İstanbul Üniv. Vet. Fak. Derg.
40 (2), 211-219, 2014
Araştırma Makalesi

J. Fac. Vet. Med. Istanbul Univ.
40 (2), 211-219, 2014
Research Article

Alterations in Electrocardiographic Parameters and Serum Cardiac Biomarkers in an Ovine Experimental Endotoxemia Model

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Geliş Tarihi / Received: 15.11.2013

ABSTRACT

In order to study the cardiac health status based on electrocardiogram recording and measurement of circulating cardiac biomarkers following the induction of endotoxemia, 5 clinically healthy 1-year old Iranian fat-tailed ewes (25±1.5 kg, bodyweight) were randomly selected and lipopolysaccharide from *Escherichia coli* serotype O55:B5 was used to induce endotoxemia in ewes at 20 µg/kg. The electrocardiograms and blood samples were taken prior and 1, 2, 3, 4, 5, 6 and 24 hours after lipopolysaccharide injection. Values of serum homocysteine, cardiac troponin I, creatine kinase isoenzyme MB and lactate dehydrogenase were assayed during the study. The rapid and significant elevation of homocysteine, cardiac troponin I, creatine kinase isoenzyme MB and lactate dehydrogenase was seen after endotoxemia induction ($P<0.05$). The results showed that T duration increased significantly after endotoxemia induction and decreased near to base line levels at 6^{th} hour after lipopolysaccharide infusion. T amplitude decreased significantly after endotoxemia induction. The significant increase in R-R, S-T and Q-T intervals were detected during endotoxemia. In conclusion, it seems that endotoxemia causes myocardial autonomic dysfunction and significant changes in ECG parameters could be interpreted in the light of concurrent cardiac biomarker changes.

Key Words: Endotoxemia, electrocardiogram, cardiac biomarkers, Iranian fat-tailed sheep

ÖZET

KOYUN DENEYSEL ENDOTOKSEMİ MODELİNDE ELEKTROKARDİYOGRAFİ PARAMETRELERİNDEKİ VE SERUM KARDİYAK BİYOMARKIRLARDAKİ DEĞİŞİMLER

Endotokseminin indüklemesini takiben elektrokardiyografi kayıtlarına ve dolaşımdaki kardiyak biyomarkerların ölçümüne dayandırılarak kardiyak sağlık durumu çalışılmıştır. Klinik açıdan sağlıklı 1 yaşındaki İran yağlı kuyruk 5 koyun (25±1,5 kg, ağırlık) rastgele seçilmiştir ve bu koyunlara endotoksemi oluşturmak amacıyla 20 µg/kg'dan *Escherichia coli* O55:B5 serotipinden lipopolisakkarid kullanılmıştır. Elektrokardiyogramlar ve kan örnekleri lipopolisakkarid enjeksiyonunun öncesinde ve 1, 2, 3, 4, 5, 6 ve 24 saat sonrasında alınmıştır. Çalışma süresince serum homocysteine, kardiyak troponin I, creatine kinase izoenzimi MB ve laktat dehidrojenaz seviyeleri ölçülmüştür. Endoksemi oluşturulduktan sonra homocysteine, kardiyak troponin I, creatine kinase izoenzimi MB ve laktat dehidrojenaz seviyelerinde hızlı ve belirgin bir yükselme ($P<0,05$) görülmüştür. Sonuçlar endotoksemi oluştuktan sonra T zamanının belirgin bir şekilde artığını ve lipopolisakkarid enjeksiyonundan 6 saat sonra alt çizgisiye kadar

düşmüştür. T amplitüdü endotoksemi oluştuktan sonra belirgin şekilde düşer. Endotoksemi süresince R-R, S-T ve Q-T aralıklarında belirgin bir şekilde yükselme belirlenmiştir. Sonuç olarak, endotoksemi myokardiyal otonomik fonksiyon bozukluğuna neden olur ve EKG parametrelerinde belirgin değişimler aynı zamanda yapılan kardiyak biyomarkerlar ile yorumlanabilir.

Anahtar Kelimeler: Endotoksemi, elektrokardiyografi, kardiyak biyomarkerlar, İran yağlı kuyruklu koyunu

Introduction

Bacterial lipopolysaccharide (LPS) causes endotoxemia which considered making most pathophysiological reactions. Endotoxemia interferes with several animals' physiological systems including cardiovascular functions and its effects in sheep are well discussed (Perkowski et al., 1996; Radostits et al., 2007). The majority of cardiac conduction disturbances can be detected on clinical examination; however, some may be undetected on clinical examinations and could be found only on electrocardiographic monitoring (Radostits et al., 2007). Evaluating the electrocardiogram (ECG) as the most rapid and readily available tool is useful for measuring and recording the heart electrical activity in exquisite details. There is limited literature on the normal electrocardiographic parameters (Ahmed and Sanyal, 2008), physiological (Pourjafar et al., 2011; 2012) and pathological (Mir et al., 2007) alterations of the heart electrical activities in sheep and goats, but based on the best of our knowledge, there are no reports on alterations of heart electrical activity on ovine experimental endotoxemia.

Assessing the values of circulating cardiac isoenzymes and biomarkers can often provide valuable information regarding the cardiovascular health status 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 endotoxemia can disturb the cardiac physiological functions, it may be hypothesized that cardiac isoenzymes and biomarkers alter during endotoxemia. These diagnostic isoenzymes and biomarkers are therefore valuable tools used in the early detection of cardiac problems as a result of ischemia, injury or inflammation (Radostits et al., 2007).

The present experiment was designed to study the cardiac health status based on ECG recording and measurement of circulating Hcy, cTnI, CK-MB and LDH following the induction of endotoxemia by *Escherichia coli* lipopolysaccharide serotype O55:B5 in Iranian fat-tailed sheep.

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 probable internal and external 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 endotoxemia 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 endotoxemia

The schematic diagram of the present experimental design is represented in figure 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 and appetite. Thawed LPS was diluted in 250 milliliter of normal saline and infused intravenously at the rate of 10 ml/kg/hour. Fluid therapy was performed in all animals over 120 minutes after LPS injection by dextrose 5% plus sodium chloride 0.45% at the rate of 20 ml/kg/hour.

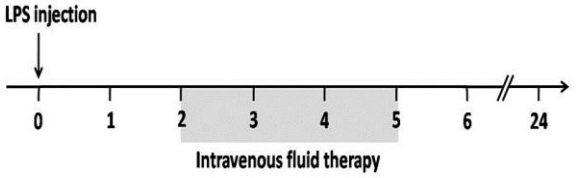

Figure 1. Schematic diagram of the present experimental design. Lipopolysaccharide (LPS) was injected at hour zero and intravenous fluid therapy was commenced 2 h later in Iranian fat-tailed sheep. Electrocardiographic recordings and venous blood samplings were performed at all hours shown.

Şekil 1. Deneysel çalışmanın şematik grafiği. Lipopolisakkarid (LPS) İran yağlı kuyruk koyununa sıfır zamanında enjekte edilmiştir ve 2 saat sonra intravenöz sıvı tedavisi başlanmıştır.

Electrocardiographic procedures

None of the ewes used in this study had any clinical signs of heart diseases (edema, jugular distension or pulsation and cardiac murmurs), coughing and exercise intolerance. The ECGs were recorded prior and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection. The ECGs were recorded on a bipolar base apex lead, using limb lead I. Animals were kept without any sedation and minimum restraint and then ECGs were recorded, using alligator-type electrodes which were attached to skin after cleaning it with ethanol and applying electrocardiographic jelly to improve skin contact. The positive electrode (left arm) was placed over cardiac apex on the 5th left intercostal space at the level of the elbow, the negative electrode (right arm) was placed on the left jugular furrow at the top of heart base, and the ground was placed on the dorsal spine or another site away from the heart (Radostits et al., 2007). All ECGs were obtained in a single channel electro-cardiographic machine (Kenz-line EKG 110, Suzuken Co., Ltd., Japan) with paper speed of 25 mm/sec and calibration of 10 mm equal to 1 mV. The precision of duration was 0.02 second (sec) and amplitude was 0.05 millivolts (mV).

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

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Repeated Measures ANOVA

to evaluate the changing pattern of electrocardiographic and serum biomarkers during experiment. Paired samples t-test was used to determine differences between two different times using SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The level of significance was set at $P<0.05$.

Results

Alterations of CK-MB, cTnI, Hcy and LDH at different hours during experimental endotoxemia in Iranian fat-tailed sheep are presented in Figures 5 and 6. In the present study, normal values of serum concentration of CK-MB, cTnI, Hcy and LDH were 301.33±62.81 IU/L, 0.35±0.02 ng/mL, 7.27±0.37 µmol/L and 759.33±114.17 IU/L, respectively. The rapid and significant elevation of CK-MB, cTnI, Hcy and LDH was seen after endotoxemia 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 results of the ECG changes are presented in Figures 2, 3 and 4. The results of Repeated Measures ANOVA showed that there were no significant changes in alterations patterns of P and S duration (Figure 2), P and R amplitude (Figure 3), and P-R interval (Figure 4). T duration increased significantly after endotoxemia induction and decreased near to baseline levels at 6th hour after LPS infusion (Figure 2). Changes of T amplitude were significant during endotoxemia and it decreased significantly after endotoxemia induction. Decreasing the T amplitude was continued up to 6th hour after LPS infusion and this ECG parameter increased near to baseline values at hour 24 (Figure 3). S amplitude increased significantly after endotoxemia induction and decreased near to baseline levels at 24th hour after LPS infusion (Figure 3). The significant changing patterns of R-R, S-T and Q-T intervals were observed during endotoxemia. These parameters were significantly increased after endotoxemia induction and decreased near to baseline levels at 24th hour after LPS infusion (Figure 4).

The results of clinical parameter monitoring are presented in Table 1. Heart and respiratory rate, rectal temperature and capillary refill time were increased after endotoxemia induction. After LPS infusion, weak appetite and congested mucous membranes were also detected.

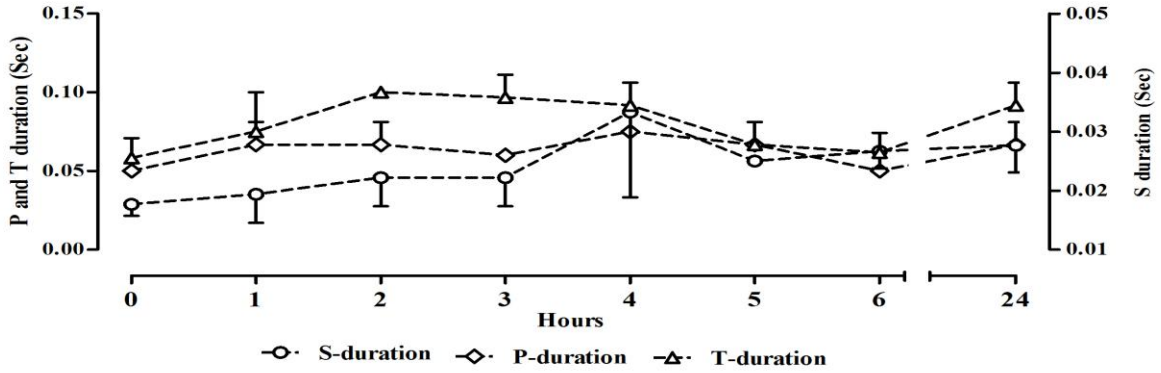

Figure 2. Alterations (mean±SEM) of duration (seconds) of P, S and T waves at different times following induction of endotoxemia in Iranian fat-tailed sheep. The significant changing pattern of T duration is seen after endotoxemia induction ($P<0.05$).

Şekil 2. İran yağlı kuyruklu koyunlardaki endotoksemi oluşumunu takiben farklı zamanlardaki P, S ve T dalgalarının süresindeki (saniye) değişimler (ortalama±SEM). T zamanındaki belirgin değişiklik endotoksemi oluşumundan sonra gözlendi ($P<0,05$).

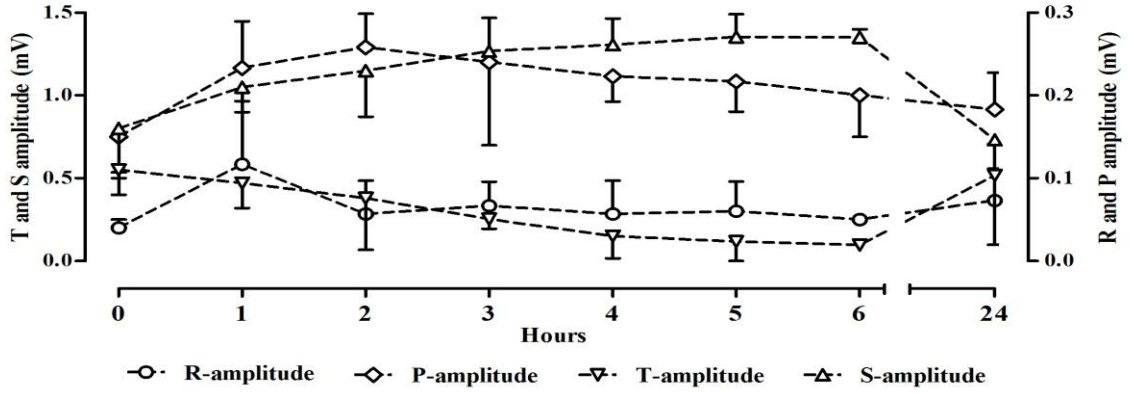

Figure 3. Alterations (mean±SEM) of amplitude (mV) of P, R, S and T waves at different times following induction of endotoxemia in Iranian fat-tailed sheep. The significant changing pattern of T and S amplitudes are seen after endotoxemia induction (P<0.05).

Şekil 3. İran yağlı kuyruklu koyunlardaki endotoksemi oluşumunu takiben farklı zamanlardaki P, S ve T dalgalarının amplitüdündeki (mV) değişimler (ortalama±SEM). T ve S amplitüdündeki belirgin değişiklik endotoksemi oluşumundan sonra gözlendi (P<0,05).

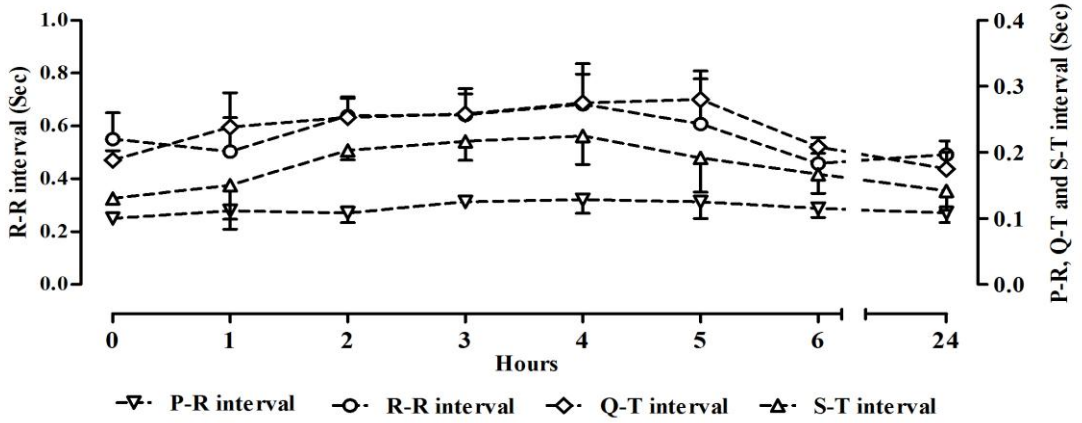

Figure 4. Alterations (mean±SEM) of duration (seconds) of P-R, R-R, S-T and Q-T intervals at different times following induction of endotoxemia in Iranian fat-tailed sheep. The significant changing pattern of R-R, S-T and Q-T intervals are seen after endotoxemia induction (P<0.05).

Şekil 4. İran yağlı kuyruklu koyunlardaki endotoksemi oluşumunu takiben farklı zamanlardaki P-R, R-R, S-T ve Q-T aralıklarının süresindeki (saniye) değişimler (ortalama±SEM). R-R, S-T ve Q-T aralıklarındaki belirgin değişiklik endotoksemi oluşumundan sonra gözlendi (P<0,05).

Discussion

Literature presents the alterations in cardiac injury biomarkers following endotoxemia induction in large animals (Green and Adams, 1992; Peek et al., 2008; Perkowski et al., 1996). However, the study on serum biochemical profile of cardiac injury biomarkers in sheep is lacking. According to our findings, serum concentrations of cTnI increased rapidly and significantly at 1st hour after endotoxemia induction ($P<0.05$) and remained at high concentrations up to 24th hour (Figure 5). Cardiac troponin is a myofibrillar protein with two diagnostically-relevant forms (cTnI and cTnT) that regulate contraction of the heart (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 and Sleeper, 2008). In the present study, increase in the cTnI immediately after LPS administration can indicate the myocardial injuries during endotoxemia. Assay of troponins constitutes the preferred biochemical marker for acute myocardial infarction (Polena et al., 2005). Increases in cTnI correlate with a wide range of animal cardiac diseases including dilated cardiomyopathy, endocardiosis, endocarditis and congestive heart failure (Serra et al., 2010). In the current study, Hcy increased rapidly and significantly at 1st hour after LPS infusion ($P<0.05$) and remained at high concentrations up to 24th hour (Figure 6). 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 considered as an independent risk factor for peripheral vascular, cerebrovascular and coronary artery diseases (Nygard et al., 1997). Significant increase in Hcy may be related to myocardial insults during endotoxemia in Iranian fat-tailed sheep. It has been reported that increased plasma and heart tissue Hcy concentrations could be considered as a risk factor in myocardium damage in conditions associated with oxidative stress (Rezaei and Dalir-Naghadeh, 2009). Significant elevations of serum CK-MB and LDH were seen at 1st hour after endotoxin infusion. High concentrations of these enzymes were detected at all hours after endotoxemia induction and remained at elevated values up to 24th hour after LPS administration (Figures 5 and 6). 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 could result in leakage of the enzymes and is considered as a valuable tool in early diagnosis of pathological conditions (Graeber et al., 1990).

In the present study, we evaluated the electrocardiographic parameters during experimental endotoxemia, in the light of above mentioned cardiac biomarkers changes in Iranian fat-tailed sheep. T wave amplitude was significantly decreased during endotoxemia. Attribution of T wave amplitude attenuation to sympathetic activity was part of the interpretation of the effects obtained by studies of cardiac changes during aversive conditioning and biofeedback control (Matyas and King, 1976). R-R interval increased significantly during endotoxemia. R-R interval variability is assessed with power spectral analysis as an index of human cardiac autonomic nervous system function (Pichot et al., 1999). The R-R interval variation on electrocardiograms has been used as an index representing the cardiac parasympathetic activity (Pfeifer et al., 1982). Increasing the R-R interval in this study may reveal the superior activities of parasympathetic functions in comparison to sympathetic nervous system activity and it seems that endotoxemia may induce myocardial autonomic dysfunction.

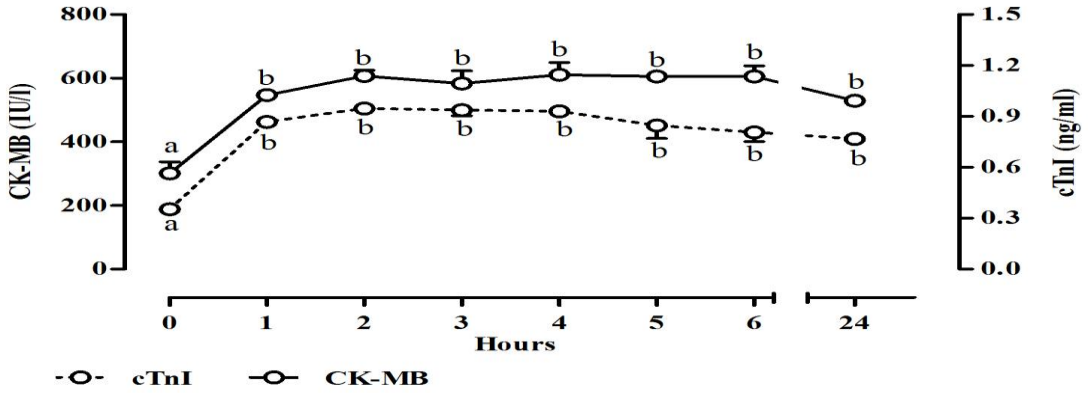

Figure 5. Alterations (mean±SEM) of serum concentration of creatine kinase isoenzyme MB (CK-MB) and cardiac troponin I (cTnI) during experimentally induced endotoxemia in Iranian fat-tailed sheep. Different letters (a and b) at each line indicate significant differences between two different hours (P<0.05).

Şekil 5. İran yağlı kuyruklu koyunlardaki deneysel endotoksemi oluşumu sırasında serum keratin kinaz izoenzim MB (CK-MB) ve kardiyak troponin I (cTnI) konsantrasyonlarındaki değişimler (ortalama±SEM). Her satırdaki farklı harfler (a ve b) iki saat arasındaki belirgin değişimleri göstermektedir (P<0,05).

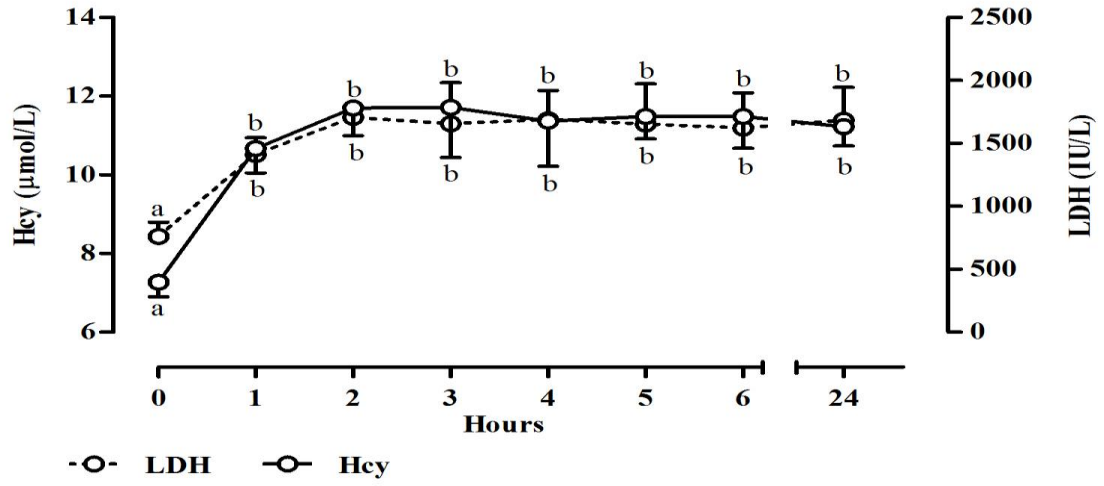

Figure 6. Alterations (mean±SEM) of serum concentration of homocysteine (Hcy) and lactate dehydrogenase (LDH) during experimentally induced endotoxemia in Iranian fat-tailed sheep. Different letters (a and b) at each line indicate significant differences between two different hours (P<0.05).

Şekil 6. İran yağlı kuyruklu koyunlardaki deneysel endotoksemi oluşumu sırasında serum homocystein (Hcv) ve laktat dehidrojenaz (LDH) konsantrasyonlarındaki değişimler (ortalama±SEM). Her satırdaki farklı harfler (a ve b) iki saat arasındaki belirgin değişimleri göstermektedir (P<0,05).

The significant increase in S-T interval was observed during endotoxemia. The S-T interval changes occur within a few minutes of the initiation of hypoxia (Belfort and Saade, 2011). Repolarization of the myocardium as reflected by the S-T interval is an energy-consuming process. It could be suggested that this changes is related to endotoxemic conditions and when there is hypoxia, the energy balance within the myocytes becomes negative and increases S-T

interval (Belfort and Saade, 2011). Significant QT prolongation in our study may result from variations of the autonomic system due to endotoxemia (Champeroux et al., 2010).

In conclusion, according to serum cardiac biomarker changes, it may be stated that myocardial insults could be induced during experimental endotoxemia after *Escherichia coli* LPS serotype O55:B5 administration in Iranian fat-tailed sheep. According to clinical parameters, it can be suggested that changes of serum cardiac biomarkers and eletrocardiographic parameters are due to induced endotoxemia and relation among clinical parameters and other evaluated factors are obvious in endotoxic sheep. Furthermore, it seems that endotoxemia causes myocardial autonomic dysfunction and significant changes in ECG parameters could be interpreted in the light of concurrent cardiac biomarker changes.

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