers for APR due to its low physiological levels, a fast incline, marked rise in concentration during APR that eases detection and a fast decline after cessation of a stimulus (Murata *et al,* 2004; Murata, 2007; Tharwat, 2020a).

In the present study, the serum concentration of the acute phase protein CRP decreased insignificantly in the halothane group 80 min after recovery from anaesthesia (T1) and significantly 48 h after anaesthesia (T2). On the contrary, the serum concentration of CRP in the isoflurane group increased significantly 80 min after recovery compared to preanaesthetic values (T0). However, 48 h later, the CRP value in the same group decreased significantly compared to 80 min of recovery. In human medicine, CRP was used as a marker of the surgical stress reduction within an enhanced recovery after surgery protocol. It was found that, the presence of complications post operation was independently associated with an increase in CRP values (Olivares *et al,* 2018). Furthermore, the CRP blood level in humans was estimated to depend on the method of anaesthesia. It was found that the smallest increase in plasma CRP was found in patients operated on under regional anaesthesia compared with those operated on under general anaesthesia (Kolomachenko, 2018). In dogs with anaesthetic protocols using sevoflurane or a combination of fentanyl, midazolam, and sevoflurane, a significant difference in serum CRP concentrations was not detected post-operatively between groups at any time point. However, serum CRP concentrations in the same study were significantly increased post-anaesthetic induction in both groups. These significant increases were attributed to surgical trauma (Saunders *et al,* 2009). Following ovariohysterectomy in female dogs due to naturally occurring pyometra, the serum concentrations of CRP in two different anaesthesia and analgesic protocols

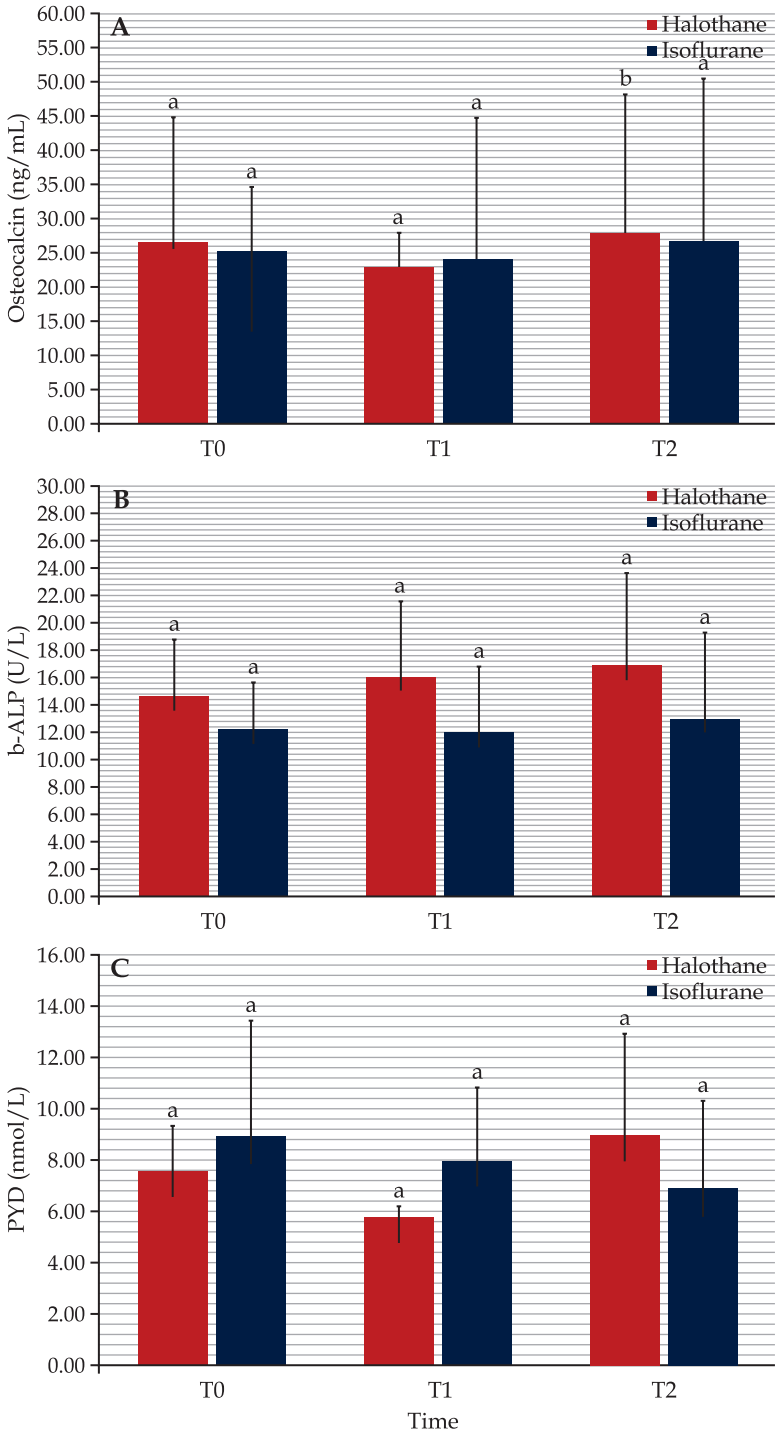

Fig 2. Serum concentrations of bone biomarkers osteocalcin (A), bone-specific alkaline phosphatase (b-ALP) (B) and pyridinoline cross-links (PYD) (C), in camels (n = 6) undergoing isoflurane and halothane anaesthesia. T0, immediately before anaesthesia; T1, 80 min of recovery; T2, 48 h after anaesthesia. Same letters did not differ significantly at P>0.05.

with and without low-dose ketamine were essentially the same before surgery, but significantly increased in the control group and decreased in ketamine group at 48 hours after surgery. It was concluded from that study that low-dose ketamine attenuated the postoperative concentration of serum CRP in dogs with pyometra compared with dogs that did not receive ketamine in the perioperative period (Liao *et al,* 2014).

The most important finding in the halothane and isoflurane groups in the present study was the sharp increase in the serum concentration of the acute phase protein Hp where it dramatically increased from 0.2±0.04 mg/L preanaesthetic (T0) in both groups to 43.4 mg/L and 20.8±4.6 mg/L (T1), respectively. Further, the Hp values in both groups declined at 48 h after anaesthesia (T2), but still significant when compared to preanaesthetic values. Postoperative changes of serum concentrations of Hp were studied in women undergoing elective hysterectomy during either general anaesthesia or epidural analgesia. Results showed that Hp was reduced during the first postoperative day, followed by a gradual, significant increase of haptoglobin to 140% above preoperative levels on day 7 after surgery (Rem *et al,* 1980).

The OC, a product of the osteoblasts, is regarded as a sensitive indicator of bone formation (Pullig *et al,* 2000). In addition to its increase that accompany skeletal growth, weight-bearing exercise induces changes in serum concentrations of OC (Eliakim *et al,* 1997). 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, 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 PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption (Thompson *et al,* 1992; Tharwat and Al-Sobayil, 2020).

The bone formation (OC, b-ALP) and bone resorption (PYD) biomarkers serum levels in this investigation did not showed any significant changes following either halothane or isoflurane general anaesthesia compared to preanaesthetic values at any test point. In response to isoflurane-induced anaesthesia in young female guinea pigs the bone metabolism biomarkers OC, but not deoxypyridinoline, increased (Tabatabaei *et al,* 2015). In another study conducted in cynomolgus monkeys, the anaesthetic isoflurane decreases ionised calcium and increased the OC secondary to the decrease in ionised calcium (Hotchkiss *et al,* 1998). In a study carried out in 36 female patients undergoing elective total hip replacement, OC as well as b-ALP concentrations decreased significantly until 72 h post-surgery. Increased cortisol secretion and other hormonal and inflammatory components of the peri-operative stress response may play a role in mediating this response (Nicholson *et al,* 2002). In another study conducted in humans scheduled for general anaesthesia due to hip fracture, serum concentrations of the bone formation biomarkers OC and b-ALP did not change significantly until 12 weeks postoperative (Biricik *et al,* 2019). It was concluded from the results of this study that isoflurane is superior to halothane as an inhalation anaesthetic in dromedary camels. Acute phase reaction occurred sharply in halothane anesthetised camels as indicated by a remarkable increase in the serum concentration of Hp (two fold increase when compared with isoflurane anesthetised camels). The bone formation and resorption biomarkers did not change significantly by either halothane or isoflurane general anaesthesia.

References

Ahmed F, Al-Sobayil F and El-Tookhy O (2015). Evaluation of halothane anaesthesia after xylazine/ketamine administration in dromedary camels (*Camelus dromedarius*). Journal of Camel Practice and Research 22:151-158.

Al-Sobayil FA (2010). Circadian rhythm of bone formation biomarkers in serum of dromedary camels. Research in Veterinary Science 89:455-9.

Al-Sobayil F, Ahmed F and El-Tookhy O (2016). Evaluation of isoflurane anaesthesia after xylazine/ketamine administration in dromedary camels. Turkish Journal of Veterinary and Animal Sciences 40:622-629.

Biricik E, Karacaer F, Gulec E, Matyar S, Ilgınel M, Bicer OS and Ozcengiz D (2019). Long term effect of general and regional anaesthesia on bone turnover markers and fracture healing in adult patients. Cukurova Medical Journal 44(2):1.

Delmas PD, Price PA and Mann KG (1990). Validation of the bone gla protein (osteocalcin) assay. Journal of Bone and Mineral Research 5:3-4.

Eckersall PD (2000). Acute phase proteins as markers of infection and inflammation: monitoring animal health, animal welfare and food safety. Irish Veterinary Journal 53:307-311.

Eckersall PD and Bell R (2010). Acute phase proteins:

Biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal 185:23-27.

Eliakim A, Raisz LG, Brasel JA and Cooper DM (1997). Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. Journal of Bone and Mineral Research 12:1708-1713.

Hotchkiss CE, Brommage R, Du M and Jerome CP (1998). The anaesthetic isoflurane decreases ionised calcium and increases parathyroid hormone and osteocalcin in cynomolgus monkeys. Bone 23:479-484.

Kolomachenko VI (2018). C-reactive protein level in plasma and drainage blood depends on the method of anaesthesia and post-operative analgesia after hip surgery. Anaesthesiology Intensive Therapy 50:117-121.

Liao PY, Chang SC, Chen KS and Wang HC (2014). Decreased postoperative C-reactive protein production in dogs with pyometra through the use of low-dose ketamine. Journal of Veterinary Emergency and Critical Care (San Antonio) 24:286-290.

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

Murata H (2007). Stress and acute phase protein response: an inconspicuous but essential linkage. Veterinary Journal 173:473-474.

Nicholson G, Bryant AE, Macdonald IA and Hall GM (2002). Osteocalcin and the hormonal, inflammatory and metabolic response to major orthopaedic surgery. Anaesthesia 57:319-325.

Olivares MC, Martínez ML, Torralba M, Fraile JRR and Martínez JCA (2018). C-reactive protein as a marker of the surgical stress reduction within an ERAS protocol (Enhanced Recovery After Surgery) in colorectal surgery: A prospective cohort study. Journal of Surgical Oncology 117:717-724.

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

Pullig O, Weseloh G, Ronneberger D, Käkönen S and Swoboda B (2000). Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. Calcified Tissue International 67:230-240.

Rem J, Nielsen OS, Brandt MR and Kehlet H (1980). Release mechanisms of postoperative changes in various acute phase proteins and immunoglobulins. Acta Chirurgica Scandinavica Supplementum 502:51-6.

Saunders AB, Hanzlicek AS, Martinez EA, Stickney MJ, Steiner JM, Suchodolski JS and Fosgate GT (2009). Assessment of cardiac troponin I and C-reactive protein concentrations associated with anaesthetic protocols using sevoflurane or a combination of fentanyl, midazolam, and sevoflurane in dogs. Veterinary Anaesthesia and Analgesia 36:449-456.

Tabatabaei N, Rodd CJ, Kremer R and Weiler HA (2015). Osteocalcin, but not deoxypyridinoline, increases in response to isoflurane-induced anaesthesia in young female guinea pigs. Journal of Bone and Mineral Metabolism 33:253-260.

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

Tharwat M and Al-Sobayil F (2015b). Serum concentrations of acute phase proteins and bone biomarkers in female dromedary camels during the periparturient period. Journal of Camel Practice and Research 22:271-278.

Tharwat M and Al-Sobayil F (2018a). Influence of electro-ejaculator on serum concentrations of acute phase proteins and bone metabolism biomarkers in male dromedary camels (*Camelus dromedarius*). Journal of Applied Animal Research 46:1226-1232.

Tharwat M and Al-Sobayil F (2018b). The impact of racing on serum concentrations of bone metabolism biomarkers in racing Arabian camels. Journal of Camel Practice and Research 25:59-63.

Tharwat M and Al-Sobayil F (2020). A review on biomarkers of bone metabolism in camels (*Camelus dromedarius*). Journal of Camel Practice and Research 27:23-29.

Tharwat M (2020a). Biomarkers of infection and inflammation in camels (*Camelus dromedarius*). Journal of Camel Practice and Research 27:159-163.

Tharwat M (2020b). *Hyalomma dromedarii* ticks induce a distinct acute phase reaction in dromedary camels. Journal of Camel Practice and Research. In press.

Thompson PW, Spector TD, James IT, Henderson E and Hart DJ (1992). Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis. British Journal of Rheumatology 31:759-761.

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

Yazwinsk M, Milizio JG and Wakshlag JJ (2013). Assessment of serum myokines and markers of inflammation associated with exercise in endurance racing sled dogs. Journal of Veterinary Internal Medicine 27:371-376.