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INFLUENCE OF 8 KM TRAINING ON CARDIAC BIOMARKERS ALONGSIDE HAEMATOBIOCHEMICAL PROFILES IN RACE CAMELS

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ABSTRACT

This study was designed to investigate the effect of 8 km training on the serum concentrations of the cardiac biomarkers troponin I (cTnI) and creatine kinase myocardial band (CK-MB) in 23 healthy racing camels (*Camelus dromedarius*). From each camel, 2 blood samples were collected; before training (T0) and within 2 h after training (T1). Serum concentrations of cTnI and CK-MB, and hematobiochemical profiles were estimated. Compared to a value of 7.21±1.9 $\times 10^9$/L pre-training, neutrophils decreased significantly to 6.2±2.2 $\times 10^9$/L post-training (P=0.05). Similarly, haemoglobin concentration decreased from 11.1±1.1 g/dL before training to 10.3±2.0 g/dL after training (P=0.0002). The MCV showed a similar pattern where it decreased from 26.0±1.3 (fl) pre-training to 24.0±3.6 (fl) post-training (P=0.01). Other haematological variables did not show any significant changes before and after training (P>0.05). The serum activity of AST increased from 85.5±12.8 U/L before training to 91.5±8.6 U/L after training (P=0.0001). Serum concentration of TP increased also from 54.2±8.7 g/L pre-training to 59.0±3.8 g/L post-training (P=0.04). On the contrary, the serum concentration of lactic acid decreased from 3.9±0.8 (mmol/L) before training to 3.3±0.4 (mmol/L) after training (P=0.004). Other biochemical variables did not show any significant changes before and after training (P>0.05). Before training the serum concentration of cTnI was 0.03±0.03 ng/mL; a value that did not differ significantly when compared to the value of 0.04±0.02 (ng/mL) after training (P=0.60). The CK-MB value differed significantly before and after training (0.47±0.1 ng/mL before training vs 0.48±0.8 ng/mL after training; P=0.004). In conclusion, the cardiac biomarker cTnI did not change significantly after training compared to baseline levels, a result that disagrees with values in camels after race. However, the CK-MB increased significantly after training compared to pre-training serum concentrations.

Key words: Cardiac biomarkers, cTnI, CK-MB, racing camels, training

In recent years, there has been increasing interest in camel racing in the Arab countries especially Gulf region. The average speed of a camel during a race is approximately 9.5 m/sec (Snow, 1992). At the beginning of the race, most camels gallop, and they change frequently between pacing and galloping during the race. Interestingly, camels can pace almost as fast as they can gallop. Many scientific investigations have focused on the training (Evans *et al,* 1992; Snow, 1992). Although the physiological adaptations of the camel have been studied extensively, changes associated with exercise have been ignored until recently (Evans *et al,* 1992).

The diagnostic and prognostic value of the cardiac biomarkers troponin I (cTnI) and creatine kinase myocardial band (CK-MB) has been studied extensively in camels as well as in other animal species (Tharwat, 2012; Tharwat *et al,* 2012; Tharwat *et al,* 2013a,b,c,d,e; Tharwat and Al-Sobayil, 2014a,b,c; Tharwat *et al,* 2014a,b; Tharwat, 2015; Tharwat and Al-Sobayil, 2015; Tharwat, 2020). The serum concentration of cTnI elevates after acute myocardial injury because of leakage from the damaged myocardial cells (O'Brien *et al*, 2006). The cTnI has also a high sensitivity and specificity in animals with diseases of cardiac and non-cardiac origin (O'Brien *et al,* 2006. The degree of increase in cTnI has been shown to correlate with the extent of myocardial damage and with survival in humans (Stanton *et al*, 2005) and animals (Oyama and Sisson, 2004; Fonfara *et al*, 2010). In human athletes, a number of studies have shown increased cTnI concentrations following high-intensity short-duration exercise and cycle-touring events (Serrano-Ostáriz *et al,* 2009; Shave *et al,* 2010; Serrano-Ostáriz *et al,* 2011). The other cardiac biomarker CK-MB has been reported to increase

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with exercise (Mamor *et al,* 1988; Rahnama *et al,* 2011). A rise in CK-MB is not always indicative of myocardial damage; it has been elevated in patients with acute skeletal muscle trauma, dermatomyositis, polymyositis, muscular dystrophy and renal failure (Erlacher *et al,* 2001).

Recently, the cTnI and CK-MB changes in race camels following 5 km race have been determined (Tharwat *et al,* 2013c). Knowledge of the effect of racing on the concentrations of the cardiac biomarkers cTnI and CK-MB is of importance when evaluating racing camels with suspected cardiac disease after recent racing or maximal exercise. However, studies on the influence of training on the serum concentrations of the cardiac biomarkers in camels is lacking. The aim of the present study was therefore to investigate the effect of 8 km training on the serum concentrations of cTnI and CK-MB alongside haematobiochemical profiles in healthy racing camels.

Materials and Methods

Animal and blood sampling

Twenty-three healthy female racing camels (*Camelus dromedarius*) aged 7.6±2.4 years and weighed 312±61 kg that participated in 8 km training were used in another experimental design but with a different protocol (Tharwat *et al,* 2013c). These animals were ensured normal complete physical examination findings, normal cardiac auscultation, normal complete blood cell counts (VetScan HM5, Abaxis, CA, USA), normal biochemistry profiles (VS2, Abaxis, CA, USA), a continuous electrocardiography recording (Kenz-Cardio 302 Suzuken Co Ltd., Nagoya, Japan), and echocardiography (SSD-500, Aloka, Tokyo, Japan) (Tharwat *et al,* 2012). Blood samples (10 mL) were collected from the jugular vein as follows; 3 mL in EDTA tubes, 2 mL in heparinised tubes and the remaining 5 mL in plain vacutainer tubes of each, immediately prior to training (T0) and within 2 h of completion of the training (T1). Sera were harvested and were aliquotted in tubes and immediately stored at -20°C pending analysis.

Haematobiochemical profiles and cardiac biomarkers assays

Haematological examinations were carried out immediately on EDTA blood samples as shown in Table 1 using an automated analyser (VetScan HM5, Abaxis, California, USA). Heparinised blood samples were used to determine the bichemical parameters as shown in Table 2 using an automated biochemical analyser (VetScan VS2, Abaxis, California, USA). The serum samples were thawed and immediately analysed for cTnI using the point-of-care analyser according to the manufacturer's instructions. The CK-MB mass measurements were performed using the Cobas 6000 C501 assay (Roche Diagnostics, Indianapolis, Indiana, USA), with an electrochemiluminescent assay. The lower limit of detection of CK-MB for this assay was 0.1 ng/mL.

Statistical analysis

Data normality was examined using the Kolmogorov–Smirnov test. The data were presented as means ± SD, and were analysed statistically using the SPSS statistical package (2009). A Student's t-test was used for comparisons between pre- and post-training values. Significance was set at $P \leq 0.05$.

Results

Table 1 summarises the haematological variables (mean±SD) in race camels before and after 8 km training, alongside the 25th, 50th, 75th and 95th and 99th percentiles. Compared to a value of 7.21±1.9 ×10^9/L pre-training, neutrophils decreased to 6.2±2.2×10^9/L post-training (P=0.05). Similarly, haemoglobin concentration decreased from 11.1±1.1 g/dL before training to 10.3±2.0 g/dL after training (P=0.0002). The MCV showed a similar pattern where it decreased from 26.0±1.3 (fl) pre-training to 24.0±3.6 (fl) post-training (P=0.01). Other haematological variables did not show any significant changes before and after training (P>0.05).

The biochemical profiles (mean ± SD) in race camels before and after 8 km training, alongside the 25th, 50th, 75th and, 95th and 99th percentiles are presented in Table 2. The serum activity of AST increased from 85.5±12.8 U/L before training to 91.5±8.6 U/L after training (P=0.0001). Serum concentration of TP increased also from 54.2±8.7 g/L pre-training to 59.0±3.8 g/L post-training (P=0.04). On the contrary, the serum concentration of LA decreased from 3.9±0.8 (mmol/L) before training to 3.3±0.4 (mmol/L) after training (P=0.004). Other biochemical variables did not show any significant changes before and after training (P>0.05).

Fig 1 illustrates the serum concentration of the cardiac biomarkers cTnI before and after training. Before training the serum concentration of cTnI was 0.03±0.03 ng/mL; a value that did not differ significantly when compared to the value of 0.04±0.02 (ng/mL) after training (P=0.60). The serum concentration of the cardiac biomarker CK-MB before and after training is illustrated in Fig 2. The CK-MB

Table 1. Haematological parameters in race camels before and after 8 km training (n=23).

Variable	Before training						After training						*P* value
	Mean ± SD	Percentile					Mean ± SD	Percentile					
		25%	50%	75%	95%	99%		25%	50%	75%	95%	99%	
WBCs ($\times10^9$/L)	12.9±3.5	9.8	12.9	14.7	16.7	16.8	11.7±2.1	10.6	11.7	14.0	15.5	15.7	0.92
Lymphocytes ($\times10^9$/L)	4.3±1.8	2.9	4.3	6.3	6.6	7.0	3.7±1.9	2.4	3.7	5.4	6.9	6.9	0.21
Monocytes ($\times10^9$/L)	0.3±0.3	0.2	0.3	0.4	0.9	1.1	0.3±0.3	0.2	0.3	0.5	0.9	1.0	0.81
Neutrophils ($\times10^9$/L)	7.21±1.9	6.4	7.2	8.5	9.4	10.0	6.2±2.2	5.1	6.1	7.8	8.1	9.0	0.05
Lymphocytes (%)	37.6±5.8	33.5	37.6	42.8	44.8	44.8	37.5±5.2	35.0	37.5	41.3	44.6	46.3	0.98
Monocytes (%)	2.3±1.7	1.9	2.3	3.1	6.3	6.7	2.1±2.1	1.8	2.1.0	4.2	7.03	7.5	0.85
Neutrophils (%)	60.3±6.8	54.3	60.3	64.0	66.5	74.0	60.0±6.4	53.3	60.0	63.1	67.8	73.4	0.96
RBCs ($\times10^{12}$/L)	9.2±1.0	8.7	9.2	10.1	10.9	11.4	8.8±2.5	8.0	8.8	10.1	12.2	15.2	0.57
Haemoglobin (g/dL)	11.1±1.1	10.7	11.1	12.6	13.4	13.7	10.3±2.0	10.0	10.3	11.4	13.4	13.5	0.0002
Hematocrit (%)	23.7±4.5	20.7	23.7	26.2	27.6	30.0	22.2±4.8	20.8	22.2	25.3	27.6	30.2	0.60
MCV(fl)	26.0±1.3	26.0	26.0	27.0	28.0	28.0	24.0±3.6	22.8	24.0	26.3	27.0	27.0	0.009
MCH (pg)	11.6±1.7	10.4	11.6	12.1	12.6	12.9	12.0±8.2	11.0	12.0	16.3	23.1	39.7	0.070
MCHC (g/dL)	44.5±7.1	40.4	44.5	47.0	49.7	53.3	48.6±25.1	42.4	48.6	58.8	80.2	128.0	0.08
Platelet count ($\times10^9$/L)	124.5±30.9	115.0	124.5	147.0	173.7	213.9	123.5±32.0	111.3	123.5	140.0	166.9	179.8	0.31

WBCs, white blood cells; RBCs, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 2. Biochemical parameters in race camels before and after 8 km training (n=23).

Variable	Before training						After training						*P* value
	Mean ± SD	Percentile					Mean ± SD	Percentile					
		25%	50%	75%	95%	99%		25%	50%	75%	95%	99%	
Albumin (G/L)	54.0±9.6	50.0	54.0	60.5	68.05	68.8	58.0±3.4	57.0	58.0	61.0	64.3	68.9	0.06
ALP (U/L)	64.8±34.5	53.3	64.8	94.5	119.3	154.3	67.0±40.5	58.0	67.0	89.5	168.7	178.5	0.53
AST (U/L)	85.5±12.8	79.8	85.5	94.5	107.2	109.4	91.5±8.6	88.5	91.5	99.0	109.2	112.2	0.0001
Calcium (mmol /L)	2.1±0.4	1.9	2.1	2.5	2.7	2.8	2.3±0.2	2.3	2.3	2.3	2.6	2.7	0.16
GGT (U/L)	7.8±1.9	7.0	7.8	8.8	11.1	11.8	8.0±2.1	7.0	8.0	8.1	11.1	11.8	0.79
Total protein (G/L)	54.2±8.7	50.0	54.2	60.5	67.2	69.4	59.0±3.8	57.8	59.0	61.0	62.4	67.7	0.04
Globulin (G/L)	2.8±1.2	2.0	2.8	3.0	4.1	5.6	3.7±1.8	1.9	3.7	4.6	5.8	6.8	0.17
BUN (mmol /L)	8.1±1.3	7.7	8.1	8.9	9.8	11.2	9.0±1.4	8.6	9.0	9.5	11.1	11.8	0.08
CK (U/L)	153.5±42.1	138.5	153.5	186.3	228.0	257.6	142.0±24.5	124.8	142.0	150.5	184.2	202.4	0.13
Phosphorus (mmol/L)	1.8±0.4	1.6	1.8	2.2	2.4	2.6	1.9±0.3	1.8	1.9	2.1	2.4	2.6	0.37
Magnesium (mmol /L)	0.9±0.2	0.8	0.9	1.1	1.3	1.3	1.04±0.1	1.0	1.1	1.1	1.2	1.3	0.18
cTnI (ng/mL)	0.03±0.03	0.02	0.03	0.05	0.08	0.09	0.04±0.02	0.03	0.04	0.05	0.06	0.07	0.60
CK-MB (ng/mL)	0.47±0.1	0.29	0.47	0.50	0.53	0.54	0.48±0.8	0.42	0.48	0.73	2.50	2.55	0.02
LA (mmol/L)	3.9±0.8	3.4	3.9	4.3	5.2	5.3	3.3±0.4	3.3	3.1	3.3	3.6	3.8	0.004

ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; BUN, blood urea nitrogen; CK, creatine kinase; cTnI, cardiac troponin I; CK-MB, creatine kinase myocardial band; LA, lactic acid.

value differed significantly before and after training (0.47±0.1 ng/mL before training vs 0.48±0.8 ng/mL after training; P=0.004).

Discussion

Significant elevations of cTnI in camel blood following racing (Tharwat *et al,* 2013c) have been observed following racing. An elevated serum concentration of cTnI has been used as a poor prognostic indicator in goats with pregnancy toxaemia (Tharwat *et al,* 2012) and in downer camels (Tharwat, 2012). In a study published recently in camels with tick infestation (Tharwat and Al-Sobayil, 2014a), it was assumed that the increased serum concentration of cTnI above 1.0 ng/ml at initial examination has a bad prognostic indicator.

Following 5 km race in dromedary camels, the serum concentration of cTnI increased significantly 2 h after race (Tharwat *et al,* 2013c). However, in present study, the serum concentration of cTnI did not change significantly before and after training (P=0.60). Results agree with a previous study in horses, where their plasma cTnI levels did not increase (P=0.48) 3-6 h after they had performed short-term high-intensity exercise for a distance of 2.0 to 2.4 km on a treadmill (Durando *et al,* 2006). The high-intensity effort of the camels during race (Tharwat *et al,* 2013c) may be a contributing factor of cTnI increase during race, but not during training. Post-exercise cTnI release and clearance were also reported in normal Standardbred racehorses. All horses experienced an increase in cTnI post-exercise, with peak occurring 2-6 h post-exercise (Rossi *et al,* 2019). In a study carried out on racing greyhounds following a 7 km race, almost all greyhounds showed increases in cTnI concentrations which were significantly higher than the pre-race concentrations (P<0.0001). However, out of the 23 racing greyhounds, only 5 showed mild increases in CK-MB concentrations but these did not significantly differ from the pre-race values (P>0.05) (Tharwat *et al,* 2013e).

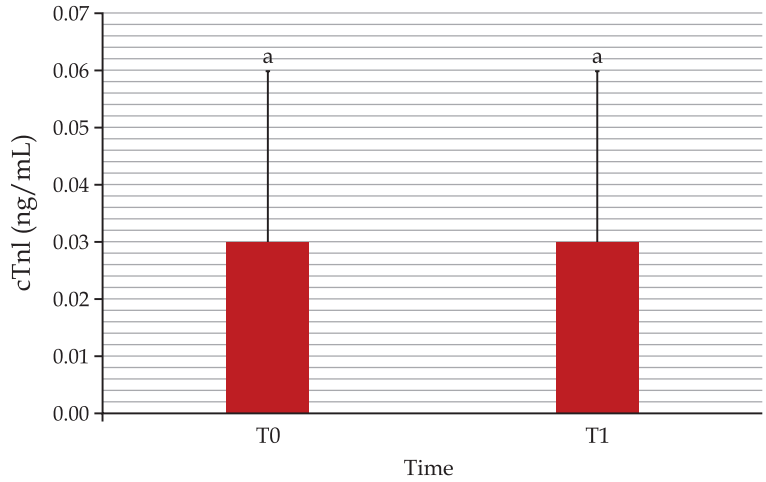

Fig 1. Cardiac troponin I (cTnI) values in camels before (T0) and 2h after 8 km training (T1). [a]Same letters did not differ significantly (P>0.05).

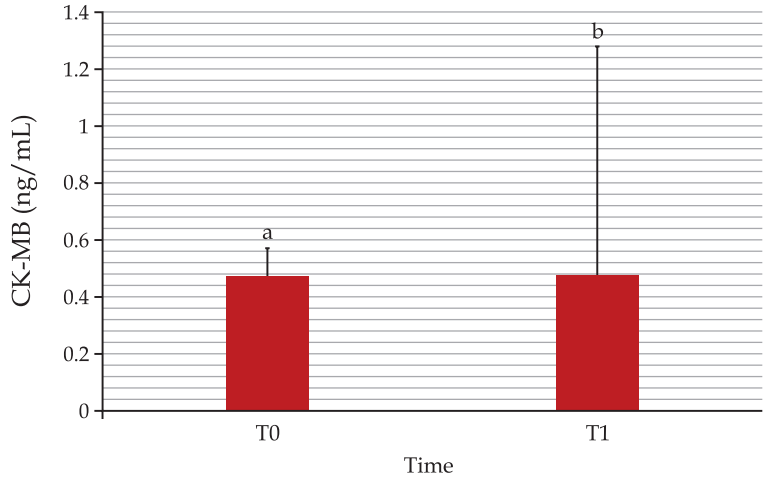

Fig 2. Creatine kinase myocardial band (CK-MB) values in camels before (T0) and 2h after 8 km training (T1). [a,b]Different letters indicate a significant difference (P<0.05).

In horses, increased concentrations of cTnI have been reported in association with endurance competition as well as after short-term maximal exercise on a treadmill for 2.0-2.4 km (Durando *et al,* 2006; Holbrook *et al,* 2006). In addition, serum cTnI concentrations were mildly elevated in some horses 1 to 14 h after racing (Nostell and Haggstrom, 2008).

In a study in standardbred racehorses, all animals experienced an increase in cTnI post-exercise, with peak occurring 2-6 h post-exercise (Rossi *et al,* 2019). In contrast, Phillips *et al* (2003) have reported that serum cTnI concentrations in race-training thoroughbred horses were not significantly different from those of pastured horses.

In the 5 km race in dromedary camels, the serum concentration of CK-MB value did not differ significantly (P=0.855) (Tharwat *et al,* 2013c). In the current study, the serum concentration of CK-MB increased significantly when compared to pre-training values (P=0.004). This result agrees well with other reports of CK-MB increase with exercise (Mamor *et al,* 1988; Rahnama *et al,* 2011). There are 3 isoforms

for the enzyme CK: BB, MM, and MB. The BB isoform is found primarily in the brain. Skeletal muscles primarily contain the MM isoform, with traces of MB (estimates of 1-4% of CK activity). Cardiac muscles also contain primarily the MM isoform, but higher amounts of MB, typically around 20% of CK activity (Moss *et al*, 1994). In a study conducted by Gojanovic *et al* (2011), no changes were observed in the serum concentration of CK-MB or cTnI as a result of whole-body vibration training.

The haematological parameters decreased significantly after training included neutrophils count, haemoglobin concentration and MCV. However, the total WBCs count did not differ significantly before and after training (P=0.92). Similarly, in racing camels with 5 km race, the WBC count did not change significantly pre- and post-race (P=0.11) (Tharwat *et al,* 2013c). Concerning the biochemical parameters, the AST activity and the TP concentration increased significantly after training (P=0.0001, P=0.04, respectively). Opposite, the serum concentration of LA decreased significantly after training (P=0.004). In a similar pattern in racing camels, the serum concentration of LA decreased significantly after race (P<0.0001). In another study in camels, lactate concentration decreased, but not significantly, after transportation for a 5-h round-trip journey (Tharwat *et al*. 2013b). Lactate is known as the end product of anaerobic glycolysis, a pathway that is of key importance during normal metabolic and athletic events (Pösö, 2002). Lactate accumulation occurs when the balance between production and consumption is breached. Instead of being regarded as a waste product, LA is now seen as a valuable substrate that contributes significantly to the energy production of the heart, muscles and even the brain. It may be used as fuel by many organ systems including the heart, liver and kidneys (Pösö, 2002; Tennent-Brown 2012). Therefore, the decreased serum concentration of LA could be due to its consumption by the muscles during training. In conclusion, the cardiac biomarker cTnI did not change significantly after training compared to baseline levels, a result that disagrees with values in camels after race. However, the CK-MB increased significantly after training compared to pre-training serum concentrations.

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