



## Daily rhythmicity of core and surface temperatures of sheep kept under thermoneutrality or in the cold

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### ABSTRACT

We compared the daily rhythms of body core temperature and surface temperature in 10 Comisana ewes housed at thermoneutrality (25 °C) and in the cold (9 °C). Core temperature was measured with a rectal probe, whereas surface temperature (in the eye, forehead, and foot) was measured by infrared thermography, at 3 h intervals for 27 consecutive hours under each condition. We found a robust rhythm of core temperature, whose amplitude doubled from 0.2 °C at thermoneutrality to 0.4 °C in the cold. The surface temperatures were all lower than core temperature and exhibited weaker but significant daily rhythmicity. At thermoneutrality, foot temperature (an indicator of vasodilation in the service of heat loss) peaked 3 h before rectal temperature. Studies on other species are needed to clarify whether the latency between the peaks of the oscillations in foot and core temperature vary in a species-specific manner and are responsible for the generation of the core temperature rhythm.

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### 1. Introduction

Repeated measurements of the body temperature of animals and human subjects over time – allowing the study of daily/circadian rhythmicity – have been conducted since at least the mid-1800s (Chossat, 1843; Davy, 1845; Hobday, 1896; Maurel, 1884; Ogle, 1866; Rattray, 1870). As summarized in occasional literature reviews over the years, much has been learned about the daily/circadian oscillation of body temperature (Aschoff, 1983; Hahn, 1989; Refinetti and Menaker, 1992; Waterhouse et al., 2005), and it is now well established that the rhythmicity is endogenously generated and has a range of oscillation of about 1–5 °C around a mean level of approximately 37.5 °C in adult mammals or 41 °C in birds (Refinetti, 2010). Yet, most studies have been limited to the measurement of core temperature, usually the temperature of the intra-abdominal cavity. Only occasionally have studies been conducted in which the oscillatory pattern of skin temperature was compared with the oscillatory pattern of core temperature (Fuller et al., 1985; Kräuchi and Wirz-Justice, 1994; Shechter et al., 2011). Similarly, almost all previous studies have been conducted while the subjects were maintained in a thermoneutral environment. Only occasionally have studies been conducted in which the influence of ambient temperature on the oscillatory pattern of core temperature was investigated (Downs and Brown,

2002; Fuller, 1984; Graf, 1980; Körtner et al., 2000; McKechnie and Lovegrove, 2001; Refinetti, 1996, 1997).

It would be valuable to know more about how the oscillatory pattern of skin temperature relates to the oscillatory pattern of core temperature. Except in hot environments, heat flows from the core to the periphery of endotherms, and one might expect oscillations in skin temperature to lag behind oscillations in core temperature. However, because dilation and constriction of peripheral blood vessels are widely used mechanisms of heat loss and heat conservation, it is possible that oscillations in skin temperature, at least in certain regions of the body, may actually precede oscillations in core temperature and serve as the mechanism that produces the oscillation in core temperature. This latter possibility has been documented in a few studies in monkeys (Fuller et al., 1985) and humans (Kräuchi and Wirz-Justice, 1994; Shechter et al., 2011). Also, it is to be expected that the temperature of the environment may affect the oscillatory pattern of core temperature. Enhanced amplitude of the daily oscillation of core temperature in cold environments has been demonstrated in several species, such as pigeon (Graf, 1980), thirteen-lined ground squirrel (Refinetti, 1996), squirrel monkey (Fuller, 1984), and tree shrew (Refinetti, 1997), although not in laboratory rat (Roussel et al., 1976; Yang and Gordon, 1996), gray mouse lemur (Aujard and Vasseur, 2001), or Syrian hamster (Refinetti, 1997).

In the present study, we investigated the daily oscillation of core temperature and surface temperature at three different body locations in sheep maintained either under thermoneutrality or in

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a cold environment. Better understanding of the relationship between skin and core temperature oscillations, and its dependence on ambient temperature, can facilitate understanding of the process by which the daily/circadian rhythm of body temperature is generated in this and other species of homeothermic animals (Refinetti, 2010).

## 2. Materials and methods

### 2.1. Animals

The animals used in the study were 10 young adult non-pregnant Comisana ewes (*Ovis aries*, 18 months old,  $38 \pm 1$  kg). Comisana is a breed of domestic sheep from Italy. This is a wool breed but is primarily raised for milk production. The animals were housed in a covered barn under natural environmental conditions in Padua, Italy (longitude  $11^{\circ}88' 33''$  E, latitude  $45^{\circ}40'00''$  N) and were fed hay and water *ad libitum*. A supplement of 400 g of Compli Sheep (Tecnozoo, Piombino Dese, Italy) per animal per day was provided at 08:00 each day. Compli Sheep is made of barley flour, cornmeal, flour extraction of sunflower, durum wheat gluten, and wheat bran, flour of wheat, soybean flour, calcium carbonate, and molasses sugar cane. The analytical composition is 16% CP, 10% CF, 2.7% EE, 10% ash, and 0.0% sodium. Protocols of animal husbandry and experimentation followed applicable regulations in Italy and South Carolina.

### 2.2. Data collection

Core and surface temperatures were measured from each animal at 3 h intervals for 27 consecutive hours on two occasions: under thermoneutral conditions (late spring, ambient temperature  $25 \pm 2$  °C, relative humidity 66%, sunrise at 5:20, sunset at 21:15) and in the cold (late winter, ambient temperature  $9 \pm 2$  °C, relative humidity 74%, sunrise at 6:30, sunset at 18:20). The mild weather in northern Italy and adequate ventilation in the barns assured a small daily variation in ambient temperature during the days of the study (2 °C). This 2 °C variation over a whole day is known to be negligible regarding core temperature (Refinetti, 2010) and is expected to have a rather small effect on skin temperature when compared to the effect of the 16 °C difference in mean ambient temperature (25 °C vs. 9 °C).

Core temperature was estimated by rectal temperature, measured at 8 cm past the sphincter with a digital thermometer (model HI-92740, Hanna Instruments, Bedfordshire, UK) with resolution of 0.1 °C. Surface temperature (of the right eye, forehead, and interdigital area of the right front foot) was measured with an infrared imaging system (ThermaCam P25 Model, Flir Systems, Boston, MA, USA), which was calibrated to ambient temperature and absorptive conditions on each sampling day. The eye was chosen as a body site with relatively high surface temperature, the forehead as a site with lower surface temperature, and the foot as a peripheral site with relatively high thermal lability.

Rectal temperature and surface temperature were measured simultaneously, and all measurements at a given time point were completed in approximately 5 min (30 s per ewe). The animals were kept in the barn for the whole day. Night-time data collection was conducted under dim red light.

To reduce the effects of environmental factors on thermographic readings, all images were scanned within the barn and at the same distance (1 m) from the subject. The settings of the camera were as follows: range of temperature 7–34 °C; emissivity of skin: 0.93; reflected air temperature (Triff): 20 °C; distance between camera and skin surface (Dist): 0.7 m; and field of view (FOV): 23°. The detector consisted of a focal plane array (FPA)

uncoiled microbolometer with the following specifications:  $320 \times 240$  pixels resolution, thermal sensitivity of 0.08 °C (at 30 °C), spatial resolution (IFOV) of 1.3 mrad, spectral range between 7.5 and 13  $\mu\text{m}$  accuracy  $\pm 2$  °C. Automatic corrections based on user input were conducted for reflected ambient temperature, distance, relative humidity, and atmospheric transmission. The ewes were unshorn in both stages of the study, as shearing is conducted once a year in the early summer. All thermographic images were analyzed for average and maximal temperature by ThermaCam Researcher Basic Software (Flir Systems).

### 2.3. Data analysis

The data collected from each animal constituted eight time series with nine data points each (four time series at thermoneutrality and four time series in the cold). Each time series was evaluated for rhythmicity by repeated-measures analysis of variance (Model III ANOVA) and by the cosinor procedure (Nelson et al., 1979; Refinetti et al., 2007). Three rhythmic parameters were determined for each time series: mesor (mean level), amplitude (half the range of excursion), and acrophase (time of peak). The cosinor procedure uses an *F* test to evaluate whether the amplitude of a cosine wave fitted to the data is significantly greater than zero (Refinetti et al., 2007). Amplitude not significantly greater than zero implies absence of rhythmicity.

## 3. Results

The mean values of rectal temperature for the nine data points in the two environmental conditions are shown in the top panels of Fig. 1 (thermoneutral, 25 °C) and Fig. 2 (cold, 9 °C). Daily rhythmicity, with a peak 7–9 h before sunrise, is visible and is reflected in a significant effect of time of day on rectal temperature ( $F_{8,153} = 4.333$ ,  $p < 0.001$ ). Neither the effect of ambient temperature ( $F_{1,153} = 0.544$ ,  $p > 0.10$ ) nor the effect of the interaction between ambient temperature and time of day ( $F_{8,153} = 0.454$ ,  $p > 0.10$ ) is significant. Significant daily rhythmicity of the mean values was confirmed by cosinor analysis both at 25 °C ( $F_{2,6} = 15.666$ ,  $p = 0.005$ ) and at 9 °C ( $F_{2,6} = 63.489$ ,  $p < 0.001$ ). The peak time of the daily oscillation was delayed by about 2 h in the cold, possibly because of the difference in photoperiod (see Figs. 1 and 2).

Fig. 3 shows a representative thermographic image of a sheep's head. Brighter areas denote higher surface temperatures. The eyes are visibly warmer than the forehead. The mean results for the three surface temperatures in the thermoneutral (25 °C) environment are shown in the three lower panels of Fig. 1, whereas the mean results for the three surface temperatures in the cold (9 °C) are shown in the three lower panels of Fig. 2. All three variables exhibited daily oscillation (ANOVA main effect of time of day – eye:  $F_{8,153} = 3.151$ ,  $p < 0.01$ ; forehead:  $F_{8,153} = 10.320$ ,  $p < 0.001$ ; foot:  $F_{8,153} = 2.485$ ,  $p < 0.05$ ), although the oscillatory strength and the timing in relation to the light–dark cycle varied from one variable to another and depended on the environmental temperature. These dependencies were clarified by cosinor analysis.

Cosinor analysis was conducted for each individual time series, and group differences in the three parameters (mesor, amplitude, and acrophase) were compared with ANOVAs. As summarized in Fig. 4, there was a significant effect of body site on the mesor ( $F_{3,63} = 765.795$ ,  $p < 0.0001$ ), with the temperature of the rectum being much higher than the surface temperatures. Ambient temperature also affected the mesor ( $F_{1,63} = 8.126$ ,  $p = 0.006$ ), although this effect was mostly restricted to the foot, as confirmed by the presence of a significant interaction effect ( $F_{3,63} = 2.874$ ,  $p = 0.042$ ). The amplitude of the daily oscillation was also

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