The Journal of Animal & Plant Sciences, 25(4): 2015, Page: 1072-1080
ISSN: 1018-7081

ALTERED SERUM ENZYMES AND BIOCHEMICAL LEVELS IN ARABIAN RACING CAMELS WITH BONE FRACTURES

N. S. Alshamsi[+], T. S. Ksiksi and S. S. Ashraf[*]

College of Science, UAE University, Alain, U. A. E.; [+]Management of Scientific Center and Presidential Camels, Alain, United Arab Emirates
[*] Corresponding author's email address: salman.ashraf@uaeu.ac.ae

ABSTRACT

Analysis of blood serum enzymesis an important and routine diagnostic tool commonly used to assess the well-being and metabolic status of racing camels. Unfortunately there are no well defined gender-specific published reference ranges for serum enzymes for healthy, lame and fractured camels. In this study, we report baseline reference range values for Glucose, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Gamma-glutamyltransferase (GGT), Albumin (ALB), Creatinine, Blood Urea Nitrogen (BUN), Intact Parathyroid Hormone (N-PTH), Osteocalcin (OC), Undercarboxylated Osteocalcin (UCOC) and Total Iron binding capacity (TIBC) in healthy (n=60), lame (n=31) and bone-fractured (n=20) racing camels. In addition to documenting gender-specific reference ranges for various biochemical parameters, we report for the first time significant differences in these parameters between healthy, lame and bone-fractured camels. Our results show that of the 15 parameters analyzed, 13 of the hematological parameters in females and 11 parameters in male camels showed significant differences between at least one of the groups – healthy vs. lame, lame vs. fractured, or healthy vs. fractured. In conclusion, the present study clearly shows that some serum enzymes and biochemical levels are altered in racing camels with bone impairment and fractures.

Key words:

INTRODUCTION

Racing camels are culturally and economically important animals, especially in the Middle East (Charles 1982). Unfortunately, until recently, not much is known and published about the various factors that can affect bone health in these animals. We had previously carried out a detailed hematological analysis of healthy, lame and bone-fractured camels and published statistical analysis (gender-based) of differences in 13 parameters between these three racing camel groups (Alshamisi, Ksiksi, and Ashraf 2013). Our results showed that a statistical model could be derived from the routine hematological analysis of camel blood which could help in predicting 'at-risk' camels, before they develop bone fractures. Another routine laboratory analysis that can be carried out on blood samples are serum enzyme levels as well as various other biochemicals. There are several groups that have reported reference values for diagnostic enzymes in camel blood, but they are mostly more than 25 years old studies, they are not very detailed and most importantly, they are only carried out on healthy camels. In this report, we present data from 15 different enzyme and biochemicals from serum of a large number of healthy (n=60), lame (n=31) and bone-fractured (n=20) racing camels. The parameters that we report here and their significance (in brief) are described below:

Glucose: Glucose is a measured parameter that is used to evaluate carbohydrate metabolism(Washington and Hoosier 2012). It is also known to affect bone metabolism in both mice and humans (Harris *et al*. 2003). There is extensive evidence which links diabetes with low bone formation in humans (Hofbauer *et al*. 2007; Leidig-Bruckner, Ziegler, and others 2001; Bouillon *et al*. 1995; Krakauer *et al*. 1995) and animals (Shyng, Devlin, and Sloan 2001; Verhaeghe *et al*. 1990) alike.

Alkaline phosphatase (ALP): Blood serum alkaline phosphatase (ALP) is a hydrolytic enzyme which removes phosphate from different molecules. It is mainly secreted by osteoblasts which play a vital role in the metastatic process of bone destruction (Roodman 2004). Alkaline phosphatase is considered a marker for osteoblastic activity (Dimai *et al*. 1998). It has been shown that ALP serum levels increase in bone metabolism (Alata *et al*. 2002).

Aspartate aminotransferase (AST): Aspartate aminotransferase (AST), catalyzes the reversible transfer of an -amino group between aspartate and glutamate (Washington and Hoosier 2012). It is a vital enzyme in amino acid metabolism. AST is commonly measured clinically as a marker for liver disease. Both the gender and age of racing horses are known to significantly affect creatine kinase (CK) and AST activities(Harris *et al*. 1990). Moreover, a high AST/ALT ratio and a low PLT

count can predict liver cirrhosis in chronic HCV infection (Pohl *et al*. 2001).

Lactate Dehydrogenase (LDH): Lactate dehydrogenase (LDH) is an enzyme known to catalyze the conversion of pyruvate to lactate, with the conversion of NADH to NAD^+ (Washington and Hoosier 2012). A high LDH level is an indicator of organ infarction and significant cell death, which includes cellular cytoplasmic loss. There are several disease conditions which are linked with elevated levels of LDH such as hepatitis, shock, hypoxia, extreme hypothermia, and meningitis (Washington and Hoosier 2012).

Creatine Kinase (CK): Creatine kinase (CK) is an enzyme known to catalyze the phosphorylation of creatine (Washington and Hoosier 2012). Creatine kinase levels increase subsequent to exercise in humans (Noakes and others 1987) and animals (Shumate *et al*. 1979). Creatine kinase is also recommended for use as an indicator for lameness via carpal osteochondral fragmentation in horses (Chaney *et al*. 2004).

Gamma-glutamyltransferase (GGT): Gamma ( )-glutamyltransferase (GGT) is an enzyme known to contribute to the extracellular catabolism of glutathione (GSH) (Grundy 2007) and is an important tool for the diagnosis of liver disorders. The measurement of urine GGT levels may be regarded as a useful marker for individuals who may have increased bone turnover, which increases the risk for bone fractures (Asaba *et al*. 2006).

Albumin (ALB): Albumin (ALB) is a protein with the highest concentration levels found in blood plasma (Soni and Margarson 2004). Albumin is formed exclusively in the liver and serves as a transport and binding protein for calcium, fatty acids, bilirubin, hormones, vitamins, trace elements, and drugs (Soni and Margarson 2004). Decreased serum albumin concentrations can be a consequence of liver disease. Decreased serum albumin may also be a result of malnutrition or of a low protein diet.

Creatinine: Creatinine is a product of the break-down of creatine phosphate in muscle (Washington and Hoosier 2012). It is used as an indicator of kidney function. Serum creatinine is increased by deficient glomerular filtration. It also increases by necrosis or atrophy of the skeletal muscle, hyperthyroidism, infections, burns, or fractures.

Blood Urea Nitrogen (BUN): Urea is a break-down product of protein metabolism (Washington and Hoosier 2012). Concentrations of blood urea nitrogen are used to test for kidney function. The concentration level of BUN can be affected by circadian rhythm, diet, liver function, hydration, and intestinal absorption (Blood Urea Nitrogen (BUN) 2012). A low concentration of blood urea nitrogen (BUN) may be due to low protein intake, liver failure, or treatment with anabolic steroids.

Calcitonin: Calcitonin is a hormone known to control calcium metabolism by inhibiting bone resorption and thus has been shown to increase bone quality in various animal models used within the studies (Wallach *et al*. 1999). In human studies, calcitonin is shown to increase lumbar vertebral cortical thickness(Mosekilde, Danielsen, and Gasser 1994). Postmenopausal women have been shown to have lower calcitonin levels in comparison to premenopausal females (Reginster *et al*. 1990).

Intact Parathyroid Hormone (N-PTH): Parathyroid Hormone is one of the most important hormones in both calcium and phosphate metabolism. Many studies have shown that the parathyroid hormone improves bone growth (Pettway *et al*. 2008; Dempster *et al*. 1993). One of the ways in which it stimulates an increased blood calcium concentration is by stimulating bone resorption. An alternative way is by reducing renal calcium excretion and increasing renal phosphate excretion. It also promotes bioactive vitamin D production by kidneys, which in turn, enhances calcium and phosphate absorption by the small intestine. Parathyroid hormone has many fragments. Intact PTH is mainly known as the biologically active form of PTH (Murray *et al*. 2005), while carboxyl-terminal or C-terminal PTH is known as the inactive form of PTH. The inactive C-terminal PTH is, however, present in the circulation in large amounts. PTH (1–34), which is one of the intact forms of PTH, is now approved by the Food and Drug Administration (FDA) as a primary anabolic therapy used in the treatment of severe osteoporosis in both women and men (Tashjian Jr and Gagel 2006). A multitude of studies have demonstrated that the “Intact PTH” therapy does in fact improve bone formation in humans (Noakes and others 1987; Shumate *et al*. 1979; Chaney *et al*. 2004; Grundy 2007; Whitfield 2001; Asaba *et al*. 2006; Dempster *et al*. 1993; Thomas 2006; Neer *et al*. 2001; Jiang *et al*. 2003; Ejersted *et al*. 1995; Orwoll *et al*. 2003) and animals (Dobnig and Turner 1997). The C-terminal to intact PTH ratio is important in Ca^{2+} ion metabolism (Nguyen-Yamamoto *et al*. 2001).

Osteocalcin (OC) and Undercarboxylated Osteocalcin (UCOC): Osteocalcin (OC) is a calcium-binding protein which is known to be an important protein in bone mineralization (Ivaska *et al*. 2005). It is a 49 amino acid polypeptide found exclusively in bone tissue that undergoes vitamin-K dependent gamma-carboxylation of three glutamic acid residues (Ng *et al*. 2004). In case of vitamin-K deficiency, Osteocalcin remains as undercarboxylated Osteocalcin (UCOC), which is not functional in binding calcium. A number of studies have shown that vitamin-K deficiency leads to the impairment in the carboxylation of Osteocalcin, the increase of under

carboxylated Osteocalcin content, and consequently an increase in bone fracture risk (Kim *et al*., 2010). Furthermore, undercarboxylated Osteocalcin (UCOC) is negatively correlated with femoral neck bone mineral density and bone fractures in the elderly (Fewtrell *et al*. 2008). The expression of the OC gene (and hence OC protein) depends on vitamin D, which is considered a major regulator of calcium metabolism (Vergnaud *et al*. 1997). To date, only one investigation has reported on osteocalcin in camels (Al-Sobayil 2010).

Total Iron binding capacity (TIBC): Iron is an essential dietary element as it is involved in various metabolic activities within the body such as oxygen sensing and transport, electron transfer, and catalysis (Papanikolaou and Pantopoulos 2005). Iron plays a significant role in bone metabolism (Katsumata *et al*. 2006), as iron deficiency decreases bone formation and resorption (Medeiros *et al*. 2002; Katsumata *et al*. 2009). Iron deficiency is also negatively associated with bone mineralization of the osteoblast cells (Parelman *et al*. 2006). Iron functions as a cofactor for many enzymes and is involved mainly in collagen synthesis (Ilich and Kerstetter 2000). Some studies have demonstrated that an overload of iron ingestion or intake may accumulate in bones, which may result in the development of bone diseases, such as osteoporosis in rats (Isomura *et al*. 2004). The dietary intake of iron is also associated with bone mineral density in postmenopausal women (Harris *et al*. 2003; Maurer *et al*. 2005).

MATERIALS AND METHODS

The study was conducted on racing camels, both females and males, ranging in age between one and four years. The camels were selected from different racing camps owned by the Presidential Affairs in Abu Dhabi Emirate, UAE.

Animals were fed on a ration of fresh clover, dry clover, barley, camel milk, pre-mix feeds, multi- vitamins and mineral. All camels had free access to drinking water. Three groups of camels were identified: healthy racing camels, racing camels with lameness, and racing camels with bone fractures. The three groups were referred to as normal, lame and fractured; respectively thereafter. Bone fracture was diagnosed by a veterinary doctor through a field examination and when necessary, an X-ray. Lameness was diagnosed by visual observations, if camels were limping while walking; they were classified as limping camels. Camels in the control groups consisted of apparently healthy animals. All groups were similar in age and gender (95% were between 1-4 years). Camels were split into three gender specific groups with a total of 111 individuals as outlined in Table1.

Sample collection and storage: Blood samples were collected in the morning hours, except in cases of fractures, when blood samples were collected immediately after the fracture was diagnosed.

Table 1. The three groups of racing camels used in this study.

	Number of animal	Gender
Healthy	35	Females
	25	Males
Lame	19	Females
	12	Males
Fractured	14	Females
	6	Males

BD Vacutainer Serum Separation Tubes or SST (Becton Dickinson, USA) were used to collect blood samples. Serum was obtained from SST tubes after centrifugation at 3,000 rpm for 10 minutes. Routine clinical chemistry tests were performed on these serum samples the same day, and remaining serum was aliquoted and kept at -80°C.

Measurements of various parameters:

Enzymes and biochemicals: For determination of clinical biochemistry parameters, the standard laboratory instrument, Dimension RxL Max (Siemens, USA) was used (Mendu *et al* 1007). It is a discrete, random access, microprocessor-controlled, integrated instrument, which is routinely used to measure a variety of analytes, including enzyme activities in body fluids (Dimension RxL Max Integrated Chemistry System n.d.). All reagents, calibrators and controls were obtained from Siemens. Calibration for each test was performed as per instrument schedule and two level controls were run daily for each test.

The parameters measured using the Dimension RxL system were:

A. Proteins: albumin, total protein (TP)

B. Enzymes: alkaline phosphatase (ALP) (EC 3.1.3.1), aspartate aminotransferase (AST) (EC 2.6.1.1), creatine kinase (CK)(EC 2.7.3.2), Gamma-glutamyltransferase (GGT)(E.C. 2.3.2.9), lactate dehydrogenase (LDH) (E.C. 1.1.1.27)

C. Other tests: blood urea nitrogen (BUN), creatinine, and glucose.

Calcitonin and Intact Pyrethroid Hormone (N-PTH) Measurement: Calcitonin and intact PTH were analyzed by Liaison instrument (DiaSorin, Germany). The Liaison instrument is a fully automated system which is commercially available to determine various bone markers. All reagents (magnetic particles, luminescence-labeled tracer, calibrators, diluents, and assay buffer)

were obtained from the manufacturer and used as per the supplied instruction. Data reduction was based on a master curve with a two-point recalibration method.

Measurement of Carboxylated (OC) and Undercarboxylated Osteocalcin (UCOC): Carboxylated (OC) and Undercarboxylated (UCOC) Osteocalcin were analyzed using enzyme-linked immunoassay kit (EIA) (Takara, Japan). The OC and UCOC Kits are a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal antibodies to detect OC or UCOC two-step procedure. One of the mouse monoclonal antibody is immobilized onto the micro titer plate and blocked against non-specific binding. Samples and standards are incubated in the micro titer plate. After one hour incubation, the plate is washed and second anti-OC or anti-UCOC labeled with peroxidase (POD). The bound POD-linked second OC or UCOC antibody is detected by the reaction between POD and substrate (H_2O_2 and tetramethylbenzidine) which results in color development with intensity proportional to the amount of OC or UCOC in the samples and standards. The amount of OC or UCOC can be quantitated by measuring the absorbance using an EIA plate reader. Accurate sample concentrations of osteocalcin can be determined by comparing their specific absorbance values with those obtained for the standards plotted on a standard curve.

The software package SPSS (v. 19) was used for all statistical analyses reported here.

Controls used in the analyses: In Dimension RxL Max instrument; two levels of controls were used daily - DADE Dimension RxL Max instument chemistry controls (TRU-Liquid moni-trol level 1B5150-1 and TRU-Liquid monitrol level 2 B5150-2 – both from Siemens, USA). The absorbance was also checked daily by absorbent test (DF73). A mixture of homogenization of blood samples serum were used for routine internal quality control. Two levels of standard spiking (at 0.25mg/l and 0.5mg/l) were also routinely used. In addition, proficiency testing from Center de Toxicologie du Quebec, Inter laboratory comparison program for metals in biological materials, PC-S-E was also carried out. Calcitonin II-Gen chemiluminescent immunoassay was used as a means of checking reliability of assay runs of Calcitonin (DiaSorin, 310651). For carboxylated (OC) and undercarboxylated Osteocalcin (UCOC) determination, immunoassay kits were used and samples were analyzed in duplicates.

RESULTS AND DISCUSSION

In spite of the importance and prevalence of racing camels in the Middle East, detailed biochemistry parameters are not widely published for these animals. In fact, only a handful of published reports can be found, and even they are fairly dated, not detailed and not performed on bone-health compromised camels. Therefore, we carried out a systematic analysis of various biochemical parameters in the serum of a relatively large numbers of healthy, lame and fractured racing camels (both genders). The reference values of various enzymes and biochemistry parameters in the three groups (healthy, lame, and fractured) of female and male racing camels shown in Tables 2 and 3. A comparison of the various parameters of the healthy camels show that for the most part, our reference values agree with those previously published by other groups. For example, the glucose and GGT values in our healthy racing camel groups were very similar to the values in Kuwaiti racing camels (Mohamed and Hussein 1999) and Sudan camels (Eldirdiri, Suliman, and Shommein 1987). Similarly, the results of BUN and TP match those of the Kuwaiti racing camels and Arabian camels in Iran (Mohamed and Hussein 1999; Alsaad 2009). AST values in our camels were also similar to Arabian camels in Iran (Alsaad 2009). However, breeding camels in Algeria (Aichouni *et al*. 2010), and Kuwaiti racing camels had less AST (Mohamed and Hussein 1999). AST values in our normal female and male camels were 99.9±21.4 IU/I and 100.7±16.2IU/I; respectively, whereas, AST level were 74.5±15 IU/I and 69.8 ± 23.11 IU/I in breading camels in Algeria, and Kuwaiti racing camels; respectively. The LDH values in females and males were similar to the values which is published in Sudan (Eldirdiri, Suliman, and Shommein 1987). On the other hand, the mean value of creatinine in our healthy racing females and males were 139.3±28.9 µmol/l and 124.8±16.7 µmol/l; respectively, which is higher than the mean value of Kuwaiti racing camels and lower than the average values of breeding camels in Algeria. Kuwaiti racing camels had 174.15 ±29.17 µmol/l of creatinine. While breeding camels in Algeria had 96.05±2.51 µmol/l of creatinine. The mean value of ALP and CK was also higher in Kuwaiti racing camels than our mean values in healthy racing camels. Kuwaiti racing camels had mean value of 95.12±36.8 IU/l and 143.634±79.63 IU/l for ALP and CK; respectively. Our mean value of ALP and CK in normal female racing camels group was 190.9±89.2 IU/l and 105.1±113.1 IU/l; respectively. While in males, ALP and CK mean vales were 193.3±29.2 IU/l and 84.2±96.2 IU/I; respectively. On the other hand, the average CK values were lower in breeding camels (96.05±2.51 IU/I) than our results. Some studies show a seasonal variation in chemical parameters (Salman and Afzal 2004; Kataria and Bhatia 1991). TP, albumin, CK and creatinine values are stable during summer and winter(Salman and Afzal 2004). However, BUN and iron are higher in winter. Some papers conclude that LDH activity is higher in summer (Salman and Afzal 2004) while other papers reported that LDH activity is higher in winter (Kataria and Bhatia 1991). The activity of AST, and ALP in

higher during hot conditions (Kataria and Bhatia 1991). The activity of AST and ALP in males is higher than females (Kataria and Bhatia 1991). In this study we have very similar results for AST and ALP in females and males racing camels.

Statistical (t-test) analyses of significant differences in these biochemistry parameters (enzymes and biochemicals) showed that there were significant in 13 out of 15 parameters between at least one of the groups - healthy vs. lame, lame vs. fractured, or healthy vs. fractured in female racing camels (Table 2). While in males, 10 out of 15 parameters were significantly different between at least one of the three groups (Table 3). This is a very interesting and novel finding, as such a comparative study has not been previously carried out and clearly shows that even camels that have not yet developed bone fractures (i.e. lame camels) have significant biological and metabolic differences as compared to healthy animals. And of course, some of these differences in enzyme levels and other biochemicals are even more pronounced in the bone-fractured racing camels. At this point, we are unable to assess the "cause and effect" relationship, i.e. whether these observed differences in biochemical parameters in the three groups are due to compromised bone health (lameness or fracture) or if the poor bone conditions in these animals cause the observed biochemical disturbances. Nevertheless, this preliminary study clearly shows that there are several factors that need to be carefully studied in the future in the hope of possibly developing diagnostic tools to identify racing camels at risk of developing bone fractures.

Acknowledgments: The authors would like to thank all the veterinary doctors in the Management of scientific Center and Presidential camels –Alain for providing camel blood samples. Additionally, the kind support from the MSc program in Environmental Sciences at the UAEU is graciously acknowledged.

Table 2. Mean, standard deviations and statistical analyses in various biochemistry parameters in the three female camel groups.

Parameters	Mean ± SD			Significance (p<0.05)		
	Normal	Lame	Fractured	N. vs. L.	N. vs. F.	L. vs. F.
Glucose (mmol/l)	6.2±0.6	6±0.9	10.6±4.1	No	Yes	Yes
BUN (mmol/l)	5.3±1.4	5.8±2.4	6.8±2.1	No	Yes	No
Creatinine (µmol/l)	139.3±28.9	126.5±18.2	150.0±37.0	No	No	Yes
TP (g/l)	65.0±3.5	63.4±3.4	67.9±5.1	No	Yes	Yes
Albumin (g/l)	29.5±2.2	29.0±2.4	31.2±3.3	No	No	Yes
GGT (IU/l)	17.2±2.9	20.1±3.2	17.3±3.3	Yes	No	Yes
ALP (IU/l)	190.9±89.2	138.0±36.4	111.9±51.9	Yes	Yes	No
AST (IU/l)	99.9±121.4	89.5±15.4	300.2±204.3	No	Yes	Yes
CK (IU/l)	105.1±113.1	55.1±30.4	1513.3±1307.6	No	Yes	Yes
LDH (IU/l)	452.1±52.6	424.1±99.5	674.9±199.2	No	Yes	Yes
TIBC (µg/dl)	213.1±28.4	201.6±21.4	200.6±58.9	No	No	No
Calcitonin II (pg/ml)	2.4±1.1	3.0±2.7	4.2±3.5	No	No	No
N Tact PTH (pg/ml)	12.8±7.9	29.9±23.1	18.4±14.2	Yes	No	No
OC (ng/ml)	1.8±1.1	1.9±0.8	0.7±0.5	No	Yes	Yes
UCOC (ng/ml)	4.3±1.2	3.9±3.0	1.4±1.4	No	Yes	No

Table 3. Mean, standard deviation sand statistical analyses in various biochemistry parameters in the three female camel groups.

Parameters	Mean ± SD			Significance (p<0.05)		
	Normal	Lame	Fractured	N. vs. L.	N. vs. F.	L. vs. F.
Glucose (mmol/l)	6.1±0.5	6.1±1.0	9.6±2.5	No	Yes	Yes
BUN (mmol/l)	6.7±1.1	7.3±1.4	6.8±1.6	No	No	No
Creatinine (μmol/l)	124.8±16.7	127.6±37.5	139.5±12	No	Yes	No
TP (g/l)	63.2±3.0	62.3±3.0	67.0±2.3	No	Yes	Yes
Albumin (g/l)	29.2±1.9	28.8±2.0	31.8±2.6	No	Yes	Yes
GGT (IU/l)	20.5±3.2	19.5±2.7	19.2±4.0	No	No	No
ALP (IU/l)	193.3±29.2	132.5±39.9	86.5±19.2	Yes	Yes	Yes
AST (IU/l)	100.7±16.2	103.5±25.5	255.7±74.7	No	Yes	Yes
CK (IU/l)	84.2±96.4	55.8±17.5	1390.3±951.5	No	Yes	Yes
LDH (IU/l)	407.0±43.8	358.4±46.9	493.0±66.5	Yes	Yes	Yes
TIBC (μg/dL)	201.6±18.1	192.9±27.2	220.8±33.4	No	No	Yes
Calcitonin II (pg/ml)	1.6±0.5	2.2±1.2	6.1±6.9	No	Yes	No
N Tact PTH (pg/ml)	32.1±22.8	30.3±19.2	28.1±20.8	No	No	No
OC(ng/ml)	2.1±2.2	1.0±1.0	0.7±0.3	No	No	No
UCOC (ng/ml	4.5±1.7	2.3±0.9	0.5±0.3	Yes	Yes	No

REFERENCES

Aichouni, A., R. Jeblawi, A. Dellal, H. Hammou, and H. Aggad (2010) Breed Variation in Blood Constituents of the One-Humped Camel (*Camelus dromedaries*) in Algeria. J. Camelid Science 3: 19–25.

Alata , F., O. Alata , M. Metinta , O. Çolak, S. Erginel, and E. Harmanci, (2002). Usefulness of bone markers for detection of bone metastases in lung cancer patients. Clinical Biochemistry 35, 293–296.

Alsaad, K. M. (2009) Clinical, Hematological and Biochemical Studies of Anaplasmosis in Arabian One-Humped Camels (C*amelus dromedaries*). J. Anim. Vet. Adv. 8(9): 1794–1797.

Alshamisi, N. S., T. S. Ksiksi, and S. S. Ashraf (2013) Hematology Analysis as a Potential Tool to Predict Bone Fracture in Arabian Racing Camels

(*Camelus dromedaries*). J. Anim. Plant Sciences 23(3): 763–770.

Asaba, Y., K. Hiramatsu, Y. Matsui, Y., A. Harada, Y. Nimura, N. Katagiri, T. Kobayashi, T.Takewaka, M. Ito, S. Niida, and K. Ikeda (2006) Urinary g-Glutamyltransferase (GGT) as a Potential Marker of Bone Resorption. Bone 39(6): 1276–1282.

Blood Urea Nitrogen (BUN) (2012) *In* Clinical Veterinary Advisor Pp. 914–915. Saint Louis: W.B. Saunders.

Bouillon, R., M. Bex, E. Van Herck, J. Laureys, L. Dooms, E. Lesaffre, and E. Ravussin (1995) Influence of Age, Sex, and Insulin on Osteoblast Function: Osteoblast Dysfunction in Diabetes Mellitus. J. Clinical Endocrinology and Metabolism 80(4): 1194–1202.

Chaney, K, J. M. MacLeay, R. M. Enns, F. Al-Sobayil, C. Kawcek, and D. Frisbie (2004) Effects of Induced Lameness via Carpal Osteochondral Fragmentation on Plasma Creatine Kinase Activity in Horses. J. Equine Vet. Sci. 24(12): 531–534.

Charles, I. (1982) An Economic History of the Middle East and North Africa (Taylor and Francis).

Dempster, D. W., F. Cosman, M. Parisien, V. Shen, and R. Lindsay (1993) Anabolic Actions of Parathyroid Hormone on Bone. Endocrine Reviews 14(6): 690–709.

Dimai, H. P., T. A. Linkhart, S. G. Linkhart,L. R. Donahue, W. G. Beamer, C. J. Rosen, J.R.Farley, and D. J. Baylink (1998) Alkaline Phosphatase Levels and Osteoprogenitor Cell Numbers Suggest Bone Formation May Contribute to Peak Bone Density Differences between Two Inbred Strains of Mice. Bone 22(3): 211–216.

Dobnig, H., and R. T. Turner (1997) The Effects of Programmed Administration of Human Parathyroid Hormone Fragment (1–34) on Bone Histomorphometry and Serum Chemistry in Rats. Endocrinology 138(11): 4607–4612.

Ejersted, C., T.T. Andreassen, E.-M. Hauge, F. Melsen, and H. Oxlund (1995) Parathyroid Hormone (1–34) Increases Vertebral Bone Mass, Compressive Strength, and Quality in Old Rats. Bone 17(6): 507–511.

Eldirdiri, N. I., Hagir B. Suliman, and A. M. Shommein (1987) Normal Serum Activities of Some Diagnostic Enzymes in Dromedary Camel in Sudan. Veterinary Research Communications 11(3): 201–203.

Fewtrell, M. S., C. Benden, J. E. Williams, S. Chomtho, F. Ginty, S. V. Nigdikar and A. Jaffe (2008) Undercarboxylated Osteocalcin and Bone Mass in 8–12 Year Old Children with Cystic Fibrosis. J. Cyst. Fibros. 7:307–312.

Grundy, S. M. (2007) Gamma-Glutamyl Transferase. Arteriosclerosis, Thrombosis, and Vascular Biology 27(1): 4–7.

Harris, M. M., L. B. Houtkooper, V. A. Stanford, C. Parkhill, J. L. Weber, H. Flint-Wagner, L. Weiss, S. B. Going and T. Lohman (2003) Dietary Iron Is Associated with Bone Mineral Density in Healthy Postmenopausal Women. J. Nutr. 133:3598–3602.

Harris, Pat A., D. H. Snow, T. R. C. Greet, and P. D. Rossdale (1990) Some Factors Influencing Plasma AST/CK Activities in Thoroughbred Racehorses. Equine Vet. J. 22(S9): 66–71.

Hofbauer, L. C., C. C. Brueck, S. K. Singh, and H. Dobnig (2007) Osteoporosis in Patients with Diabetes Mellitus. J.Bone and Mineral Research 22(9): 1317–1328.

Ilich, J.Z., and J.E. Kerstetter (2000) Nutrition in Bone Health Revisited: A Story beyond Calcium. J. American College of Nutr. 19(6): 715–737.

Isomura, H., F. Katsutoshi, S. Kouichi, N. Inoue, T. Iizuka, G. Takebe, K. Takahashi, J. Nishihira, H. Izumi, and W. Sakamoto (2004) Bone Metabolism and Oxidative Stress in Postmenopausal Rats with Iron Overload. Toxicology 197(2): 92–99.

Ivaska, K.K., S.M. Kakonen, P. Gerdhem, K. J. Obrant, K. Pettersson and H. K. Väänänen (2005) Urinary Osteocalcin as a Marker of Bone Metabolism. Clinical Chemistry 51(3): 618.

Jiang, Y., J. J. Zhao, B. H. Mitlak, O. Wang, H. K. Genant, andE. F. Eriksen (2003) Recombinant Human Parathyroid Hormone (1–34)[teriparatide] Improves Both Cortical and Cancellous Bone Structure. J. Bone and Mineral Res. 18(11): 1932–1941.

Kataria, N., and J. S. Bhatia (1991) Activity of Some Enzymes in the Serum of Dromedary Camels. Research in Veterinary Science 51(2): 174–176.

Katsumata, S., R. Katsumata-Tsuboi, M. Uehara, and K. Suzuki (2009) Severe Iron Deficiency Decreases Both Bone Formation and Bone Resorption in Rats. J. Nutrition 139(2): 238–243.

Katsumata, S., R. Tsuboi, M. Uehara, and K. Suzuki (2006) Dietary Iron Deficiency Decreases Serum Osteocalcin Concentration and Bone Mineral Density in Rats. Bioscience, Biotechnology, and Biochemistry 70(10):2547-2550

Kim, M, Kim, H, and Sohn, C (2010) Relationship between Vitamin K Status, Bone Mineral Density, and Hs-CRP in Young Korean Women 4(6): 1–5.

Krakauer, J. C., M. J. McKenna, N. F. Buderer, D. S. Rao, F. W. Whitehouse, and A. M. Parfitt (1995) Bone Loss and Bone Turnover in Diabetes. Diabetes 44(7): 775–782.

Leidig-Bruckner, G. and R. Ziegler (2001) Diabetes Mellitus: A Risk for Osteoporosis? Experimental and Clinical Endocrinology and Diabetes 109: 493–514.

Maurer, J., M. M. Harris, V. A. Stanford, *et al*. (2005) Dietary Iron Positively Influences Bone Mineral Density in Postmenopausal Women on Hormone Replacement Therapy. J. Nutrition 135(4): 863–869.

Medeiros, D. M., A. Plattner, D. Jennings, and B. Stoecker (2002) Bone Morphology, Strength and Density Are Compromised in Iron-Deficient Rats and Exacerbated by Calcium Restriction. The J. Nutrition 132(10): 3135–3141.

Mendu, D. R., P. R. Chou, and S. J. Soldin (2007) An Improved Application for the Enzyme Multipled Immunoassay Technique for Caffeine, Amikacin, and Methotrexate Assays on the Dade-Behring Dimension RxL Max Clinical Chemistry System: Therapeutic Drug Monitoring 29, 632–637.

Mohamed, H. A., and A. N. Hussein (1999) Studies on Normal Haematological and Serum Biochemical Values of the “Hijin”racing Camels (Camelus Dromedarius) in Kuwait. Veterinary Research Communications 23(4): 241–248.

Mosekilde, L., C. C. Danielsen, and J. Gasser (1994) The Effect on Vertebral Bone Mass and Strength of Long Term Treatment with Antiresorptive Agents (estrogen and Calcitonin), Human Parathyroid Hormone-(1-38), and Combination Therapy, Assessed in Aged Ovariectomized Rats. Endocrinology 134(5): 2126–2134.

Murray, T. M., L. G. Rao, P. Divieti, and F. R. Bringhurst (2005) Parathyroid Hormone Secretion and Action: Evidence for Discrete Receptors for the Carboxyl-Terminal Region and Related Biological Actions of Carboxyl-Terminal Ligands. Endocrine Reviews 26(1): 78–113.

Neer, R. M., C. D. Arnaud, J. R. Zanchetta, R. Prince, G. A. Gaich, J. Y. Reginster, A. B. Hodsman, E. F. Eriksen, S. Ish-Shalom, H. K. Genant, O. Wang, and B. H. Mitlak (2001) Effect of Parathyroid Hormone (1-34) on Fractures and Bone Mineral Density in Postmenopausal Women with Osteoporosis. New England J. Med. 344(19): 1434–1441.

Ng, A.H.M., G. Hercz, R. Kandel, and M.D. Grynpas (2004) Association between Fluoride, Magnesium, Aluminum and Bone Quality in Renal Osteodystrophy. Bone 34(1): 216–224.

Nguyen-Yamamoto, L., L. Rousseau, J. H. Brossard, R. Lepage, and P. D’amour (2001) Synthetic Carboxyl-Terminal Fragments of Parathyroid Hormone (PTH) Decrease Ionized Calcium Concentration in Rats by Acting on a Receptor Different from the PTH/PTH-Related Peptide Receptor. Endocrinology 142(4): 1386–1392.

Noakes, T. D (1987) Effect of Exercise on Serum Enzyme Activities in Humans. Sports Medicine (Auckland, NZ) 4(4): 245.

Orwoll, E. S., W. H. Scheele, S. Paul, S. Adami, U. Syversen, A. Diez-Perez, J-M Kaufman, A. D. ClancyandG. A. Gaich (2003) The Effect of Teriparatide [human Parathyroid Hormone (1–34)] Therapy on Bone Density in Men with Osteoporosis. J. Bone and Mineral Res. 18(1): 9–17.

Papanikolaou, G., and K. Pantopoulos (2005) Iron Metabolism and Toxicity. Toxicology and Applied Pharmacology 202(2): 199–211.

Parelman, M., B. Stoecker, A. Baker, and D. Medeiros (2006) Iron Restriction Negatively Affects Bone in Female Rats and Mineralization of hFOB Osteoblast Cells. Experimental Biology and Medicine 231(4): 378–386.

Pettway, G. J., J. A. Meganck, A. J. Koh, *et al*. (2008) Parathyroid Hormone Mediates Bone Growth through the Regulation of Osteoblast Proliferation and Differentiation. Bone 42(4): 806–818.

Pohl, A., C. Behling, D. Oliver, M. Kilani, P. Monson, and T. Hassanein (2001) Serum Aminotransferase Levels and Platelet Counts as Predictors of Degree of Fibrosis in Chronic Hepatitis C Virus Infection. The American J. Gastroenterology 96(11): 3142–3146.

Reginster, J.Y., R. Deroisy, M.P. Lecart,N. Sarlet, M. A. Fontaine, A. Albert, and P. Franchimont (1990) Calcitonin and Postmenopausal Bone Loss. Experimental Gerontology 25(3–4): 349–356.

Roodman, G. D. (2004) Mechanisms of Bone Metastasis. New England J. Medicine 350(16): 1655–1664.

Salman, R., and M. Afzal (2004) Seasonal Variations in Hematological and Serum Biochemical Parameters in Racing Camels. J. Camel Sci. 1: 62–65.

Shumate, J. B., M. H. Brooke, J. E. Carroll, and J. E. Davis (1979) Increased Serum Creatine Kinase after Exercise. Neurology 29(6): 902–902.

Shyng, Y. C., H. Devlin, and P. Sloan (2001) The Effect of Streptozotocin-Induced Experimental Diabetes Mellitus on Calvarial Defect Healing and Bone Turnover in the Rat. International J. Oral and Maxillofacial Surgery 30(1): 70–74.

Al-Sobayil, F. A. (2010) Circadian Rhythm of Bone Formation Biomarkers in Serum of Dromedary

Camels. Research in Veterinary Science 89(3): 455–459.

Soni, Neil, and M. Margarson (2004) Albumin. Where Are We Now? Current Anaesthesia and Critical Care 15(1): 61–68.

Tashjian Jr, A. H., and R. F. Gagel (2006) Teriparatide [Human PTH (1-34)]: 2.5 Years of Experience on the Use and Safety of the Drug for the Treatment of Osteoporosis. J. Bone and Mineral Research 21(3): 354–365.

Thomas, T (2006) Intermittent Parathyroid Hormone Therapy to Increase Bone Formation. Joint Bone Spine 73(3): 262–269.

Vergnaud, P., P. Garnero, P. J. Meunier, G. Breart, K. Kamihagi, and P. D. Delmas (1997) Undercarboxylated Osteocalcin Measured with a Specific Immunoassay Predicts Hip Fracture in Elderly Women: The EPIDOS Study. J. Clin. Endocrinology and Metabolism 82(3): 719.

Verhaeghe, J., E. Van Herck, W. J. Visser, A. M. Suiker, M.Thomasset, T. A. Einhorn, E. Faierman and R. Bouillon (1990) Bone and Mineral Metabolism in BB Rats with Long-Term Diabetes. Decreased Bone Turnover and Osteoporosis. Diabetes 39(4): 477–482.

Wallach, S, G Rousseau, L Martin, and M Azria (1999) Effects of Calcitonin on Animal and in Vitro Models of Skeletal Metabolism. Bone 25(5): 509–516.

Washington, Ida M., and Gerald Van. Hoosier (2012) Chapter 3 - Clinical Biochemistry and Hematology. *In* The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents Pp. 57–116. Boston: Academic Press.

Whitfield, J. B. (2001) Gamma Glutamyl Transferase. Critical Reviews in Clinical Laboratory Sciences 38(4): 263–355.