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Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters

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Abstract Transportation is often considered as one of the main causes of stress raising considerable interest, both in economic and animal welfare terms. The objective of the current study was to determine physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical factors during summer conditions. Ten Iranian dromedary camels, five males and five females, were selected for the study. The study was conducted on three consecutive days in August 2008. At first day, blood samples were collected at 08:30 A.M., 09:30 A.M. and 01:30 P.M. to determine any possible variation in individual measurements due to diurnal changes or as a result of food and water deprivation for 5 h. Travel commenced on day 2 at 08:30 A.M. for 5 h, with a total of about 300 km traveled. At second day, blood samples were collected immediately before loading, at 08:30 a.m., after 1 h transport, at 09:30 A.M., and on the end of transportation, after unloading, at 01:30 P.M. Final blood sample was taken 24 h after arrival. In the current study no significant difference was observed in any parameter between sexes at each sampling time. The data related to day before transport had no significant differences between different times except for values obtained for cortisol that at 01:30 P.M. showed a significant decrease in comparison with data at 08:30 and 09:30. Circulating cortisol, T_4, T_3 and fT_4 levels was significantly higher after transportation compared with pre-transport values and returned to basal values within 24 h after transport. Transportation had effects on metabolism as demonstrated by increase in serum concentrations of glucose, NEFA, and urea nitrogen. Serum concentrations of glucose, NEFA, and urea nitrogen returned to basal values in final bleeding at 24 h after transport termination. In the current study transportation had no significant effects on serum concentrations of fT_3, triglycerides, cholesterol, β-hydroxybutyrate, albumin and total protein. Taken together, the results obtained for short road transportation of dromedary camels showed a strong physiological response and provide some biomarkers for stress detection in this species. Further research to validate these potential biomarkers is necessary.

Keywords Dromedary camel · Transportation stress · Cortisol · Thyroid hormones · Serum biochemical parameters

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Introduction

Transportation is an inevitable husbandry practice which animals unexpectedly encounter in the livestock industry. It is often considered as one of the main causes of stress raising considerable interest, both in economic and animal welfare standpoints (Swanson and Morrow-Tesch 2001). Furthermore, the time between removing animals from farms to the slaughterhouse is found to be associated with different forms of injuries sustained by the animals. This often results into increased morbidity and mortality, poor meat and skin quantity, consequently a substantial economic loose (Minka and Ayo 2007).

During transport, animals are exposed to a variety of potential stressors such as motion of the vehicle, noise, vibrations, centrifugal forces, rapidly changing light conditions, heat, cold, poor air quality, deck height, mixing of unfamiliar groups, poor road conditions and the possible lack of water and feed (Hurtung 2003). Signs of transportation stress have been demonstrated in different animal species by, for example, increased adrenal cortical activity (Ruiz-de-la-Torre et al. 2001), decreased humoral immunity (Machenzie et al. 1997), increased morbidity and mortality due to infectious diseases in the few weeks following transportation (Hurtung 2003; Chirase et al. 2004). Several clinical, biochemical, hormonal, and immunological effects of transportation stress have been documented in farm animals. (Broom 2003; Fazio and Ferlazzo 2003; Fazio et al. 2008). Indeed it is well established that different animal species and even animals of the same species but different genetic backgrounds respond differently to the same stressor (Hall et al. 1998).

The dromedary camel is one of the most important domestic animals in the arid and semi arid regions as it is equipped to produce high quality food at comparatively low costs under extremely harsh environments. The camel has great tolerance to high temperatures, high solar radiation and water scarcity. It can survive well on sandy terrain with poor vegetation and may chiefly consume feeds unutilized by other domestic species (Kadim et al. 2009). Camel husbandry in Iran is almost localized in southern provinces, while its slaughter and meat consumption is done in most parts of this country. Sale and slaughter are the most usual reasons for transporting camels in Iran. Handling, transport, mixing and overcrowding are considered as predisposing factors to pneumonia in camels (Wernery and Kaaden 2002). While there is substantial study on the effects of handling and transportation of cattle, horse, pig, sheep, goat, alpaca and various wildlife species (Broom 2003; Fazio and Ferlazzo 2003; Fazio et al. 2008), no work has been carried out to assess the effects of stress in transported dromedary camels. Indeed it was thought of interest to assess if these desert animals would respond differently to the stressful stimulus of transportation. This study was carried out to investigate the impact of road transportation on cortisol, thyroid hormones and blood chemistry in Iranian dromedary camels. These physiological parameters have been proposed as sensitive indices of physiological stress response in animals that encountered welfare problems such as handling and transport (Broom and Johnson 1993).

Materials and methods

Animals and transportation

Ten clinically healthy Iranian dromedary camels (*Camelus dromedarius*), five males and five females, ranging in age from 3 to 4 years and weighing about 300 kg were selected for the study. The camels had been reared at the Camel Research Institute in Yazd province of Iran, which is supervised by an experienced veterinarian. Preliminary procedures (handling, physical restraint, loading, and unloading) were undertaken by the same staff and blood sampling was always carried out by the same operator. The study was conducted on three consecutive days in August 2008. The camels had similar feeding and watering conditions *ad libitum* except between 08:30 A.M. and 01:30 P.M., on day 1, and also during the journey, on day 2, that there was no feed or water available. Transportation of the camels was conducted at the second day between Camel Research Institute in Baphgh toYazd and back to Institute, which took 5 h (about 300 km). Animals were captured, loaded and placed in an open truck with stocking density about 1 m^2 per animal. Road trip was made at a speed of 55–65 km/h on an asphalt smooth road. Environ-

mental temperature and relative humidity during the journey was 32–36°C and 17–25%, respectively.

Blood sampling and processing

At first day, blood samples were collected at 08:30 A.M., 09:30 A.M. and 01:30 P.M. to determine any possible variation in individual measurements due to diurnal changes or as a result of food and water deprivation for 5 h. At second day, blood samples were collected at three different times: immediately before loading at 08:30 A.M. (T1), after 1 h transport, at 09:30 A.M. (T2), and on the end of transportation, immediately after unloading, at 01:30 P.M. (T3). Final blood sample was taken 24 h after arrival (T4).

Blood samples were collected from jugular vein under aseptic conditions directly into test tubes without any anticoagulant and were kept in ice until serum was separated within 2 h of collection by centrifugation at 4°C for 10 min at 3000 rpm. Any haemolysed samples were discarded. Serum samples were stored at — 20°C until analyzed.

Serum triiodothyronine (T_3), thyroxine (T_4), fT_3 (free triiodothyronine) and fT_4 (free thyroxine) were measured by radioimmunoassay (RIA) method (kits available from Immunotech Company, Immunotech-Radiova, Prague, Czech Republic). The areas of validation for T_3, T_4, fT_3 and fT_4 assays included limits of detection, and precision in the standard curve following sample dilution, inter- and intra- assay coefficients of variation results were considered. Intra- and inter- assays for T_4 and T_3 were found to be below 6.2%, 8.6%, 3.3% and 8.6% respectively. For fT_4 and fT_3 the values were found to be below 6.5%, 7.2%, 3.5% and 7.0%, respectively. Cortisol was assayed using RIA kits (Orion, Diagnostica, Finland). Serum total protein was performed by the Biuret method, albumin by the bromcresol green method, urea nitrogen by the diacetyl monoxime method. The serum was analysed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood 1994), for triglyceride by the enzymatic procedure of McGowan et al. (1983) and for glucose by using glucose oxidase method. The β-hydroxybutyrate and non-esterifed fatty acids (NEFA) were measured by kinetic enzymatic and colorimetric methods, respectively using β-hydroxybutyrate and NEFA kits (Randox Laboratories, Crumlin, Antrim, UK).

Statistical analysis

The data are presented as mean±standard error (SE) in SI units.The data from first day, which animals were not transported yet, were analysed separately. The data related to physiological measurements immediately before transportation (T1), 1 h after transport initiation (T2), on arrival in the Institute (T3) and 24 h after arrival were statistically analysed together. A two way (time, gender) repeated measures analysis of variance (RMANOVA) was applied for statistical analysis. The level of significance was set at $P<0.05$. Significances between means were assessed using the least-significant-difference procedure. All calculations were performed using SPSS/PC software.

Results

The blood concentrations of cortisol, thyroid hormones and metabolic factors related to first day measurements are shown in Table 1. The data related to measurements taken before, during and after transportation are shown in Table 2. No significant difference was observed in any parameter between sexes at all sampling times. Time x gender interaction was not significant for all parameters. The data related to day before transport had no significant differences between different times except for values obtained for cortisol that at 13:30 showed a significant decrease (26.36±2.51 nmol/l) in comparison with data at 08:30 (40.04±3.43 nmol/l) and 09:30 (36.07± 3.48 nmol/l). Mean circulating cortisol level was increased by 140% at T2 and by 173% at T3 in comparison with pre-transport basal values. T_4 and T_3 showed significant increase after road transportation and decreased to basal values after 24 h sampling. The increase in serum concentrations after transportation was significant for fT_4, but not for fT_3.

In the current study, transportation had effects on metabolism as demonstrated by increase in serum concentrations of glucose, NEFA, and urea nitrogen during and after the journey. Glucose concentrations were increased by approximately117% at post-transport (7.46±0.34 mmol/l at T3) compared to

Table 1 Mean (± SE) of hormone levels and serum biochemical factors in dromedary camels (n=10, five males and five females) in three time periods at day before transport

Parameter	08:30 a.m.	09:30 a.m.	01:30 p.m.
Cortisol (nmol/l)	40.04±3.43 [a]	36.07±3.48 [a]	26.36±2.51 [b]
T_4 (nmol/l)	152.40±7.85	164.50±9.16	161.20±11.90
T3 (nmol/l)	2.13±0.22	2.05±0.24	2.34±0.29
fT4 (pmol/l)	19.67±1.78	20.45±2.75	18.04±1.81
fT3 (pmol/l)	2.19±0.27	2.13±0.33	2.20±0.33
Glucose (mmol/l)	6.39±0.48	6.16±0.42	5.78±0.37
Triglycerides (mmol/l)	0.45±0.04	0.37±0.04	0.40±0.05
Cholesterol (mmol/l)	1.05±0.06	0.98±0.06	1.02±0.07
NEFA(mmol/l)	0.67±0.09	0.58±0.09	0.47±0.04
β-hydroxybutyrate (mmol/l)	0.11±0.01	0.11±0.02	0.09±0.01
Urea nitrogen (mmol/l)	12.12±0.95	10.76±0.96	12.40±1.34
Albumin (g/l)	37.40±1.90	38.50±2.00	37.70±2.00
Total protein (g/l)	64.50±4.20	56.90±2.90	62.10±3.70

[a,b] Mean (± SE) in each row with no common superscript differ significantly (P<0.05).

pre-transport values (6.36±0.35 mmol/l at T1). NEFA concentrations were increased by approximately 130% (0.88±0.11 mmol/l) after transportation in comparison with pre-transportation values (0.68± 0.10 mmol/l). Blood urea nitrogen was increased by about 121% at post-transport (16.89±1.29 mmol/l), compared to basal values (13.98±1.09 mmol/l). Serum concentrations of glucose, NEFA, and urea nitrogen returned to basal values in final bleeding at 24 h after transport termination. As shown in Table 2, in the current study transportation had no significant effects on serum concentrations of triglycerides, cholesterol, β-hydroxybutyrate, albumin and total protein.

Discussion

Transportation is thought to be very stressful because animals are exposed to unfavorable environments and this may induce intense physiological changes. These physiological changes may reduce an animal's productivity and the quality of its products. For this many researchers has attempted to quantify the severity of the stress imposed by the various stages involved in transport and to identify acceptable conditions and methods to minimize the adverse effects of transport. The complexity and suite of behavioral and physiological changes due to stress response can differ markedly from species to species,

Table 2 Mean (± SE) of hormone levels and serum chemistry values in dromedary camels (n=10, five males and five females) before transport (T1), 1 h after transport initiation (T2), on the end of transportation (T3) and 24 h after arrival (T4)

Parameter	T1	T2	T3	T4
Cortisol (nmol/l)	38.17±3.99[a]	53.61±4.65 [b]	66.24±6.03 [b]	32.17±3.54 [a]
T_4 (nmol/l)	154.70±9.18[a]	166.4±6.52 [b]	212.5±9.16 [c]	143.00±7.62 [a]
T_3 (nmol/l)	2.09±0.24 [a]	2.77±0.44 [a]	3.73±0.35 [b]	1.88±0.20 [a]
F T_4 (pmol/l)	18.37±1.65[a]	25.65±2.15 [b]	28.17±2.68 [b]	21.66±2.20 [a,b]
f T_3 (pmol/l)	2.11±0.34	2.32±0.54	3.11±0.56	2.69±0.30
Glucose (mmol/l)	6.36±0.35 [a]	7.71±0.41 [b]	7.46±0.34[b]	6.42±0.41[a]
Triglycerides (mmol/l)	0.42±0.03	0.41±0.09	0.37±0.04	0.49±0.04
Cholesterol (mmol/l)	1.04±0.05	1.02±0.08	1.07±0.06	0.99±0.07
NEFA (mmol/l)	0.68±0.10 [a]	0.93±0.08 [b]	0.88±0.11 [b]	0.67±0.10 [a]
B-hydroxybutyrate (mmol/l)	0.11±0.01	0.13±0.02	0.10±0.01	0.14±0.03
Urea nitrogen (mmol/l)	13.98±1.09[a]	17.20±1.51 [b]	16.89±1.29[b]	12.74±1.04[a]
Albumin (g/l)	38.30±2.10	39.29±1.91	39.00±1.10	36.60±1.70
Total protein (g/l)	61.20±4.30	56.86±4.25	58.50±3.80	55.40±5.20

[a,b,c] Mean (± SE) in each row with no common superscript differ significantly (P<0.05)

individual to individual and stressor to stressor and can vary according to prior experience and hormonal status (Cook et al. 2000). Different parameters have been used to assess animal welfare during transport, but this can only be properly evaluated if a number of measures are considered (Grandin 1997).

Camels are animals exposed to a wide array of physiological and pathological stressors in harsh grazing condition. However they acquire various biochemical adaptive patterns to withstand these conditions. In the current study circulating levels of cortisol, thyroid hormones and serum chemistry parameters were investigated in relation to transportation stress in dromedary camels.

Endocrine responses constitute an integral component of the stress response. Stress can affect hormonal control of metabolism, reproduction, growth, and immunity. Since hormone signaling plays a vital role in the maintenance of homeostasis, virtually every endocrine system responds in some fashion to specific stressors. The overall effect on the animal's adaptive response to stress is an integration of multiple, and often interactive, hormone response that directly affect physical health and well-being (Materri et al. 2000).

The glucocorticoid content in blood is a good index for the reaction of animals to any environmental challenge (Tarrant and Grandin 2000). Stressors such as weaning (Mohamed 2006), dehydration (Kataria et al. 2000) and drought (Kataria and Kataria 2004) have been shown to cause significant increase in cortisol level in dromedary camel. Increased cortisol level has been also reported after mating in pluriparous dromedary camels, so probably mating can be considered as a stress factor in dromedary camel (Elias and Weil 1989). Higher cortisol concentration is probably required to meet the energy crisis during physical stress to the animals (Kataria and Kataria 2004).

In the current study, the results concerning pattern of cortisol levels following transportation stress in dromedary camels agree with data previously obtained in many species, including cattle, horse, sheep, goat, swine and various wildlife species (Fazio et al. 2005; Mohammadi et al. 2007; Odore et al. 2004; Fazio et al. 2008) and show induction of the hypothalamus-pituitary-adrenal axis due to transportation stress in dromedaries. Based on the results of this study it is possible to conclude that serum cortisol concentration may be a useful indicator of transportation stress in dromedary camels. This study indicated no effect of sex on serum cortisol level in camels that is consistent with previously reported data in dromedary camels (Mohamed 2006; Kataria and Kataria 2004).

The mean serum basal concentrations of thyroxine (T_4) in Iranian dromedary camels were in agreement with the values reported earlier (Nazifi et al. 1999, 2009; Zia-ur-Rahman et al. 2007). The mean serum basal concentrations of triiodothyronine (T_3) in the serum of Iranian dromedary camels were in agreement with the values reported earlier (Nazifi et al. 1999, Nazifi et al. 2009), but higher than values reported by other workers (Bengoumi et al. 1999) and lower than values reported by Zia-ur-Rahman et al. (2007). Our results indicated no effect of sex on circulating iodothyronines levels in dromedary camels that is different from results of Bengoumi et al. (1999) who showed female dromedaries have lower T_3 and T_4 concentrations.

Thyroid hormones are known to be important modulators of developmental processes and general metabolism (Kaneko et al. 1997), and by their effect on energy, nitrogen, minerals and water metabolism are related to the adaptation of mammals to their environment (Greer and Saloman 1974). Blood levels of thyroid hormones have been used as a measurement of stress response (Siegel and Gross 2000). Consensual increases of total and free iodothyronine levels and their correlations after long distance road transport were obtained for Limousine calves (Fazio et al. 2001) and cattle (Fazio et al. 2005). A significant increase in T3 serum levels was recorded in stallions for distances between 60 and 120 km, of T4 levels for distances of 60–120 km and 240 and 300 km and of fT_4 levels for distances between 120 and 240 km, as compared to basal values, as well as a general increase of fT_3 levels. A positive and significant correlation between T_3 and fT_3 was found for distances between 240–300 km, and between T_4 and fT_4 for distances less than 60 km (Fazio and Ferlazzo 2003). In Omani goats the thyroid hormones did not change significantly due to transportation stress (Al-Kindi et al. 2005). Increased serum T_3 and T_4 concentrations due to thermal stress in dromedary camels have been recorded (Nazifi et al. 1999). Yagil et al. (1978) also reported that in one-humped camel, the thyroid was stimulated in summer when water was available, but was inhibited after dehydration. This

inhibition assists in the preservation of body water by decreasing pulmonary water loss and reducing basic metabolism.

Results of this study show the effect of transport stress on circulating iodothyronines levels in dromedary camels. These results suggest that transport stress induced an increase of activity of the hypothalamus-hypophysis-thyroid axis. In the current study transportation stress had no effect on hydration status of the animals because no significant changes observed in serum total protein and albumin at pre- and post-transport samples. Thus increase in thyroid hormones can be attributed to transport stress itself. This increase may reflect increased energy demands of animals due to stress situation. Thyroid hormones increase metabolism in almost all cells of the body. If carbohydrates and fats are insufficient for energy, thyroid hormones cause a rapid degradation of proteins for energy. If, however, adequate substrates for energy are available, thyroid hormones can enhance the rate of protein synthesis (Hyyppa 2005).

The impact of stress on metabolism can be characterized as a gradient response with some positive correlation between the magnitude of the stress challenge(s) and the change in metabolism (Elasser et al. 2000). Changes in various blood metabolites have been studied in relation to transportation stress in farm animals (Kannan et al. 2007). Increase in plasma levels of glucose and NEFA have been reported in goat due to transportation stress (Kannan et al. 2007). Wensvoort et al. (2004) showed that 5 days fasting cause significant increase in NEFA concentration in dromedary camel. Based on the results of the current study, glucose and NEFA concentrations increased significantly following transportation stress. Fluctuations in plasma concentrations of glucose and NEFA in stressful conditions is closely related to adrenal function. Glucocorticoids secreted by this gland play an important role in gluconeogenesis by stimulating the liver to convert fat and protein to intermediate metabolites that are ultimately converted to glucose for energy. Glucocorticoids also support this response by potentiation of the synthesis and action of epinephrine, a catecholamine released by the adrenal medulla during the stress response (Materri et al. 2000). Epinephrine induces excessive glycogenolysis in liver and muscle owing to stimulation of a phosphorylase. In muscle, as a result of absence of glucose-6-phosphatase, glycogenolysis ensues with the formation of lactate; wherase in liver glucose is the main product, leading to an increase in blood glucose (Murray et al. 1996). Lipolytic response to adrenaline in stressful conditions leading to mobilization of lipid stores and increased NEFA concentration that is a good indicator of body fat utilization. NEFA concentration in the present study at basal pretransport state was greater than values obtained by Wensvoort et al. (2004) in dromedary camel. Mean β-hydroxybutyrate concentration obtained in the present study at basal state was lower than values reported by Wensvoort et al. (2004) and greater than values reported by Chandrasena et al. (1979). However according to recorded results of Chandrasena et al. (1979) and our results, β-hydroxybutyrate concentration in camel is lower than reference ranges recorded for other ruminants, for instance 0.41±.03 in cow and 0.55±.04 in sheep (Kaneko et al. 1997), because in dromedary camel the activity of the enzyme β-hydroxybutyrate dehydrogenase in both the rumen epithelium and the liver is low, and the rumen epithelium is devoid of papillae which would greatly reduce the surface area available for metabolic functions. In addition, the camel lacks the omasum which is reported to produce some keton bodies (Chandrasena et al.1979).

According to the results of this study, β-hydroxybutyrate, did not change significantly due to 5 h transportation stress in dromedary camels. Wensvoort et al. (2004) showed that 5 days fasting in dromedary camel did not cause any significant change in β-hydroxybutyrate concentration and concluded that camelids have a unique ability to prevent or postpone the pathological state of ketosis.

Triglycerides and cholesterol did not change significantly due to transportation stress. Rajion et al. (2001) also reported no effects of transportation stress on cholesterol levels in goats.

Increased urea nitrogen following transport in this study is in line with previous reports in other transport stressed species (Hurtung 2003). Any process which increases protein catabolism will tend to increase the levels of blood urea, and these also tend to increase when the levels of cortisol increase, and also with food deprivation (Finco 1997). Increased urea nitrogen following fasting had been reported in dromedary camel (Wensvoort et al. 2004), but drought stress had no significant effect on urea level (Kataria and Kataria 2004).

Transport stress has been reported to cause dehydration and may manifest itself as a hyperproteinemia (Schaefer et al. 1992). In the current study albumin and total protein did not change significantly due to stress, suggesting no dehydration due to 5 h transportation stress in dromedary camel. Drought stress also had no significant effect on total protein concentration in dromedary camel (Kataria and Kataria 2004). No occurrence of dehydration signs during transportation of dromedary camel in relatively high temperatures might reflect camel's ability to utilize internal water due to the physiological behavior of its internal systems (Mehrotra and Gupta 1989).

The levels of glucose, NEFA and urea nitrogen had returned to pretransport levels after 24 hours, indicating that general metabolism of animals had largely returned to normal by this time. Disagreements between literature in mean basal concentrations of different serum parameters mentioned above in dromedary camels may be due to differences in time of sampling, season, ambient temperature and water balance, quality of nutrition, average age of the groups sampled and measurement techniques used.

The data related to cortisol levels on day before transport show that circadian variations might affect cortisol status in dromedary camel. There is some controversy about the occurrence of circadian variation in cortisol concentrations. In the horse, bull, sheep as well as pig the occurrence of circadian rhytms is generally acknowledged (Rijnberk and Mol 1997). We could not obtain data related to cortisol diurnal rhytms in dromedary camel. However it is clear that stress, independently of circadian variations, may activate the hypothalamus-pituitary-adrenal axis (Rijnberk and Mol 1997). The data related to other factors on day before transport was not changed significantly between different times. This might show that 5 h deprivation of food and water in summer conditions had no effect on mentioned parameters in dromedary camels and observed changes in the following day might be related to transportation stress itself.

In conclusion, we have determined that transportation stress in dromedary camels alter concentrations of cortisol, thyroid hormones and physiological variables of metabolism in the circulation that, taken together, may be effective biomarkers of transportation stress in this species. Alleviating stress factors in transported animals should therefore be a prime concern for camel welfare and health. Transport is inevitably associated with a stress response but this can be avoided by the adequate handling and management. Therefore, the use of stress indices merits consideration for identifying methods to minimize the adverse effects of transport.

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