Bulgarian Journal of Veterinary Medicine (2013), **16**, No 2, 123–132

CORRELATIONS AMONG CARDIAC BIOMARKERS IN SHEEP WITH EXPERIMENTALLY INDUCED ENDOTOXAEMIA

A. CHALMEH, M. POURJAFAR, KH. BADIEI & S. NAZIFI

Department of Clinical Sciences, School of Veterinary Medicine,
Shiraz University, Shiraz, Iran

Summary

Chalmeh, A., M. Pourjafar, Kh. Badiei & S. Nazifi, 2013. Correlations among cardiac biomarkers in sheep with experimentally induced endotoxaemia. *Bulg. J. Vet. Med.*, **16**, No 2, 123–132.

Five clinically healthy 1-year old Iranian fat-tailed ewes (25±1.5 kg body weight) were randomly selected and lipopolysaccharide from *Escherichia coli* serotype O55:B5 was infused intravenously to induce endotoxaemia at 20 μg/kg. Blood samplings and serum separations were performed prior and 1, 2, 3, 4, 5, 6 and 24 hours after lipopolysaccharide injection and values of serum homocysteine, cardiac troponin I, creatine kinase isoenzyme MB and lactate dehydrogenase were assayed. A rapid and significant elevation of all studied parameters was seen after endotoxaemia induction. There were positive correlations among homocysteine, cardiac troponin I, creatine kinase isoenzyme MB and lactate dehydrogenase at all hours after endotoxaemia induction. The results of the current experimental study provide evidence for associations among these biomarkers and their changes during endotoxaemia and the data can be useful for assessing suspected cases of myocardial diseases and its changes may be of diagnostic and prognostic values.

Key words: cardiac biomarkers, cardiac enzymes, endotoxaemia, myocardial injuries, sheep

INTRODUCTION

Bacterial lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria causes endotoxaemia which considered making most pathophysiological reactions. Endotoxaemia interferes with several animal physiological systems such as cardiovascular functions and its effects in sheep are well discussed (Perkowski *et al.*, 1996; Radostits *et al.*, 2007). Endotoxaemia in sheep interferes with cardiovascular function at 3 phases. At phase 1 during the first hour, hypovolaemia occurs and cardiac output decreases leading to decrease lymph flow and pulmonary artery pressure. The decreased volume is the result of fluid movement from the vascular compartment to the interstitial space consequent to a microvascular pressure increase. During phase 2 these variables tend to return toward their baseline values. Phase 3 begins several hours later and shows many of the same changes that were observed in phase 1. This late fall in cardiac output in phase 3, also the result of a diminished vascular volume, occurs secondarily to extravasular fluid movement as a consequence of both an elevated microvascular pressure and an increased permeability to protein (Traber *et al.*, 1981).

Evaluating the values of circulating enzymes and serum biochemical analysis can often provide valuable information regarding the cardiovascular healthiness and sickness in animals (Coodley, 1970). Several researchers mentioned that when there are damages to the myocardium, the circulating levels of homocysteine (Hcy) (Ciaccio *et al.*, 2008), cardiac troponin I (cTnI) (Radostits *et al.*, 2007) and enzymes such as creatine kinase isoenzyme MB (CK-MB) (Kaneko, 1989) and lactate dehydrogenase (LDH) (Bassit *et al.*, 2010) are elevated. Since the endotoxaemia can affect the cardiac physiological functions, it may be hypothesised that cardiac isoenzymes and biomarkers alter during endotoxaemia.

Hcy is a sulfur-containing amino acid which is found in blood and produced in the metabolism of the essential amino acid methionine (Ciaccio *et al.*, 2008). Epidemiological studies have shown that too high serum concentrations of Hcy are related to a higher risk of coronary heart disease, stroke, peripheral vascular disease and deep venous thrombosis (Weikert *et al.*, 2005; Kullo *et al.*, 2006). Several studies have suggested that cTnI has become especially important in early diagnosis of myocardial damages in large animals (Radostits *et al.*, 2007; Tunca *et al.*, 2009). Moreover, cTnI level is a more specific marker than CK-MB for diagnosing myocardial necrosis (Başbuğan *et al.*, 2010); however, myocardium is one of the richest sources of CK-MB. Therefore, it is the most widely used serum enzyme determination in cardiac diseases of large animals (Kaneko, 1989). LDH catalyses the reversible oxidation of pyruvate to lactate. Multiple forms of LDH enzymes in several tissues have been reported but LDH is found to be a general indicator for the existence and severity of acute or chronic myocardial tissue damages (Bassit *et al.*, 2010). LDH is not organ-specific and may be of value in order to diagnose the cardiac problems in conjunction with other cardiac biomarkers (Coodley, 1970). These diagnostic biomarkers and enzymes are therefore valuable tools used in the early detection of cardiac problems as a result of ischemia, injury or inflammation (Radostits *et al.*, 2007).

There are several studies on the effects of the endotoxaemia on cardiovascular biomarkers in large animals (Green & Adams, 1992; Perkowski *et al.*, 1996; Peek *et al.*, 2008) but knowledge regarding the analysis of circulating biochemical profile of cardiac parameters undergoing endotoxaemia in sheep is rare. Therefore, the present experimental study was designed to clarify and study the cardiac biomarkers and enzymes following the induction of endotoxaemia by *Escherichia coli* serotype O55:B5 based on measurement of circulating Hcy, cTnI, CK-MB and LDH in Iranian fat-tailed sheep. The results of the present study also reveal the relationship among these parameters during endotoxaemia and their changing patterns in this breed.

MATERIALS AND METHODS

Animals

The present experiment was performed after being approved by the Ethics Committee of School of Veterinary Medicine, Shiraz University. Five clinically healthy 1-year old Iranian fat-tailed ewes (25±1.5 kg, bodyweight) were randomly selected for the project in April 2011. All animals were maintained in Laboratory Teaching Barn of Agricultural College of Shiraz University, Badjgah region (latitude of 29° 32' N and longitude 52° 35' E, 1810 m above sea level), south of Iran. Four weeks before commencing experiments,

each sheep received albendazole (15 mg/kg, orally; Dieverm®600, Razak Pharmaceutical Co, Tehran, Iran) and ivermectin (0.2 mg/kg, subcutaneously; Erfamectin®1%; Erfan Pharmaceutical Co, Tehran, Iran) to control internal and external probable parasites. All ewes were maintained in open-shed barns with free access to water and shade. The ration included mainly alfalfa hay, corn silage, corn and barley.

Chemicals and drugs

Phenol extracted lipopolysaccharide (LPS) from *Escherichia coli* serotype O55:B5 (Sigma-Aldrich®; product No. L2880) was used to induce endotoxemia in ewes at 20 μg/kg. This endotoxin was diluted in sterile phosphate-buffered saline (PBS) and divided into 5 equal doses each containing 500 μg endotoxin and stored at –80°C until endotoxaemia induction. For each animal, each dose was thawed and infused intravenously as described below. The intravenous fluid used in the present experiment was dextrose 5% plus sodium chloride 0.45% (Shahid Ghazi Pharmaceutical Co., Tabriz, Iran).

Induction and treatment of endotoxaemia

The schematic diagramme of the present experimental design is presented on Fig. 1. A 16 gauge 5.1 cm catheter was secured in the left jugular vein and used for blood samplings, endotoxin and fluids infusions. All 5 ewes were evaluated clinically before and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection. Clinical parameters monitored during experiments included rectal temperature, heart and respiratory rates, mucous membrane color, capillary refill time, appetite and fecal consistency. Thawed LPS was diluted in 250 mL normal saline and infused intravenously at the rate of 10 mL/kg/hour. Fluid therapy was performed in all animals over 120 min after LPS injection by dextrose 5% plus sodium chloride 0.45% at the rate of 20 mL/kg/h. Blood glucose was monitored in all animals, using a rapid response glucose meter device (Accucheck Active®, Roche, Germany) to prevent hypoglycemia.

Blood sampling and serological assays

Blood samples were collected from all ewes through the fixed catheter prior and 1, 2, 3, 4, 5, 6 and 24 h after LPS injection in plain tubes. Immediately after collections, sera were separated by centrifugation (for 10 minutes at 3,000×*g*) and stored at −22 °C until assayed.

Values of serum CK-MB and LDH were measured with Integra 800 auto-analyzer (Roche-Cobes, Switzerland). Levels of serum cTnI were determined by ELISA equipment (ELISA Reader®-DAS Italy) and calculated with commercial test kit as instructed by the manufacturer (Troponin I kit-DRG Diagnostic). Serum Hcy levels

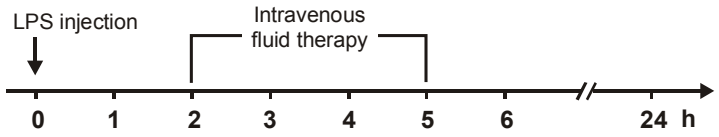

Fig. 1. Schematic diagramme of the experimental design. Lipopolysaccharide (LPS) was injected at zero hour and intravenous fluid therapy was commenced 2 hours later in Iranian fat-tailed sheep and continued for 180 minutes. Venous blood sampling was performed at all demonstrated hours.

were determined by ELISA using commercial kit (Homocysteine AXIS, Catalog no: 802865065).

Statistical analyses

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Pearson's correlation test to detect the relationship among studied parameters at each hour, separately, using SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). In the present study, the Pearson's correlation coefficient greater than 0.8 was considered as strong, whereas, a coefficient lesser than 0.5 described as weak. Paired samples *t*-test was used to determine differences between two different times during the current experimental study. The level of significance was set at $P<0.05$.

RESULTS

Alterations of CK-MB, cTnI, Hcy and LDH at different hours during experimental endotoxaemia in Iranian fat-tailed sheep are presented in Fig. 2 and 3, on which baseline levels are presented at hour zero. The rapid and significant elevation of CK-MB, cTnI, Hcy and LDH was seen after endotoxaemia induction ($P<0.05$). The results of paired samples *t*-test showed that amounts of CK-MB, cTnI, Hcy and LDH at 24th hour were significantly higher than baseline values at hour zero ($P<0.05$).

The correlations among CK-MB, cTnI, Hcy and LDH prior to and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection are presented in Table 1. There were no significant correlations before endotoxaemia induction. The results of the present experimental study showed that CK-MB, cTnI, Hcy and LDH at all hours after endotoxaemia induction were positively correlated together. LDH was correlated significantly with cTnI at all hours after LPS infusion.

All sheep were considered permanent survivors, alive and healthy after all experiments.

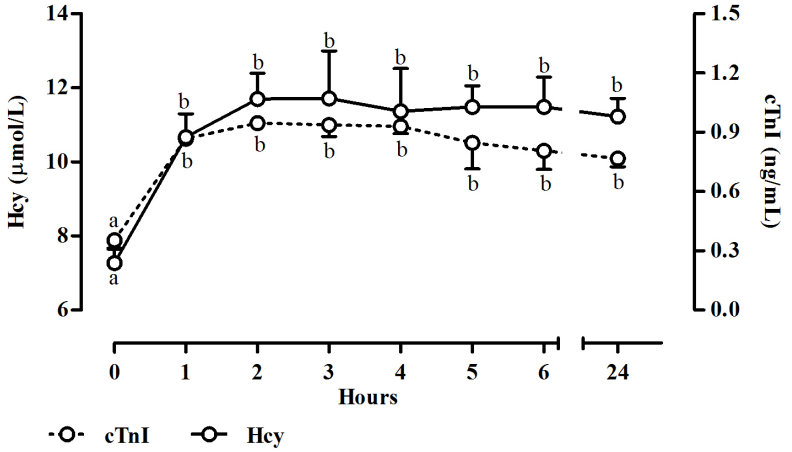

Fig. 2. Alterations of serum concentrations of homocystein (Hcy) and cardiac troponin I (cTnI) during experimentally induced endotoxemia in Iranian fat-tailed sheep. Different letters (a and b) at each line indicate significant differences between two different hours ($P<0.05$).

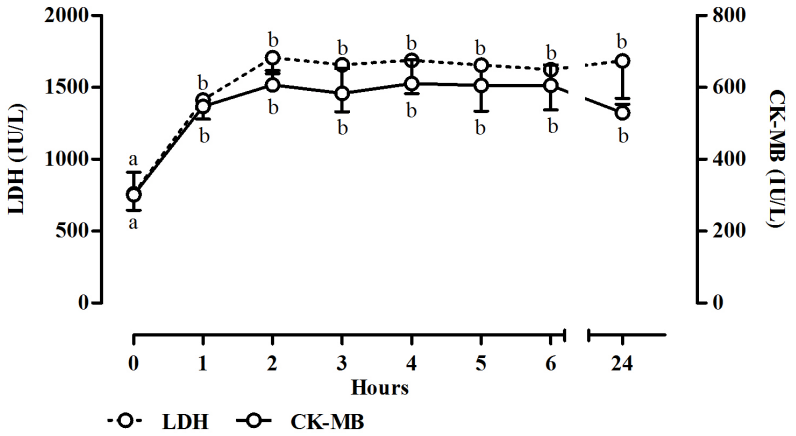

Fig. 3. Alterations of serum concentrations of creatine kinase isoenyme MB (CK-MB) and lactate dehydrogenase (LDH) during experimentally induced endotoxemia in Iranian fat-tailed sheep. Different letters (a and b) at each line indicate significant differences between two different hours (P<0.05).

DISCUSSION

There are several researches which demonstrate the alterations in cardiopulmonary physiology during experimental endotoxaemia in large animals and include increases in heart rate, pulmonary artery pressure, left ventricular contractility, chamber stiffness, mechanical efficiency alongside decreases in left ventricular stroke work, mean systemic blood pressure, and cardiac output (Olson & Brown, 1985; Kuhl *et al.*, 1988; Constable, 1999). Furthermore, some literatures present the alterations in cardiac injury biomarkers following endotoxaemia induction in large animals (Green & Adams, 1992; Perkowski *et al.*, 1996; Peek *et al.*, 2008). However, the study on serum biochemical profile of cardiac injury biomarkers in sheep is lacking.

In the present study, normal serum concentration of cTnI was 0.35±0.02 ng/mL in Iranian fat-tailed sheep. According to our findings, serum concentrations of cTnI increased rapidly and significantly at 1st hour after endotoxaemia induction (P<0.05) and remained at high concentrations to hour 24 (Fig. 2). Cardiac troponin is a myofibrillar protein with two diagnostically-relevant forms (cTnI and cTnT) that regulate contraction of the heart. cTnI binds to actin and inhibits its interactions between actin and myosin. Cardiac troponin is released from injured myocardiocytes into the circulation within hours (Polena *et al.*, 2005). In recent years, the development of cardiac troponins as the gold standard, sensitive and specific biochemical markers of myocardial injuries have aided the diagnosis and management of myocardial injuries (Wells & Sleeper, 2008). In the present study, increasing the cTnI immediately after LPS administration can indicate the myocardial injuries during endotoxaemia. The serum concentrations of cardiac troponin correlate well with histopathological changes in the myocardium, extent of cardiac injury, clinical signs and prognosis (Wells & Sleeper, 2008). Assay of troponins constitutes the preferred biochemical marker for

Table 1. Correlations among cardiac biomarkers in Iranian fat-tailed sheep with experimentally induced endotoxemia at different periods after endotoxaemia induction

Baseline (hour 0)		CK-MB	LDH	cTnI
	LDH	–0.932		
	cTnI	–0.788	0.958	
	Hcy	0.315	0.049	0.335
Hour 1		CK-MB	LDH	cTnI
	LDH	0.215		
	cTnI	0.217	0.907*	
	Hcy	0.027	0.982*	0.970*
Hour 2		CK-MB	LDH	cTnI
	LDH	0.553		
	cTnI	0.810	0.842	
	Hcy	0.774	0.956*	0.255
Hour 3		CK-MB	LDH	cTnI
	LDH	0.616		
	cTnI	0.778	0.974*	
	Hcy	0.094	0.726	0.552
Hour 4		CK-MB	LDH	cTnI
	LDH	0.893*		
	cTnI	0.952*	0.988*	
	Hcy	0.591	0.891*	0.810
Hour 5		CK-MB	LDH	cTnI
	LDH	0.727		
	cTnI	0.157	0.964*	
	Hcy	0.994*	0.648	0.264
Hour 6		CK-MB	LDH	cTnI
	LDH	0.991*		
	cTnI	0.873	0.931*	
	Hcy	0.875*	0.800	0.526
Hour 24		CK-MB	LDH	cTnI
	LDH	0.584		
	cTnI	0.805	0.951*	
	Hcy	0.314	0.588	0.310

CK-MB: creatine kinase isoenzyme MB; LDH: lactate dehydrogenase; CtnI: cardiac troponin I; Hcy: homocysteine. *statistically significant correlations at $P<0.05$.

increases in cTnI correlate with a wide range of animal cardiac diseases including dilated cardiomyopathy, endocardiosis, endocarditis and congestive heart failure, as well as with various forms of severe respiratory disease (Serra *et al.*, 2010), acute myocardial infarction (Polena *et al.*, 2005). In the present experimental study, cTnI had positive and significant correlation coefficients with LDH at all hours after endotoxaemia induction which indi-

cate the direct relationship between these two cardiac biomarkers (Table 1).

In the current study, serum concentration of Hcy was 7.27±0.37 µmol/L at base line levels. This factor increased rapidly and significantly at 1st hour after LPS infusion ($P<0.05$) and remained at high concentrations to hour 24 (Fig. 2). Hcy is a highly reactive amino acid derived from methionine metabolism, and is known to produce endothelial cell injury in experimental animals (Harker *et al.*, 1983) and cell culture (Wall *et al.*, 1980). Elevated total serum Hcy has been stated as an independent risk factor for peripheral vascular, cerebrovascular and coronary artery diseases (Nygard *et al.*, 1997). According to the results of Hcy alterations in the present experiment, it may be stated that there were myocardial problems and injuries during endotoxaemia in Iranian fat-tailed sheep. Studies showed that increased plasma and heart tissue Hcy concentrations could be considered as a risk factor in myocardium damage in condition associated with oxidative stress (Rezaei & Dalir-Naghadeh, 2009). Several meta-analyses have shown an association between total plasma Hcy concentration and cardiovascular diseases (Hankey & Weikelboom, 1999). In the current experimental study, Hcy had positive relationships with all other parameters at all hours after endotoxaemia induction (Table 1).

According to our results, normal values of CK-MB and LDH were respectively 301.33±62.81 IU/L and 759.33± 114.17 IU/L at hour zero. In the current experimental study the significant elevations of serum CK-MB and LDH were seen at 1st hour after endotoxin infusion ($P<0.05$, Fig. 3). High concentrations of these enzymes were detected at all hours after endotoxaemia induction and remained at elevated values to 24th hour after LPS administration (Fig. 3).

CK-MB and LDH are cytoplasmic enzymes with a high activity in heart, skeletal muscle, liver, kidney, and red blood cells. These enzymes are indicators of a higher level of cellular damage and their increased activity is a consequence of their increased release from the damaged cells and a reflection of metabolic changes in the inflamed tissues especially in the heart (Graeber *et al.*, 1990). The damage to the skeletal or heart musculature results in a considerable increase in the level of serum CK-MB and LDH due to the fact that the bulk of the vessels throughout the body could be considered as an ample reservoir of enzymes liable to be released and detected during pathological situations. Thus, any damages to the vasculature that could result in leakage of enzymes, could thus be considered as a valuable tool in early diagnosis of pathological conditions (Graeber *et al.*, 1990).

Ohman *et al.* (1982) stated that LDH activity rises slowly after myocardial injuries and becomes maximal after the CK-MB elevations but according to our results serum concentrations of LDH were increased along with the CK-MB during this experimental study. Determinations of LDH activity have been used diagnostically to determine whether acute myocardial infarction occurred in the days before a patient was evaluated (Adams *et al.*, 1993). Measurement of cTnI is clearly more sensitive than the LDH cutoff value for retrospective diagnoses of acute myocardial injuries. Resolution of this problem has been advanced by the development of techniques that separate CK into its three isoenzymes containing MM, MB, and BB (Van der Veen & Willebrands, 1966). Separation and quantification of MB isoenzyme, which is found almost exclusively in heart muscle, provides a

more specific indicator of acute myocardial infarction than total CK alone. CK-MB as a cardio-specific enzyme has been introduced as a sensitive marker of myocardial injury (Roe *et al.*, 1972). Recent studies report that although the sensitivity of cTnI is comparable to that of CK-MB, its specificity seems to be higher (Adams *et al.*, 1994). In the diagnosis of acute myocardial infarction, the measurement of elevated levels of CK-MB and LDH are well known (Jaffe *et al.*, 1984).

In the present experimental study, LDH had positive and significant correlation coefficients with cTnI at all hours after endotoxaemia induction ($P<0.05$). According to the results of Pearson's correlation test there were positive correlation coefficients among CK-MB, LDH, cTnI and Hcy at all hours after LPS administration (Table 1).

In conclusion, it may be stated that myocardial injuries can be induced during experimental endotoxaemia after *E. coli* serotype O55:B5 administration in the Iranian fat-tailed sheep. The data provided here is the first report on health assessment of cardiac biochemical parameters in Iranian fat-tailed sheep. Moreover, it can be mentioned that serum concentrations of cTnI, Hcy, CK-MB and LDH are elevated immediately after *E. coli* serotype O55:B5 endotoxaemia induction in the Iranian fat-tailed sheep. Furthermore, the current experiment showed direct relationships among all studied cardiac isoenzymes and biomarkers during experimentally induced endotoxaemia in sheep. Finally, our results provide evidence for associations among these biomarkers and their changes during endotoxaemia and the data can be useful for assessing suspected cases of myocardial diseases and its changes may be of diagnostic and prognostic values.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Adams, J. E., D. R. Abendschein & A. S. Jaffe, 1993. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990's? *Circulation,* **88**, 750–763.

Adams, J. E., G. A. Sicard, B. T. Allen, K. H. Bridwell, L. G. Lenke, V. G. Davila-Roman, G. S. Bodor, J. H. Ladenson & A. S. Jaffe, 1994. Diagnosis of perioperative myocardial infarction with measurements of cardiac troponin I. *The New England Journal of Medicine,* **330**, 670–674.

Başbuğan, Y., Z. Ağaoğlu & N. Yüksek, 2010. An investigation on serum troponin concentration in healthy ruminants. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* **16**, 641–645.

Bassit, R. A., C. H. Pinheiro, K. F. Vitzel, A. J. Sproesser, L. R. Silveira & R. Curi, 2010. Effect of short-term creatine supplementation on markers of skeletal muscle damage after strenuous contractile activity. *European Journal of Applied Physiology,* **108**, 945–955.

Ciaccio, M., G. Bivona & C. Bellia, 2008. Therapeutical approach to plasma homocysteine and cardiovascular risk reduction. *Journal of Therapeutics and Clinical Risk Management,* **4**, 219–224.

Constable, P. D., 1999. Acute endotoxemia increases left ventricular contractility and diastolic stiffness in calves. *Shock,* **12**, 391–401.

Coodley, E. L., 1970. Diagnosic Enzymology. 4th edn, WB Saunders Co, Philadelphia, USA.

Graeber, G. M., G. P. Clagett, R. E. Wolf, P. J. Cafferty, J. W. Harmon & N. M. Rich, 1990. Alterations in serum creatine kinase and lactate dehydrogenase. Association with abdominal aortic surgery, myocardial

infarction and bowel necrosis. *Chest,* **97**, 521–527.

Green, E. M. & H. R. Adams, 1992. New perspectives in circulatory shock: pathophysiologic mediators of the mammalian response to endotoxemia and sepsis. *Journal of the American Veterinary Medical Association,* **200**, 1834–1841.

Hankey, G. J. & J. Weikelboom, 1999. Homocysteine and vascular disease. *The Lancet,* **354**, 407–413.

Harker, L. A., J. M. Harlan & R. Ross, 1983. Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. *Circulation Research,* **53**, 731–739.

Jaffe, A. S., B. T. Garfinkel, C. S. Ritter & B. E. Sobel, 1984. Plasma MB creatine kinase after vigorous exercise in professional athletes. *American Journal of Cardiology,* **53,** 856–858.

Kaneko, J. J., 1989. Clinical Biochemistry of Domestic Animals. 3rd edn, New York, USA, Academic Press.

Kuhl, P. G., J. M. Bolds, J. E. Loyd, J. R. Snapper & G. A. Fitzgerald, 1988. Thromboxane receptor mediated bronchial and hemodynamic responses to bovine endotoxemia. *American Journal of Physiology,* **254**, R310–R319.

Kullo, I. J., G. Li, L. F. Bielak, K. R. Bailey, P. A. Peyser, S. T. Turner & S. L. Kardia, 2006. Association of plasma homocysteine with coronary artery calcification in different categories of coronary heart disease risk. *Mayo Clinic Proceedings,* **81**, 177–182.

Nygard, O., J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad & S. E. Vollset, 1997. Plasma homocysteine levels and mortality in patients with coronary artery disease. *The New England Journal of Medicine,* **337**, 230–236.

Ohman, E. M., K. K. Teo, A. H. Johnson, P. B. Collins, D. G. Dowsett, J. T. Ennis & J. H. Horgan, 1982. Abnormal cardiac enzyme responses after strenuous exercise: Alternative diagnostic aids. *British Medical Journal (Clinical Research Edition),* **285**, 1523–1526.

Olson, N. C. & T. T. Jr. Brown, 1985. Effects of endotoxemia on lung water and hemodynamics in conscious calves. *American Journal of Veterinary Research,* **46**, 711–718.

Peek, S. F., F. S. Apple, M. A. Murakami, P. M. Crump & S. D. Semrad, 2008. Cardiac isoenzymes in healthy Holstein calves and calves with experimentally induced endotoxemia. *Canadian Journal of Veterinary Research,* **72**, 356–361.

Perkowski, S. Z., P. J. Sloane, J. A. Jr. Spath, T. H. Elsasser, J. K. Fisher & M. H. Gee, 1996. TNF-alpha and the pathophysiology of endotoxin-induced acute respiratory failure in sheep. *Journal of Applied Physiology,* **80**, 564–573.

Polena, S., K. H. Shen, E. Mamakos, P. J. Chuang, M. Sharma, P. Griciene, A. A. Ponomarev, J. Gintautas & R. Maniar, 2005. Correlation between cardiac enzyme elevation and the duration of cardiopulmonary resuscitation. *Proceedings of the Western Pharmacology Society,* **48**, 136–138.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff & P. D. Constable, 2007. Toxemia and endotoxemia. In: *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*, 10th edn, Elsevier, pp. 53–60, 399–438.

Rezaei, S. A. & B. Dalir-Naghadeh, 2009. Association of plasma and heart homocysteine and blood malondialdehyde with cardiovascular diseases induced by acute selenium deficiency in lambs. *Small Ruminant Research,* **83**, 22–28.

Roe, C. R., L. E. Limbird, G. S. Wagner & S. T. Nerenberg, 1972. Combined isoenzyme analysis in the diagnosis of myocardial injury: Application of electrophoretic methods for the detection and quantitation of the creatine phosphokinase MB isoenzyme. *Journal of Laboratory and Clinical Medicine,* **80**, 577–590.

Serra, M., S. Papakonstantinou, M. Adamcova & P. J. O'Brien, 2010. Veterinary and

toxicological applications for the detection of cardiac injury using cardiac troponin. *The Veterinary Journal,* **185**, 50–57.

Traber, D. L., T. H. Adair & T. Jr. Adams, 1981. Hemodynamic consequences of endotoxemia in sheep. *Circulation and Shock,* **8**, 551–561.

Tunca, R., H. M. Erdogan, M. Sozmen, M. Citil, A. K. Devrim, S. Erginsoy & E. Uzlu, 2009. Evaluation of cardiac troponin I and inducible nitric oxide synthase expressions in lambs with white muscle disease. *Turkish Journal of Veterinary and Animal Sciences,* **33**, 53–59.

Van der Veen, K. J. & A. F. Willebrands, 1966. Isoenzymes of creatine phosphokinase in tissue extracts and in normal and pathological sera. *Clinica Chimica Acta,* **13**, 312–316.

Wall, R. T., J. M. Harlan, L. A. Harker & G. E. Striker, 1980. Homocysteine-induced endothelial cell injury *in vitro*: A model for the study of vascular injury. *Thrombosis Research,* **18**, 113–121.

Weikert, C., K. Hoffmann, J. Dierkes, B. C. Zyriax, K. Klipstein-Grobusch, M. B. Schulze, R. Jung, E. Windler & H. Boeing, 2005. A homocysteine metabolism-related dietary pattern and the risk of coronary heart disease in two independent German study populations. *Journal of Nutrition,* **135**, 1981–1988.

Wells, S. M. & M. Sleeper, 2008. Cardiac troponins. *Journal of Veterinary Emergency and Critical Care,* **18**, 235–245.

Paper received 21.01.2013; accepted for publication 18.03.2013

Correspondence:

Dr. Aliasghar Chalmeh
Department of Clinical Sciences,
School of Veterinary Medicine,
Shiraz University, Shiraz, Iran
tel.: +98 711 6138700
e-mail: achalmeh81@gmail.com