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Effect of endurance load exercise on physio-biochemical and hormonal parameters of single-humped camels (*Camelus dromedarius*) at high altitude

SUNIL KUMAR[1✉], DOLKER LAMO[1], GEETA GAHLAWAT[1], VIJAY K BHARTI[1] and KRISHNA KUMAR[1]

DRDO-Defence Institute of High Altitude Research, Leh, Ladakh UT 194 101 India

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ABSTRACT

The present study was carried out for 7 days on four adult low-lander single-humped camels to know the effect of endurance load exercise on physiological, biochemical, hormonal, and inflammatory cytokines at high altitude. A significant 1.5 to 3 fold increase was observed in physiological responses, viz. the respiration and heart rate on the 1st and 7th day after the load endurance exercise. Further, serum triglycerides levels were significantly increased on the 7th day after the load endurance exercise, whereas other biochemical parameters were unaffected. However, hormones and inflammatory cytokines responses, viz. cortisol, cardiac-troponin (C-troponin), interleukin-6 (IL-6), tri-iodothyronine (T3), thyroxine, thyroid-stimulating hormone (TSH), and tumor necrosis factor-α (TNF-α) were significantly increased on 1st and 7th day after the endurance exercise. These physio-biochemical changes during load endurance exercise indicated that low-lander single-humped camels have low endurance and are under physiological stress in high altitude conditions. Thus, the present study has brought new primary data and information on physio-biochemical parameters of the single-humped camel at high altitude. This data may help identify suitable camels for load-carrying and other logistics at high altitude areas.

Keywords: Endurance, High-altitude, Hormones, Ladakh, Physio-biochemical, Single-humped camel

Ladakh region is a high altitude area and has very harsh climatic conditions and rocky-gravel rough terrains that badly impact the deployment of pack animals and all-terrain vehicles (ATV). Thus, people are dependent on high endurance native pack animals, viz. pony, yak, and double-humped camel, for load carrying, riding, and other logistics (Lamo *et al*. 2020). Native Zanskar ponies used in these areas can carry only 60 to 70 kg of weight, so they are unsuitable for heavy load carrying and other logistics (Vivek *et al*. 2017). Therefore, it is essential to enhance the capability of native people in these difficult terrains of high altitude through alternative pack animals having a better capacity to carry the load and low cost of maintenance. Interestingly, camels are hardy animals as they can work and carry weight up to 200 to 350 kg comfortably in the extremes of altitude and climatic conditions. Camels have unique adaptability to oxidative stress conditions as depicted by their heavy load carrying capacity, exercise endurance, reproduction, and disease resistance despite poor nutrition at high altitude (Mohammed 2000, Quajd and Kamel 2009, Roy and Tiwari 2010, Wu *et al*. 2014). Therefore, camel can be deployed in different operational environments, including the high altitude of different regions worldwide, for load carrying and patrolling. Hence, a camel could be a good substitute for ponies for load carrying and patrolling in border areas of the Ladakh region (Roy and Tiwari 2010, Makhdoomi *et al*. 2013). However, the native double-humped camel population is small; hence, the exotic low-lander single-humped camel may be a suitable alternative for double-humped camel at high altitude (Vyas *et al*. 2015, Lamo *et al*. 2020). Though, the low-lander single-humped camel is yet to be evaluated for load endurance performances at high altitude.

The high altitude region has hypobaric-hypoxia, high UV radiation, cold stress, low humidity, extreme temperature variation of -20°C to 35°C, and difficult terrains. These harsh climatic conditions lead to oxidative stress, impaired immune system, poor growth, and reproductive health in livestock at high altitude (Monge and Leon-Velarde 1991, Bharti *et al*. 2016, Giri *et al*. 2019, Xin *et al*. 2019). Further, the most critical stress factor at high altitude for lowlander animals is hypobaric-hypoxia, which is considered severe physiological stress which affects their metabolic functions and makes them prone to high altitude-related diseases (Giri *et al*. 2017, Vivek *et al*. 2017, Kalia and Bharti 2018, Verma *et al*. 2018, Kumar *et al*. 2019, Lamo *et al*. 2020). In the hypobaric-hypoxia state, oxygen diffusion to the vascular system becomes very difficult, affecting cellular functions, energy metabolism, and later endurance performance of animals in the cascade manner (Horscroft and Murray 2014, Sperlich *et al*. 2017).

Present address: [1]DRDO-Defence Institute of High Altitude Research, Leh, Ladakh UT. ✉Corresponding author email: as108611@gmail.com

The single-humped camel is not found in high altitude areas; hence if required for logistic use, they must be transported, acclimatized, and trained for load-carrying before deployment. Therefore, the processes concerning their performance and health parameters need to be standardized. Furthermore, an in-depth study is required to understand their physiology and evaluate their feasibility for riding and load-carrying to meet the logistic needs of the native people at high altitude. Therefore, analysis of physiological responses, liver, and kidney function biochemical parameters, along with some hormones and inflammatory cytokines can be used to evaluate endurance performance in pack animals.

With this backdrop, this study was undertaken to investigate the physiological, biochemical, and hormonal changes before and after the endurance load exercise of a single humped camel at Ladakh. So far, no studies have been conducted to evaluate the effect of endurance on the physiology of single-humped camels in high-altitude regions.

MATERIALS AND METHOS

Experimental animals and place of study: The present study was conducted at DRDO-Defence Institute of High Altitude Research (DIHAR), Leh, UT Ladakh, India, a high altitude place located at 11,500 feet MSL. The necessary permission was obtained from the Institute Animal Ethics Committee (IAEC). All the good animal welfare and veterinary care practices were followed during field load exercise, animal handling, and sampling. A total of four (2 males and 2 females) 54-56 months old adult lowlander single-humped camels of either sex were procured from ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India and transported to the experimental place from March-April 2017 for acclimatization to high altitude and load endurance studies. The limitation of this study was the small sample size due to constraints of animal unavailability, ethical issues, and logistics in this difficult terrain for this kind of experiment. However, this is the first such study that brought important primary data, which will be the basis of future studies on the larger sample size. Although differences in male and female exercise capacity have been studied extensively in rodent species with debatable findings, few studies indicated higher exercise capacity in females (Konhilas *et al*. 2004, McMullan *et al*. 2016, Sun *et al*. 2018). However, camels are induced ovulator and ruminants, which may have different effects on endurance load exercise in females and males.

The camels were transported in stages from Bikaner (Plain areas) to Kashmir (Moderate hilly areas), then to Ladakh, India (High altitude area), and reared for two years for proper acclimatization before performing the present exercise. The readymade concentrate ration and hay lucerne were fed as per the standard ration scale of the institute for high altitude, whereas *ad lib.* drinking water was provided. All the camels were clinically monitored for behaviour and health during the entire period of study.

Endurance performance trials: This experiment was conducted for seven days during the summer season under an ambient temperature (18±3°C). For this endurance trial, all the four adult camels were packed with the permissible lower limit of constant weight of 200 kg load of sand over a gunny bag made saddleback. This load quantity was fixed as per @ 35-40% body weight and comfort of animals considering welfare as per the pilot study. All these camels were allowed to travel on a flat track up to 3 km, which took 85±15 min with an average speed of 35.28 m/min to complete the track.

The load endurance exercise period (Treatment group) was compared with before the load period (Control group), as no-load was given before the start of the experiment. Further, all the parameters were compared with the value of single-humped camels in plain areas from a published literature report. Moreover, considering the statistical requirement, not more than 25% of individual value should be on the group's mean (Casler 2015). Thus four or more number of replicates is a sufficient sample size for a large animal like a camel.

Collection of the blood sample: All the camels showed normal behaviour during handling. After careful restraining, about 6-8 ml of blood was collected from the jugular vein of each camel on the first and seventh day before and after the load endurance exercise. After that, serum was isolated and transported to DIHAR (DRDO) Base Laboratory, Chandigarh, for various biochemical, cytokines and hormonal parameters analysis.

Analysis of physiological parameters: Physiological parameters, viz. heart rate (HR), respiration rate (RR), and rectal temperature (RT) were recorded just before initiating the trials and immediately after completion of the load-carrying exercise at Leh-Ladakh. Heart rate was measured using a stethoscope in breaths per minute (bpm), and the respiration rate was recorded in beats per minute (bpm) by observing the movement of the belly and nostrils for one minute. The electronic digital thermometer measured rectal temperature until it gave the beep sound.

Analysis of biochemical parameters: To investigate the biochemical changes of before and after load-carrying trials, the following assays were carried out in serum, viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, urea, uric acid, creatinine, glucose, magnesium (Mg), triglycerides, calcium (Ca) and total protein by using commercially available kits as per manufacturer instruction using serum biochemistry analyzer (BS-120 Mindray Medical Pvt. Ltd, Shenzhen).

Analysis of hormonal parameters and inflammatory cytokines responses: All hormonal parameters, viz. cortisol, cardiac-troponin-1 (c-Tn1), interleukin-1 (IL-1), interleukin-6 (IL-6), triiodothyronine (T-3), thyroxine (T-4), thyroid-stimulating hormone (TSH), and tumor necrosis factor-α (TNF-α) were analyzed using ELISA-based assay methods by commercially available kits.

Statistical analysis: Data obtained from the experiments were expressed as mean±standard error of the mean

(Mean±SEM) and statistically analyzed using the One Way Analysis of Variance (Tukey's HSD method) to compare the difference of mean values before and after load trials. Statistical analysis was performed using Sigma plot software (version 12.5), and $p<0.05$ were significant.

RESULTS AND DISCUSSION

The present study investigated single humped camels' load-carrying capacity at high altitude. The preliminary results showed no clinical signs of ortho-muscular and gait difficulties. None of the camels showed any discomfort and rectal straining during loading and completed the entire load-carrying track without interluding. All the animals showed normal social behaviour, and none of them showed irritant behaviour, viz. legs kicking, biting, spitting, body shaking, etc., during load endurance exercise.

Physiological responses: Physiological parameters, viz. heart rate, respiration rate, and rectal temperature were recorded just before trials (control) and immediately after completing trials on the first and seventh day. HR showed a 1.5 fold increase in all the camels on the first and seventh days after load trials (Table 1; $p<0.05$), whereas RR showed a 3 and 1.5 fold increase during the first and 7-day afterload, respectively. No significant increase in rectal temperature was observed on the first day after the loading exercise, although a slight increase in temperature was recorded on the seventh day after the load trials. Physiological responses are an essential indicator of animal adaptation to specific physiological status under a particular environment, which plays a crucial role in body homeostasis (Mohammed *et al*. 2007). The endurance load exercise creates stress conditions that induce neuroendocrine changes in the body, thereby increasing the sympathetic nervous system activity (Hackney 2006). This might have caused a significant increase in heart rate in low-lander Dromedarian camels at high altitude. Further, this high HR and RR could be due to an exercise-induced increase in blood flow towards the periphery, including skeletal muscle, for heat dissipation through the skin to meet the enhanced energy supplies (Reimers *et al*. 2018). These changes create hypoxic conditions in the tissue, which increases the oxygen demand; hence increased RR is observed to meet high blood oxygen demand (Moon *et al*. 2016). The rectal temperature was unaffected on the first day, whereas it significantly increased on the seventh day after the load. However, no changes were recorded in rectal temperature compared to first day. This could be due to the acclimatization of single-humped camels to the prevailing environment and experimental exercise regime.

Biochemical changes: The changes in biochemical parameters help in evaluation of camel health and load-carrying performance. The biochemical changes indicated no significant difference in total protein, AST, ALT, albumin, glucose, creatinine, Ca, Mg, urea, and uric acid in all the camels on the first and seventh day after the load trial (Table 2; $p>0.05$). However, there was a significant difference in triglycerides level on the seventh day after load trials ($p<0.05$) (Table 2). Liver metabolism is a vital physiological process to maintain body homeostasis through optimization of enzymatic and cellular function (Aragon and Younossi 2010). The high serum triglycerides levels might be because of endurance load response hepatocytes and release of lipid in the blood that circulates in the body to provide instant energy to muscles that are much needed during exercise.

Hormonal responses: It is a well-known fact that adrenal and thyroid secretary hormones play a significant role in thermoregulation, metabolic function, reproduction, and homeostasis in livestock, including camels (Bengoumi *et al*. 1999, Saeb *et al*. 2009, Youssef *et al*. 2015). Hence, hormonal changes were investigated before and after load-carrying to understand the endocrine role in homeostasis during endurance load exercise in single-humped camels at high-altitude. The mean serum concentrations of cortisol, T-3, T-4, and TSH were significantly ($p<0.05$) increased in all the single-humped camels during the first and seventh day after the load trial (Table 3). Cortisol is a steroid hormone released into circulation in response to stress and low blood glucose concentrations (Lektib *et al*. 2016). Hence, an increase in oxidative stress during endurance load trials could be responsible for increasing cortisol levels in the present study. The thyroid hormones, viz. T3, T4, and TSH play a vital role in body metabolism and antioxidant enzyme activities in camels (Ahmad *et al*. 2007). So, excessive generation of reactive oxygen species during endurance exercise might have caused the increased serum thyroid hormone levels. Thyroid hormones have a specific role in regulating mitochondrial respiration, which is the main site of oxidative phosphorylation and necessary antioxidant enzymes (superoxide dismutase and glutathione peroxidase) function. Hence, these hormone level increased after exposure to stress conditions.

Inflammatory cytokines responses: The mean serum concentrations of c-Tn1, IL-6, and TNF-α were significantly ($p<0.05$) increased at first and seventh day, whereas IL-1 was significantly ($p<0.05$) increased at seventh

Table 1. Physiological parameters (Mean±SEM) of low-lander single-humped camel during endurance load exercise at high-altitude

Physiological parameter	1^{st} Day (BL*)	1^{st} Day (AL#)	7^{th} Day (BL*)	7^{th} Day (AL#)
Heart rate (bpm)	39.0 ± 1.29^{a}	66.0 ± 4.32^{b} (1.5 fold increase)	47.5 ± 2.87^{a}	75.0 ± 3.69^{b} (1.5 fold increase)
Respiration rate (bpm)	10.5 ± 0.50^{a}	31.5 ± 2.50^{c} (3.0 fold increase)	15.75 ± 0.95^{a}	21.5 ± 1.50^{b} (1.5 fold increase)
Rectal temperature (°C)	37.10 ± 0.12^{b}	37.35 ± 0.12^{b}	36.80 ± 0.15^{a}	37.15 ± 0.26^{b}

*BL, Before Load; #AL, After Load; SEM, Standard error of mean; ^{a,b,c}Mean values bearing the same superscripts in a column do not differ significantly ($p>0.05$).

Table 2. Biochemical parameters (Mean±SEM) of low-lander single-humped camel during endurance load exercise at high-altitude

Biochemical parameter	1st Day (BL*)	1st Day (AL#)	7th Day (BL*)	7th Day (AL#)
Albumin (g/dl)	2.65±0.39	3.17±0.60	3.02±0.41	3.25±0.34
Calcium (mg/dl)	7.10±1.22	8.68±0.63	7.63±0.92	8.02±.0.30
Creatinine (mg/dl)	1.07±0.62	1.03±0.24	1.03±0.50	1.95±0.31
Alanine aminotransferase (U/L)	6.80±1.91	6.28±2.18	7.57±3.80	9.15±1.9
Aspartate aminotransferase (U/L)	49.38±5.6	62.08±6.12	59.10±6.3	65.95±8.87
Triglycerides (mg/dl)	29.43±5.43[a]	33.53±5.43[a]	33.17±3.8[ab]	45.55±3.89[b]
Magnesium (mg/dl)	1.63±0.35	1.3±0.27	1.83±0.42	1.56±0.21
Total protein (g/dl)	6.94±0.19	6.47±0.46	6.49±0.23	6.62±0.46
Uric acid (mg/dl)	0.23±.047	0.20±0.28	0.35±0.04	0.22±0.062
Urea (mg/dl)	40.93±3.04	52.84±5.81	52.86±7.71	52.31±3.84
Glucose (mg/dl)	61.84±7.70	65.69±5.47	43.84±10.92	68.02±8.04

*BL, Before Load; #AL, After Load; SEM, Standard error of mean; [a,b]Mean values bearing the same superscripts in a column do not differ significantly (p>0.05).

day after the load trial (Table 3). The environmental and metabolic stress during endurance trials is responsible for increasing pro-inflammatory markers, i.e. IL-6, IL-1, and TNF-α (Bernecker *et al*. 2013, Jahromi *et al*. 2014). So, load endurance exercise in the present study might have caused up-regulation of serum TNF-α, IL-1, and IL-6. These conditions, if prolonged, can be potential triggers to increase the depletion of glycogen levels in muscles (Petersen and Pedersen 2005). Similarly, IL-6 cytokine has also been known to induce hepatic glucose output and lipolysis in skeletal muscles in response to endurance exercise (Pedersen *et al*. 2001). Cannon *et al*. (1989) have elucidated that IL-1 cytokines are rapidly cleared from circulation after endurance exercise but remain visible in tissues for a extended period. In the present study, the levels of IL-1 cytokines remain unchanged during the first day of load trials, which might be due to the rapid clearance of IL-1 from blood circulation. However, their level significantly increased on the seventh day after the load trial.

The c-Tn1 is a sensitive cardiac biomarker of cardiac muscle injury (Tharwat *et al*. 2013a). Hence, c-Tn1 monitoring may help in the detection of any cardiac disease or injury. In the present study, c-Tn1 concentration was increased on the first and seventh days after load endurance exercise trials. This could be due to more cardiac stress in hypoxic conditions at high altitudes, as c-troponin has shown high specificity of cardiac muscles' (Tharwat *et al*. 2013b). However, Akwe *et al*. (2018) reported the non-cardiac origin of c-Tn1 after strenuous physical exercise. After seven days of continuous load trials, the c-Tn1 level returned to normal, and there was no significant difference between the first and seventh day before load trials data (Table 3). However, the c-Tn1 level showed adverse changes after load trials on the first and seventh day. These findings indicate that exercise-induced stress if prolonged, may cause cardiac injury. Hence, a sufficient rest period may be required after load-carrying by these animals at high altitude.

In conclusion, the endurance load exercise resulted in adverse changes in physiological responses and cytokines and hormonal levels in the low-lander single-hump camel at high altitude. This is the first study on physio-biochemical changes in single-humped camels during endurance load exercise at high-altitude, which may be considered as primary data to understand the effect of endurance exercise in camels at high altitude. However, future studies should be focussed on a large number of low-lander single-humped camels. Further, their progeny born at high altitude should also be studied for molecular parameters related to adaptation, immunity, and inflammation in a different season. This study may also help in the screening of low-lander single-humped camels for emergency deployment to high-altitude for load-carrying capacity and other logistics,

Table 3. Hormonal parameters (Mean±SEM) of low-lander single-humped camel during endurance load exercise at high-altitude

Hormone	1st Day (BL*)	1st Day (AL#)	7th Day (BL*)	7th Day (AL#)
Cortisol (ng/ml)	204.98±4.88[a]	256.06±5.86[b]	216.35±2.97[a]	256.35±5.13[b]
C-troponin (ng/ml)	25.49±0.43[a]	29.71±0.63[b]	24.06±0.48[a]	29.18±0.96[b]
Interleukin-1 (ng/ml)	2.56±0.033[a]	2.68±0.063[a]	2.59±0.058[a]	2.74±0.018[b]
Interleukin-6 (ng/ml)	2.20±0.04[a]	2.67±0.03[b]	2.19±0.05[a]	2.51±0.08[b]
Tri-iodothyronine (ng/ml)	28.02±0.8[a]	34.89±0.85[b]	29.26±0.86[a]	34.64±0.44[b]
Thyroxin (ng/ml)	780.11±20.53[a]	906.78±33.43[c]	798.28±19.57[a]	886.57±24.2[bc]
TSH (mlU/ml)	21.44±0.97[a]	26.06±0.23[c]	22.34±0.36[a]	24.56±0.35[bc]
TNF-α (ng/ml)	31.55±0.91[a]	38.24±1.53[b]	32.71±1.69[a]	41.31±0.55[b]

*BL, Before Load; #AL; After Load; C-troponin, Cardiac troponin; TSH, Thyroid-stimulating hormone; TNF-α, Tumor necrosis factor-alpha; SEM, Standard error of mean; [a,b,c]Mean values bearing the same superscripts in a column do not differ significantly (p>0.05).

which would be helpful for meeting the local people's demands and give insights to the veterinary physician for the examination of camel health.

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