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DAILY RHYTHMS OF THE BODY TEMPERATURE AND SOME HAEMATOCHEMICAL PARAMETERS IN DONKEY

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Key words: chronophysiology; circadian rhythm; body temperature; haematochemical parameters; *Equus asinus*

The presence of functional rhythmic variations is a well-demonstrated phenomenon at all levels of a physiological organization and especially in the functioning of cell components in tissues and organic systems. The circadian rhythmicity of livestock has received very little attention in chronobiological literature. Taking this little knowledge as a starting-point, the authors studied seven Ragusana breed donkeys in order to observe the circadian periodicity of their body temperature and some of their haematochemical parameters. A periodic statistical model was applied to the average values that were obtained. These results showed circadian rhythms of body temperature, glucose, triglycerides, phospholipids, total lipids, NEFA and total cholesterol.

DNEVNI RITMI TELESNE TEMPERATURE IN NEKATERIH HEMATOLOŠKIH PARAMETROV PRI OSLIH

Ključne besede: kronofiziologija; cirkadiarni ritem; telesna temperatura; hematološki parametri; *Equus asinus*

Variacije funkcionalnega ritma so dobro pojasnjen pojav na vseh stopnjah fiziološkega organiziranja in še posebej pri delovanju celičnih komponent v tkivih in organskih sistemih. Ontogeneza cirkadiane ritmičnosti pri živini je v kronobiološki literaturi deležna zelo majhne pozornosti. Upoštevaje to dejstvo kot izhodišče, so avtorji proučili sedem oslov pasme ragusana ter opazovali cirkadiano periodičnost telesne temperature in nekaterih hematokemičnih parametrov. Dobljene povprečne vrednosti so obdelali s periodičnim statističnim modelom. Dobljeni rezultati so

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pokazali cirkadiani ritem telesne temperature, glukoze, trigliceridov, fosfolipidov, celotnih lipidov, NEFA (neestrificiranih maščobnih kislin) in celotnega holesterola.

Introduction

Living matter and evolving organisms are exposed to the earth's revolution around the sun and its subsequent periods of day and night, of light and darkn, and the periodic changes in the length of the day and dark span the climatic changes of season (12). Various of biological variables oscillates in organisms, including behaviour, physiological functions and biochemical factors. If any event within a biological system recurs at approximately regular intervals, we can assume that this is a biological rhythm. The predominant rhythms in nature are daily, e.-g. rest and activity, body temperature and the concentrations of both hormones in blood and ions in urine etc. The rhythms are not merely passive responses to the daily alternation betweenf light and darkness as they also persist in non-periodic environments. An especially important topic in physiological research is the study of the biological rhythms of body temperature because it involves the integrated effort of two large groups of researchers: those interested in the regulation of body temperature and those interested in the mechanisms of biological timing. The multifrequency animal's time structure represents a challenge for sampling and interpreting laboratory measurements. The daily variations of the body temperatures of endothermic animals are not only influenced by changes in their physical activity and metabolic levels (17), but are also synchronized to the daily changes in light intensity, temperature, and other environmental factors (5). Rhythmic events in the frequency range of ultradian, circadian and circannual rhythm in the horse (6, 11), cow (8), sheep and goats (9, 10) and rabbit (15) have been described. In the study of haematochemical parameters. Despite the relative abundance of reviews on the biological rhythms of domestic animals' body temperatures (1, 12, 13, 14, 16), little information is available about this daily activity in donkey (4). Additionally, there is no literature available describing the circadian rhythms of the body temperature or the haematochemical parameters in this species. On the basis of this knowledge, and as a continuation of our research, the body temperature measurements and blood concentration levels of some haematochemical metabolites were determined under normal schedules conditions in an attempt to gain an insight into the mechanisms of the circadian rhythms of body temperature and energetic metabolisms.

Material and methods

Seven Ragusana breed donkeys, being on average 10 years of age, were used in our study.The animals were all clinically healthy and in individually housed in stalls with a natural photoperiod and environmental temperature. For 30 days before the study, the animals followed the same daily pattern of activity. The study was carried out in spring in Sicily (Italy) and was held in the spring, which had a natural photoperiod consisting of approximately 12 hours of sunlight and 12 hours of darkness per day. In line with standard farming practices, the animals had free access to water and were fed hay and oats three times per day (at 07.00, 12.00 and 17.00). The body-temperature measurements were taken every four hours with a digital thermometer whose probe was inserted 15 cm into the rectum. The digital thermometer, which had a recording time of 15 seconds, was equipped with a HI 92704 (Hanna Instruments) printer and dad a four-channel scale ranging from -50 °C to 150 °C, with a ± 0.2 °C degree of precision. Blood samples were collected at the same time as the body-temperature measurements through jugular intravenous catheters. Immediately after sampling, the glucose, triglycerides, phospholipids, total lipids, NEFA and total cholesterol concentrations were determined by means of both a standard kit (SEAC, Florence, Italy) and a spectrophotometer (model DU-40, Beckman Instruments, Fullerton, CA). The environmental temperature and humidity inside the stalls were measured using a Hygrothermograph ST-50 (Sekonic Corporation), which had both a semiconductor sensor and a high molecular electro-static capacity sensor. It could measure temperature ranging from -20 °C ~ 50 °C with a ± 1.0 °C accuracy and humidity levels ranging from 20 ~ 90 Rh % with ± 3 Rh % accuracy.

Statistical analysis

As the intragroup variance was not significant, the statistical analysis was carried out on the average values of the data obtained at six different points in time apart. We applied a trigonometrical statistical model to the average values of each time-series measurement so as to describe the periodic phenomenon analytically. The procedure used the cosine function: $f(t) = M + A \cos(\omega t + \phi)$ as a model for biological rhythms, where f (t) was the value at time t of the function defined by parameters M (mesor = value about which oscillation occured), A (amplitude = half the difference between the highest and lowest values), ω (angular frequency = degrees/unit time, with 360° representing a complete cycle) and ϕ (acrophase = timing of higher point, in degrees). The cycle duration (and hence ω) was given on the basis of either prior knowledge or a reasonable assumption.

In a time-series of data to be analysed, consisting of j = 1, 2,…, n and the measurements $y_1, y_2, \ldots, y_n$ taken at times $t_1, t_2, \ldots, t_n$, as long as there are at least 4 different time points, the t_j in a given series need not be all different, thus allowing for the replication of measurements at a given time point. The interval between the time points does not need to be fixed, but should be reasonably uniform throughout the cycle (7).

The single Cosinor procedure, as described by Nelson, was applied to the results obtained (7). This method is used for data that can be described by the fitting of a mathematical model. It represents the crest time of the cosine curve best fitting to the data and it may be expressed in (negative) degrees as the lag from the acrophase reference (360° = 1 period) or in calendar time units (hours for the circadian rhythm).

Results

Table 1: Average values (± SD) of the body temperature and of the haematochemical parameters, sampled at different time points (4 hours apart) in 7 donkeys

Parameters (mmol/l)	TIME					
	01.00	05.00	09.00	13.00	17.00	21.00
Body temperature (°C)	38.8±0.2	38.6±0.1	38.5±0.2	38.7±0.2	38.8±0.1	38.9±0.1
Glucose	3.60±0.46	3.52±0.22	3.41±0.22	3.59±0.35	3.74±0.19	3.88±0.26
Triglycerides	0.39±0.08	0.37±0.09	0.34±0.06	0.38±0.06	0.40±0.07	0.42±0.08
Phospholipids	1.77±0.22	1.69±0.18	1.90±0.26	1.79±0.22	2.25±0.94	1.76±0.14
Total lipids (g % ml)	3.29±0.76	3.27±0.60	3.18±0.15	3.32±0.43	3.46±0.40	3.52±0.35
NEFA (µEq/l)	140.6±30.0	138.7±26.2	143.7±32.5	141.5±29.7	145.6±43.6	141.2±32.1
Total cholesterol	2.63±0.14	2.63±0.16	2.62±0.20	2.55±0.31	2.77±0.23	2.42±0.26

Table 1 shows mean values and standard deviation in the donkeys' body-temperature and haematochemical parameters (glucose, triglycerides, phospholipids, total lipids, NEFA and total cholesterol) at the different points in time. The application of the periodic model and the statistical analysis of the "Cosinor" enabled us to define the periodic parameters and their acrophases (expressed in hours). The body temperature shows a circadian rhythm with an adapting index (A.I.) = 0.96, a statistical significance (F) = 1 % and an acrophase at 20.24; glucose shows a circadian rhythm with an adapting index (A.I.) = 0.94, with a statistical significance (F) = 1 % and an acrophase at 19.52: The triglycerides show a circadian rhythm with an adapting index (A.I.) = 0.94, with a statistical significance (F) = 1 % and an acrophase at 20.20, and finally, the total lipids show a circadian rhythm with an adapting index (A.I.) = 0.92, with a statistical significance (F) = 1 % and an acrophase at 19.20.

Table 2: The periodic parameters in 7 donkeys: mesor (M), with the confidence interval (C.I.) at 95 %, amplitude (A) and acrophase (µ) expressed in hours with the confidence interval (C.I.) at 95 %

Parameter	M	C.I. 95%	A	Φ	C.I. 95%
Body temperature (°C)	38.72	(38.67-38.76)	0.18	$20.^{24}$	$(17.^{48}$ - $23.^{00})$
Glucose (mmol/l)	3.62	(3.56 - 3.69)	0.20	$19.^{52}$	$(16.^{24}$ - $23.^{20})$
Triglycerides (mmol/l)	0.38	(0.37 - 0.39)	0.03	$20.^{20}$	$(17.^{08}$ - $23.^{32})$
Total lipids (g % ml)	3.34	(3.28 - 3.40)	0.15	$19.^{20}$	$(14.^{52}$ - $23.^{48})$

Table 2 shows the mesor, with the confidence interval (C.I.) at 95 %; the amplitude, expressed in the same unit as the relative mesor; the acrophase, calculated with the singular Cosinor method and expressed in hours, together with the confidence interval at 95 %, for the periodic parameters. Figure 1 shows the rhythm parametersobtained by "Cosinor" procedure.

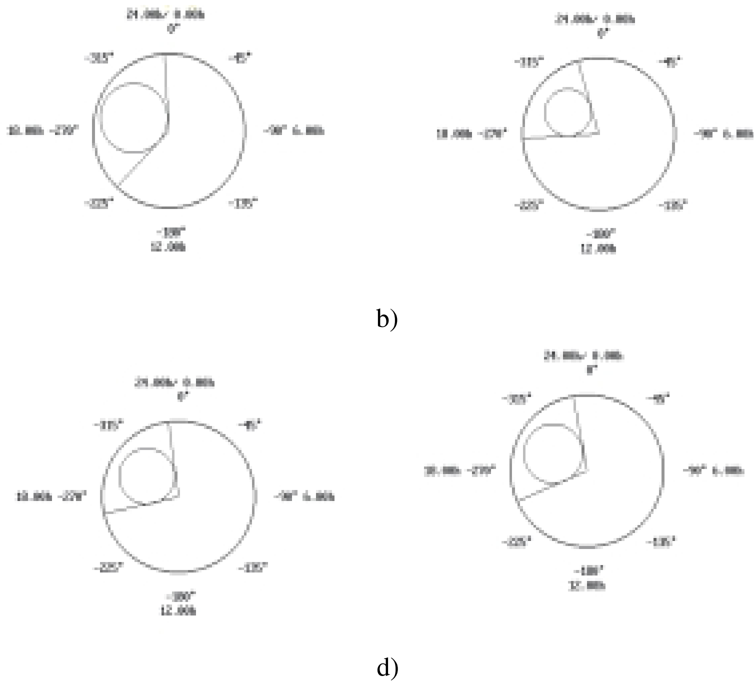

Figure 1: Cosinor of: a) Body temperature; b) Glucose; c) Triglycerides; d) Total lipids, in the donkey

Discussion

The acrophases of the body temperature and the blood concentrations of glucose, triglycerides and total lipids, occurred in the dark of night between 19.20 and 20.24. This led us to hypothesize about the existence of a correlation between the body temperature and some of the energetic-metabolite acrophases. Additionally it showed that there is a temporal correlation between the different physiological systems in donkey too. The amount and composition of lipids circulating in the blood of domestic animals is dependent upon a number of physiological variables (2). In sheep the metabolic level also changes seasonally with a sinusoidal curve (3). According to Blaxter and Boyne (1982) such a variation would not be due to oscillations in food intake since the animals they used were given maintenance feeding throughout the experiment; the variation in metabolic rate was not related to food intake, therefore a different zeitgeber is implied (5). In practice, however, blood metabolites are poor indicators of nutritional and physiological status, since homeostasis is a basic biological control mechanism developed by living organisms to prevent gross chemical imbalances (2). Intensive sampling, as demonstrated in our study on body temperatures and haematochemical parameters, can reveal the presence of circadian variations. Therefore, in the knowledge that numerous endogenous and exogenous factors are very important variables that influence the main parameters characterizing the periodic function, it is possible to define the temporal organization of domestic animals concerning certain parameters of great physiological interest. The study of biological rhythms is important as the state of health of the single animal and that of the population as a whole are correlated. The living organism is characterized by extreme variability: in fact the majority of physiological variables follow an oscillating rhythmic pattern. The determination of the spectrum of frequencies typical of biological rhythms has made it possible to give a specific foundation to the dynamic concept of well-being (12). Furthermore, the study of the modifications produced in such rhythms as a result of given stimuli caused by environmental factors makes it possible to study the capacity of reaction and adaptation of animals to the environment, as well as pathological reactions, and hence improve their output by intervening upon the environment and breeding techniques.

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