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Daily rhythms of physiological parameters in the dromedary camel under natural and laboratory conditions

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Abstract Camels are well adapted to hot arid environments and can contribute significantly to the economy of developing countries in arid regions of the world. Full understanding of the physiology of camels requires understanding of the internal temporal order of the body, as reflected in daily or circadian rhythms. In the current study, we investigated the daily rhythmicity of 20 physiological variables in camels exposed to natural oscillations of ambient temperature in a desert environment and compared the daily temporal courses of the variables. We also studied the rhythm of core body temperature under experimental conditions with constant ambient temperature in the presence and absence of a light-dark cycle. The obtained results indicated that different physiological variables exhibit different degrees of daily rhythmicity and reach their daily peaks at different times of the day, starting with plasma cholesterol, which peaks 24 minutes after midnight, and ending with plasma calcium, which peaks 3 hours before midnight. Furthermore, the rhythm of core body temperature persisted in the absence of environmental rhythmicity, thus confirming its endogenous nature. The observed delay in the acrophase of core body temperature rhythm under constant conditions suggests that the circadian period is longer than 24 hours. Further studies with more refined experimental manipulation of different variables are needed to fully elucidate the causal network of circadian rhythms in dromedary camels.

Keywords: Core body temperature; Circadian rhythm; *Camelus dromedarius*; Heat exposure; Nycthemeral rhythm; Rhythm robustness

1. Introduction

The dromedary camel (*Camelus dromedarius*) is an important livestock species uniquely adapted to hot arid environments by its ability to reduce water loss through feces, urine, and evaporation (Bekele et al., 2013; Ben Goumi et al., 1993; Robertshaw and Zine-Filali, 1995; Schmidt-Nielsen et al., 1956). With increasing human population and inadequate food production in Africa and parts of Asia, it is important to develop semi-arid and arid rangelands through appropriate livestock production systems, and the camel is a natural choice (Schwartz and Dioli, 1992). In addition to being well-adapted to hot arid environments, the camel can serve the food supply chain with milk and meat, can serve the textile industry with wool and hair, can serve the transportation industry by providing transport of humans and goods, and can serve the agricultural industry as a traction animal. Increased utilization of camels has been suggested in many countries with arid regions such as Ethiopia (Mehari et al., 2007), Kenya (Guliye et al., 2007), India (Mehta et al., 2009), and Pakistan (Ahmad et al., 2010).

Daily oscillation in the levels of physiological variables in animals has been described for a multitude of variables, including locomotor activity, body temperature, heart rate, blood pressure, hormonal secretion, and urinary excretion (Dunlap et al., 2004; Refinetti, 2016). Rhythmic parameters of individual variables have been studied in great detail, but very few studies have been conducted on the temporal relationships between the rhythms of different variables. In the camel, in particular, only a few studies have examined daily rhythmicity, and they focused on individual variables such as body temperature (Bligh and Harthoorn, 1965; Bouâouda et al., 2014; El-Allali et al., 2013), sweat rate (Abdoun et al., 2012), plasma aldosterone (Khaldoun et al., 2002), plasma melatonin (El-Allali et al., 2005), and biomarkers of bone formation (Al-Sobayil, 2010).

Although the detailed study of rhythmic properties of individual variables can provide significant advances in the understanding of individual functions, the simultaneous study of many variables is a necessary step in the path to the understanding of the multiple temporal relationships of physiological processes. Thus, in the present study, we monitored simultaneously 20 different rhythms in camels exposed to natural hot weather in Saudi Arabia. To evaluate the endogenous nature of circadian rhythmicity, we also studied the body temperature rhythm of camels maintained indoors with constant illumination and constant room temperature.

2. Materials and methods

2.1. Animals

Nine male dromedary camels (*Camelus dromedarius*) with mean body weight of 450 ± 20 kg and two years of age were used in the study. Animals were housed individually in shaded pens, fed twice a day at 0700 and 1600 hours, and had free access to clean tap water. All camels were tied up during sampling and measurement collection. Protocols of animal husbandry and experimentation followed applicable regulations in Saudi Arabia.

2.2. Experimental design and data collection

In the first experiment, four camels were housed under natural late-summer conditions (natural light in LD 12:12 and ambient temperature oscillating daily from a low of 25 °C to a high of 46 °C) and were fed twice a day at 0700 and 1600 hours with free access to drinking water. Measurements of 20 physiological variables were obtained at 3-hour intervals for 24 consecutive hours: rectal temperature, skin temperature, sweat rate, heart rate, respiratory rate, and various plasma constituents (total protein, albumin, globulin, urea, cholesterol, creatinine,

sodium, potassium, chloride, calcium, phosphorus, aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], and alkaline phosphatase [ALP]).

Rectal temperature (10 cm deep) was measured using a calibrated digital thermometer that measures temperature to the nearest 0.1°C, whereas skin temperature (on the shaved flank) was measured using an infrared thermometer (Traceable Mini IR™ Thermometer, Friendswood, Texas, USA) with resolution of 0.1°C. Measurements of rectal temperature were taken always after fecal evacuation. As a precaution, the sensor of the thermometer was placed facing the mucosal surface in order to accurately measure rectal temperature. Because camels are large and docile, the minor disturbance caused by temperature measurements does not have unintended effects on their body temperature.

Sweat rate was measured by the method based on water absorption by paper discs placed on shaved skin (Pereira et al., 2010). Heart rate and respiratory rate were measured by auscultation. Potential measurement errors caused by emotional disturbance were excluded or kept minimal by habituation of the camels to the auscultation procedures before the commencement of the experiment.

Blood samples were collected through jugular intravenous catheters (FEP G20 1 x 32 mm) into plain tubes. Sera were separated by centrifugation at 1500 g for 30 min, and then stored at -20°C until analysis. Colorimetric assays were used to spectrophotometrically quantify serum total protein (g/L), albumin (g/L), urea (mmol/L), creatinine (µmol/L), sodium (mmol/L) potassium (mmol/L), chloride (mmol/L), calcium (mmol/L), phosphorus (mmol/L), AST (U/L), ALT (U/L), LDH (U/L), and ALP (U/L) using commercial kits (Randox Laboratories Ltd., Crumlin, UK). Globulin levels (g/L) were calculated as the difference between measured total protein and albumin concentrations. Meanwhile, an enzymatic colorimetric assay was conducted

for serum cholesterol (mmol/L) using a commercial kit (Randox Laboratories Ltd., Crumlin, UK).

In the second experiment, five camels were housed indoors. Core body temperature was measured every 20 minutes with iButton data loggers (Maxim Integrated Products, San Jose, CA, USA) surgically implanted into the peritoneal cavity. During this experiment, the animals had free access to food and water and were subjected to three environmental conditions, each for 2 days: natural late-winter condition (with both a light-dark cycle [LD 12:12, 5000:1 lux] and an ambient temperature cycle [15 to 30 °C daily]), controlled temperature (with a light-dark cycle [LD 12:12, 400:1 lux] but a constant temperature of 22 °C), and constant environment (with constant light at 400 lux and constant temperature at 22 °C).

In both experiments, ambient temperature and relative humidity were recorded continuously at 10 min intervals using two HOBO data loggers (Onset Computer Corp., Wareham, MA, USA) placed inside the pens.

2.3. Data analysis

For each of the 20 variables in the first experiment, the measurements from different animals were averaged to produce a single times series with 8 equidistant time points. Each time series was analyzed by cosinor rhythmometry (Nelson et al., 1979; Refinetti et al., 2007) to identify four rhythmic parameters: mesor (mean level), amplitude (half the range of oscillation), acrophase (time of peak), and robustness (strength of rhythmicity, herewith denoted by the lower-case Greek latter ρ). The cosinor procedure assigns 100% robustness only to time series that are perfectly sinusoidal; however, natural biological noise always reduces the robustness of circadian rhythms, keeping it below 100% (Refinetti, 2004), and the strength of rhythmicity can

be estimated by the cosinor procedure even when the wave form of the rhythm is not sinusoidal (Refinetti et al., 2007).

In the second experiment, the first 5 hours of body temperature data were discarded to allow for accommodation to changing environmental conditions, and the next 40 hours were analyzed by cosinor rhythmometry. The analytical procedure includes a component of inferential statistics to calculate the probability of events as extreme as those obtained under the assumption of the null hypothesis (Nelson et al., 1979; Refinetti et al., 2007). Only events with $p < 0.05$ were considered to be statistically significant.

3. Results

The results for the five whole-organism variables in the first experiment are shown in Fig. 1. The duration of the light and dark phases of the light-dark cycle are shown at the very top, followed by the oscillation in ambient temperature. Ambient temperature started to rise from a low of 25 °C at sunrise, reached a high of 46 °C at noon, and fell slowly through the afternoon and evening. Rectal temperature of the four camels also exhibited daily rhythmicity, lagging behind ambient temperature by a few hours and rising from a low of 37.6 °C to a high of 38.4 °C. The oscillatory pattern of rectal temperature was almost as strong ($\rho = 81\%$) as that of ambient temperature ($\rho = 85\%$). Skin temperature ($\rho = 32\%$), sweat rate ($\rho = 60\%$), heart rate ($\rho = 71\%$), and respiratory rate ($\rho = 74\%$) also exhibited daily rhythmicity, even if not as robustly as rectal temperature did.

The acrophases (peak times) of all 20 physiological variables are shown in Fig. 2. Rhythms peaking earlier in the day appear at the top, whereas rhythms peaking later in the day appear at the bottom. Clearly, different rhythms peak at different times of the day and night, and the dispersion of acrophases spans 24 hours, starting with plasma cholesterol, which peaks 24

minutes after midnight, and ending with plasma calcium, which peaks 3 hours before midnight. The mesor, amplitude, and robustness of each rhythm are shown in Table 1. The weakest rhythm was that of plasma chloride ($\rho = 15\%$), the strongest rhythm was that of rectal temperature ($\rho = 81\%$), and the other 18 rhythms lay in between these two extremes of rhythm robustness.

Records of a representative camel in the second experiment are shown in Fig. 3. As was the case in the first experiment, core body temperature oscillated with a temporal pattern similar to that of ambient temperature (Fig. 3 A). Because core body temperature rose and fell in synchrony with ambient temperature, an influence of ambient temperature on core body temperature cannot be excluded based on these data alone. However, when ambient temperature was kept constant (Fig. 3 B), core body temperature continued to exhibit daily oscillation, even if with a slightly different wave form. Furthermore, core body temperature continued to oscillate even when both illumination and ambient temperature were kept constant (Fig. 3 C). Similar results were obtained with the other four camels, except that the delay in acrophase under constant light and constant temperature was not as extreme as in this animal. The average delay in acrophase for the five camels (± SEM) was 2.3 ± 0.9 hours. The animal whose data are shown in Fig. 3 was chosen because the two main parameters of its core body temperature rhythm (i.e., mean level and amplitude) represented well the parameters of the rhythms of the other animals. The differences in acrophase are expected to reflect differences in free-running period. Because the animals were studied under constant conditions for only 2 days, reliable computations of free-running circadian period cannot be made, but the consistent delay in acrophase suggests that the circadian period is on average longer than 24 hours, possibly as long as 26 hours.

The average mesor and average amplitude of the core body temperature rhythm for the five camels across the three conditions (± SEM) in the second experiment were 37.9 ± 0.12 °C and 0.3 ± 0.03 °C, which are very similar to the values of rectal temperature obtained in the first

experiment. The amplitude of the core body temperature rhythm was larger in the condition with light-dark cycle plus ambient temperature cycle (0.3 ± 0.04 °C) than in the condition with light-dark cycle without an ambient temperature cycle (0.2 ± 0.03 °C), but the difference was not statistically significant ($t(4) = 1.687$, $p > 0.10$). Robustness of the rhythm was lower when calculated for each camel individually (mean $\rho = 40\%$) than when calculated for the group as a whole ($\rho = 78\%$), but the latter value was comparable to that found in the first experiment ($\rho = 81\%$).

4. Discussion

Our results confirm previous demonstrations of robust daily rhythmicity of core body temperature in camels (Bligh and Harthoorn, 1965; Bouâouda et al., 2014; El-Allali et al., 2013). We found the core body temperature rhythm to be robust not only under a natural, large daily oscillation of ambient temperature, but also under constant ambient temperature. Under a light-dark cycle with 12 hours of light and 12 hours of darkness per day, with oscillating or constant ambient temperature, we found the core body temperature rhythm to have a mean of approximately 38.0 °C, an amplitude of approximately 0.3 °C (range of oscillation from 37.6 to 38.4 °C), and acrophase at 2 hours before lights-off. If provided with free access to drinking water, the camel is clearly a superb homeotherm, being able to maintain the same range of core body temperature under a daily variation in ambient temperature from 25 °C to 46 °C (or 15 °C to 30 °C) as under a constant mild ambient temperature of 22 °C.

Although Bligh and Harthoorn (1965) and Bouâouda et al. (2014) found the mean core body temperature of the camel to be considerably lower (around 37.0 °C) than we did (38.0 °C), El-Allali et al. (2013) obtained a mean very similar to the one we obtained (37.9 °C). The lower temperature in the study by Bligh and Harthoorn (1965) can be explained by the fact that they

measured body temperature in the camel's hump, whereas Bouâouda et al. (2014) measured temperature in muscle tissue. El-Allali et al. (2013) measured temperature in the rectum, as we did in our first experiment. The daily range of oscillation was a little narrower in our study (0.8 °C) than in the three previous studies (2.0 °C), but all four studies agreed about the occurrence of the acrophase shortly before sunset.

The robustness of the core body temperature rhythm was between 40 and 80%, depending on whether it was calculated for individual animals or for the group as a whole, which is consistent with the findings in sheep, goats, horses, and cattle (Piccione et al., 2003). When the animals were maintained in a stable environment (without a light-dark cycle or a cycle of ambient temperature), core body temperature still exhibited near-24-hour rhythmicity, thus confirming the endogenous nature of the rhythm previously documented in a large number of species (Refinetti, 2010), including the camel (El-Allali et al., 2013). Records longer than the 2 days evaluated in this study would be needed to document the existence of a self-sustaining biological clock responsible for the generation of endogenous rhythmicity. Technically, our observations serve to document an hourglass mechanism but not necessarily a pacemaker.

Because the recording under constant environmental conditions in our study lasted only 2 days, accurate computations of free-running circadian period could not be made. However, the consistent delay in acrophase suggests that the circadian period is longer than 24 hours, which is consistent with the results obtained by El-Allali et al. (2013).

A major component of our study was the simultaneous measurement of 20 variables in camels maintained under natural desert conditions. By measuring 20 variables simultaneously, we were able to compare the temporal properties of different variables. The results indicate that different physiological variables exhibit different degrees of daily rhythmicity and reach their daily peaks at different times of the day. Half the number of variables peaked during the light

phase of the light-dark cycle, whereas the other half peaked during the dark phase. This proportion is similar to the proportions previously found in sheep and horses (Piccione et al., 2005). The mean level of the measured variables was generally within the range of values reported in previous studies in camels (Aichouni et al., 2010; Ayoub and Saleh, 1998; Bekele et a., 2013; Eltahir et al., 2010; Hussein et al., 1992) with, notably, lower plasma concentrations of sodium, creatinine, ALP, ALT, AST, and LDH but higher concentrations of cholesterol, globulin, and total protein in our study.

The finding that different variables exhibit different degrees of rhythmicity is not surprising and has been previously described in laboratory rodents (Refinetti, 1999) and farm animals (Piccione et al., 2005). The finding is important, however, because of its implications concerning the issue of causality of daily rhythmicity (i.e., internal temporal order). The question raised by the findings is: If one rhythm lags behind another, is it because it is caused by the earlier rhythm? Does the circadian pacemaker generate each and every rhythm individually, or are most rhythms simply derived from a few clock-controlled rhythms? Conceptually, a rhythm with low robustness cannot be the cause of a rhythm with high robustness. Thus, in the present study, the rhythms of rectal temperature, creatinine concentration, respiratory rate, and heart rate must not be caused by any of the other 16 rhythms that we investigated (Table 1). Whether any of these four rhythms is the cause of the other three rhythms (or of the remaining 16 rhythms) cannot be determined from the data on rhythm robustness alone. Importantly, the strength of rhythmicity of a variable need not correlate with this variable's importance for the overall health of the organism. Although there is strong evidence that loss of daily rhythmicity is associated with diseased states (Goldberger et al., 1990; Keith et al., 2001; Zuurbier et al., 2015), very little is currently known about how important the rhythmicity of each individual variable is for the functioning of the organism as a whole.

The data on the distribution of acrophases also have implications for the issue of causation. Conceptually, a rhythm that phase leads another rhythm cannot be caused by it, unless the phase lead is so great that it actually constitutes a phase lag in the following cycle. Thus, in camels, the rhythm of rectal temperature cannot be the cause of the rhythm of sweat rate, skin temperature, or respiratory rate, and the rhythms of albumin or potassium concentration cannot be the cause of the rhythm of rectal temperature (Fig. 2). Further studies with experimental manipulation of different variables, however, are needed to fully elucidate the causal network of circadian rhythms.

The current findings collectively offer an insight on the endogenous nature of the core body temperature rhythm in dromedary camels. Additionally, the findings shed light, for the first time, on the circadian rhythmicity of several physiological processes under natural and experimental conditions in a camelidae species. Our findings have strengthened the knowledge of how and why these animals respond as they do under different environmental conditions. The knowledge gained from this study enhances our understanding of the circadian system of dromedary camels under different environmental conditions and may help organize previous observations about their thermophysiology, production, and husbandry, thus providing the starting point for a long-term research program that helps the development of optimal and practical management procedures with higher probability of profitable economic productivity of dromedary camels.

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Table 1. Mesor, amplitude, and robustness of the 20 physiological variables measured in dromedary camels exposed to natural environmental conditions

Parameter[1]	**Mesor**	**Amplitude**	**Robustness**
Chloride (mmol/L)	126.23	4.20	15%
ALT (U/L)	3.93	0.27	17%
Albumin (g/L)	36.45	2.19	30%
Skin temperature (°C)	35.9	0.4	32%
Potassium (mmol/L)	6.18	0.57	36%
Phosphorus (mmol/L)	1.61	0.17	39%
Cholesterol (mmol/L)	1.40	0.20	42%
Urea (mmol/L)	14.93	3.79	49%
AST (U/L)	52.71	5.49	49%
ALP (U/L)	28.52	2.55	50%
LDH (U/L)	115.19	11.87	56%
Sweat rate ($g/m^2/h$)	91.32	42.20	60%
Globulin (g/L)	60.90	3.40	64%
Sodium (mmol/L)	118.57	19.09	67%
Calcium (mmol/L)	2.60	0.28	68%
Total protein (g/L)	97.80	4.50	68%
Heart rate (bpm)	36.04	3.34	71%
Respiratory rate (bpm)	15.73	2.90	74%
Creatinine (µmol/L)	24.75	3.54	76%
Rectal temperature (°C)	38.0	0.2	81%

[1] The parameters are listed in ascending order of rhythm robustness.

Fig. 1. Ambient temperature and mean values of rectal temperature, skin temperature, sweat rate, heart rate, and respiratory rate of four male camels during 24 hours under natural late-summer environmental conditions. Error bars denote the standard error of the means. The horizontal rectangles at the top denote the duration of the natural light-dark cycle.

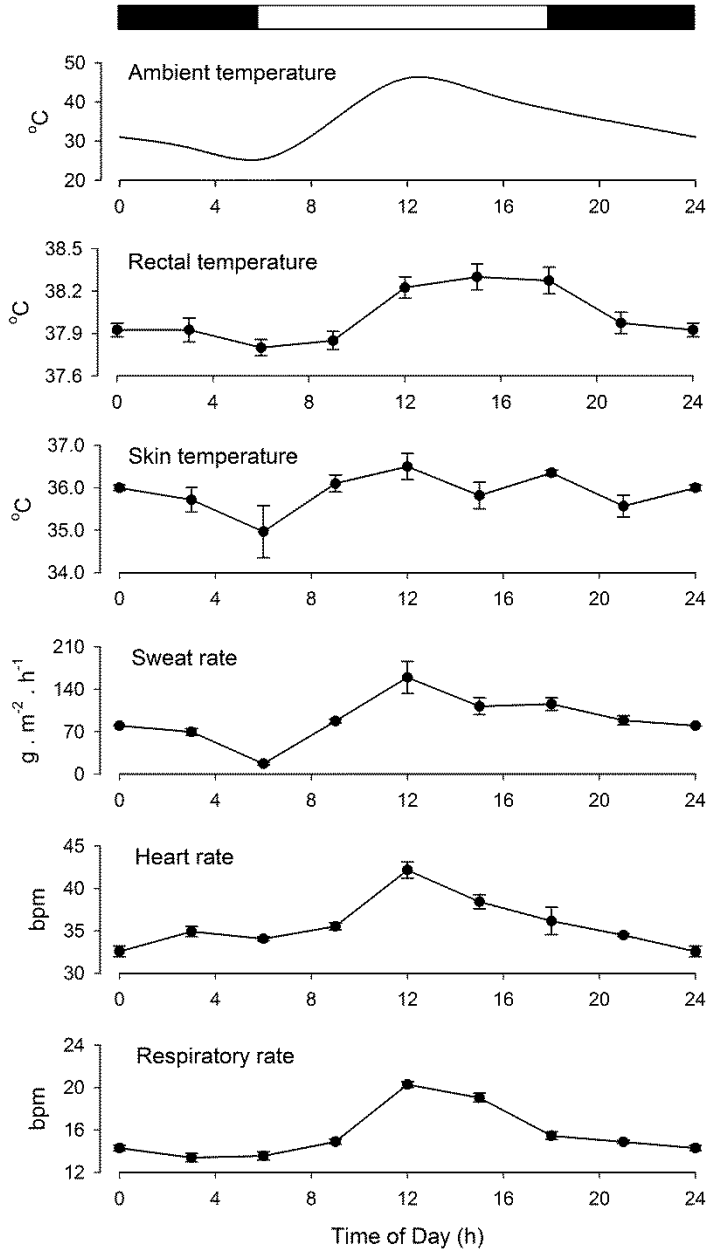

Fig. 2. Acrophases (peak times) of the 20 variables measured in the first experiment. Mean acrophases are denoted by closed circles. Error bars denote the standard errors of the means. Shaded areas indicate the dark phase of the light-dark cycle.

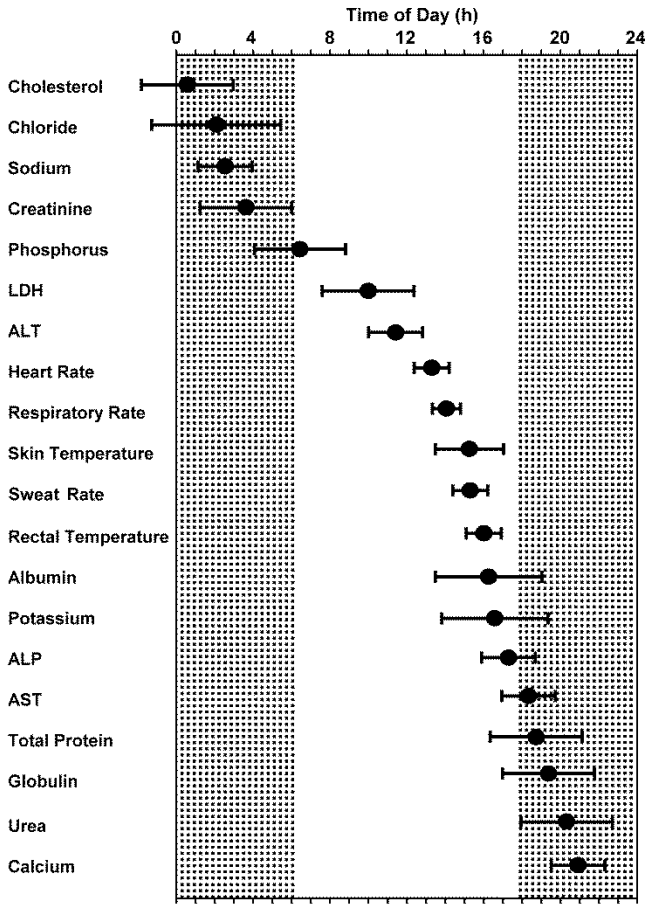

Fig. 3. Records of core body temperature of a representative camel maintained under three environmental conditions: light-dark cycle and daily oscillation in ambient temperature (A), light-dark cycle but constant temperature (B), and constant light and constant temperature (C). In each panel, T_a denotes ambient temperature and LD denotes the light-dark cycle.