Body size and the daily rhythm of body temperature in dogs

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A B S T R A C T

Practically every physiological variable exhibits daily rhythmicity. The daily rhythm of body temperature, like that of many other variables, is often weak in newborns and gains strength as the animals grow. Because of the natural association between physiological maturation and gain in body size, these two processes are naturally confounded. To differentiate between the effects of maturation and the effects of body growth, we undertook advantage of the large variation in body size that exists among different breeds of the domestic dog. We compared the body temperature rhythms of developing puppies of different dog breeds. Puppies of none of the breeds exhibited statistically significant daily rhythmicity for several days after birth. Regardless of breed or sex, rhythmicity matured over several weeks and attained a stable level by 6 weeks after birth. Body size did not seem to be an important element in the development of rhythmicity because the development was similar in three breeds that differed greatly in body size (Basset Hound, Boxer, and Neapolitan Mastiff). On the other hand, the difference in body size associated with the different breeds had a strong impact on the absolute level of body temperature regardless of age: we found a strong inverse correlation between temperature and body size among the puppies and dams of the three breeds and among 115 adult dogs from 19 different breeds ranging from 2-kg Yorkshire Terriers to 80-kg Great Danes.

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1. Introduction

Daily oscillation in physiological processes has been described for a multitude of variables, including locomotor activity, heart rate, blood pressure, hormonal secretion, urinary excretion, and body temperature (Dunlap et al., 2004; Refinetti, 2006). Rhythmicity in body temperature is an important physiological process both as a convenient and reliable marker of the operation of the biological clock (Zulley et al., 1981; Klerman et al., 2002) and as an indicator of the general health of an animal and of its energy metabolism (Cossins and Bowler, 1987; Blumberg, 2002).

The daily oscillation of body temperature is often weak in newborns and gains strength as the animals grow. The adult pattern of temperature oscillation is not fully attained until a few months after birth in rats (Kittrell and Satinoff, 1986), lambs and foals (Piccione et al., 2002), humans (Abe and Fukui, 1979), or calves (Piccione et al., 2003). The first few months of life are also the time when rapid body growth occurs, but there has been no experimental investigation of whether the maturation of temperature rhythmicity is, at least in part, causally related to the growth in body size.

Because of the natural association between physiological maturation and gain in body size, the investigation of the relationship between the body temperature rhythm and body size must take into consideration differences in body size that are not associated with maturation. Body size varies greatly among different species, so that comparison of various species might be a viable option. However, interspecies differences in physiological adaptation are likely to confound the effect of body size. To avoid this complication, we undertook advantage of the large variation of body size that exists among different breeds of a single species, the domestic dog (Canis familiaris). We compared the body temperature rhythms of developing puppies of different dog breeds. We hypothesized that puppies of large breeds would mature significantly faster than puppies of small breeds (because they grow faster than puppies of small breeds).

2. Materials and methods

2.1. Animals

The study involved a laboratory component and a veterinary clinic component, both conducted at the University of Messina’s School of Veterinary Medicine. Protocols of animal husbandry and experimentation were reviewed and approved in accordance with.
the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609 CEE.

For the laboratory component, puppies from 2 litters from each of three breeds were studied. These amounted to 10 female and 5 male puppies of a large breed (Neapolitan Mastiff), 7 female and 6 male puppies of a medium-size breed (Boxer), and 6 female and 4 male puppies of a small breed (Basset Hound). The puppies from each litter were kept with the respective dams in individual pens (140 × 200 cm²) lined with wood shavings. Light-controlling timers were set to maintain a light–dark cycle with 12 h of light and 12 h of darkness each day (lights on at 07:00). Ambient temperature was thermostatically maintained at 20 ± 2 °C. Relative humidity was kept in the range of 50–60%.

All dams were 3 years old and were fed a certified dog diet (Teklad 2021 Global Dog, Harlan Laboratory, Udine, Italy) once a day at 09:00. During early pregnancy, Mastiff dams were fed 800 g of dry food per day, Boxer dams were fed 450 g, and Basset dams were fed 200 g. The amount of food was increased as pregnancy progressed, up to 1200, 700, and 300 g per day, respectively. Maternal milk was the only source of nutrition for the puppies from birth to weaning (30 days of age). After weaning, puppies were fed dry dog food four times a day (300, 200, and 170 g for Mastiff, Boxer, and Basset puppies, respectively). Water was available ad libitum at all times.

For the clinic component of the study, 115 adult dogs of various breeds were recruited by advertisement among university students for a single outpatient examination at the veterinary clinic. Nineteen breeds were included: Yorkshire Terrier (2 females, 2 males), Miniature German Spitz (4 females, 1 male), Pekingese (3 females), Miniature Pinscher (4 females, 3 males), Pug (4 females, 2 males), Miniature Poodle (1 female, 4 males), Basset Hound (2 females, 3 males), Cocker Spaniel (3 females, 2 males), Beagle (7 females, 4 males), Pointer (4 females, 3 males), Dalmatian (4 females, 3 males), English Setter (2 females, 4 males), German Shorthaired Pointer (4 females, 4 males), Boxer (5 females, 3 males), German Shepherd (4 females, 6 males), Maremma Sheepdog (3 females, 3 males), Rottweiler (3 females, 2 males), Neapolitan Mastiff (2 females, 2 males), and Great Dane (1 female, 2 males).

2.2. Procedures

In the laboratory component of the study, puppies were individually weighed on the day of birth and every 10 days afterwards until they reached 2 months of age. Measurements of rectal temperature were conducted every 7 days from 7 to 56 days after birth. On these days, rectal temperature (3 cm deep) was recorded at 3-h intervals for 27 consecutive hours with a calibrated electronic thermometer with resolution of 0.1 °C (Model HI-92740, Hanna Instruments, Bedfordshire, UK). Measurements of rectal temperature of the dams were also conducted at 3-h intervals for 27 consecutive hours on the 45th and 60th days post-partum.

In the clinic component of the study, healthy dogs were brought by their owners to the veterinary clinic during weekdays between 12:00 and 14:30 over a period of 5 days in mid-autumn. The breed, sex, body mass, and rectal temperature of the dog were recorded after a short wait (30–45 min) to eliminate potential confounding effects of previous physical activity. The same wait time and same electronic thermometer (Model HI-92740, Hanna Instruments, Bedfordshire, UK) were used for all subjects.

2.3. Data analysis

Laboratory measurements of rectal temperature from each puppy on each sampling day produced time series consisting of 9 equally spaced data points, which were analyzed by cosinor rhythmometry (Nelson et al., 1979; Refinetti et al., 2007). Four rhythmic parameters were determined for each time series: mesor (mean level), amplitude (half the range of excursion), acrophase (time of peak), and robustness (strength of rhythmicity computed as the fraction of the variance explained by the cosine model). 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 (Nelson et al., 1979). Comparisons of group means were conducted by factorial ANOVA (Kirk, 1995).

The correlation between body mass and rectal temperature (the mean mesor of the temperature rhythm in the laboratory component of the study or the individual rectal temperature measurement in the veterinary clinic component of the study) was calculated by the method of the least squares and was tested for statistical significance with a t test (Hays, 1994).

3. Results

The mean values of body mass of puppies of the three breeds over the 2 months are shown in Fig. 1. As expected, there was a strong, statistically significant effect of breed (F2,6 = 17420, p < 0.0001) as well as of time (F6,192 = 15644, p < 0.0001). The effect of sex was smaller but still significant (F1,32 = 78.69, p < 0.0001). The difference in rates of growth of the three species was reflected in a significant effect of the interaction of breed and time (F12,192 = 3038, p < 0.0001). Even though growth was not yet approaching an asymptote by 2 months after birth, data collection was stopped at this time because, as shown below, the parameters of the body temperature rhythm had stabilized.

Fig. 2 shows representative records of rectal temperature of a Boxer puppy during development. There was no clear daily pattern on day 7, and the cosinor procedure was unable to fit a cosine function with amplitude significantly greater than 0 (F2,6 = 0.682, p = 0.544, robustness = 0%). Rhythmicity was present on day 28 (F2,6 = 6.977, p = 0.027, robustness = 56%) and
became stronger (with a smoother oscillatory pattern) on day 56 ($F_{2,175.6} = 7.224, p < 0.0001$, robustness = 98%).

The mean values of rhythm robustness of puppies of the three breeds over the 2 months are shown in Fig. 3. Robustness was very low in puppies of both sexes and of all three breeds for several days after birth but rose past the significance line within a few weeks and reached a stable value by 6 weeks after birth. Analysis of variance revealed a significant effect of time ($F_{7,224} = 99.45, p < 0.0001$) but not of breed ($F_{2,32} = 0.810, p > 0.10$) or sex ($F_{1,32} = 0.887, p > 0.10$). There was also a small interaction between breed and time ($F_{14,224} = 2.55, p > 0.10$).

Fig. 4 shows the mean values for mesor, amplitude, and acrophase. Although differences in breed were responsible for the greatest differences in mesor ($F_{2,32} = 23.24, p < 0.0001$), there was also a maturational effect, as documented by a significant effect of time ($F_{7,224} = 34.90, p < 0.0001$). Sex did not significantly affect the mesor ($F_{1,32} = 0.049, p > 0.10$). The amplitude of the body temperature rhythm significantly expanded over time ($F_{7,224} = 49.97, p < 0.0001$), and the effect of breed was small albeit significant ($F_{2,32} = 9.46, p = 0.0009$), with Mastiffs exhibiting slightly greater amplitudes than Boxers, and Boxers exhibiting slightly greater amplitudes than Bassets. Sex did not significantly affect the amplitude ($F_{1,32} = 0.001, p > 0.10$). As expected because of the absence of significant rhythmicity shortly after birth, the acrophase values were widely spread out initially but gradually converged into a narrow bend around 19:00 (the time of lights off). The effect of time was significant ($F_{7,224} = 4.64, p = 0.0002$), but there was no significant effect of either breed ($F_{2,32} = 2.35, p > 0.10$) or sex ($F_{1,32} = 0.37, p > 0.10$) on the acrophase.

To evaluate more closely the role of body size in the effect of breed on the parameters of the body temperature rhythm, correlation coefficients were computed. For each parameter of the rhythm (mesor, amplitude, acrophase, and robustness), the mean value for all 60-day-old puppies of each breed and the mean value for the dams recorded 45 and 60 days post-partum were correlated with the body masses of the animals, as illustrated in Fig. 5. There was a significant negative correlation between mesor and body mass ($r = -0.91, p = 0.01$), but none of the other parameters correlated significantly with body mass ($p > 0.10$).

Because only the mesor was found to correlate with body size, we collected data from 115 other adult dogs by taking their rectal temperature between 12:00 and 14:30, a time when temperature is expected to reflect the mesor of the daily rhythm (see Fig. 2). The data, as shown in Fig. 6, revealed a significant negative correlation between temperature and body mass ($r = -0.57, p < 0.0001$). If temperature is expressed as a power function of body mass, the function is $T = 39.22 \cdot M^{-0.004}$ (where $T$ is in °C and $M$ is in kg). With correction for non-zero offset, the function becomes $T = 1.425 \cdot M^{-0.235}$. We calculated the same functions using the mean temperatures for each of the 19 breeds instead of using all 115 individuals. Linear regression yielded a considerably better fit ($r = -0.92, p < 0.0001$), likely because of the reduced variance in body masses. The power functions were $T = 39.30 \cdot M^{-0.005}$ and $T = 1.756 \cdot M^{-0.450}$.

4. Discussion

4.1. Maturation of the body temperature rhythm

Puppies of none of the breeds exhibited statistically significant daily rhythmicity for several days after birth. Regardless of breed or sex, rhythmicity matured over several weeks and attained a

![Fig. 2. Records of rectal temperature of a representative puppy (female Boxer) on days 7, 28, and 56 after birth. The curves are cosine functions fitted by the cosinor procedure. The white and black rectangles at the top indicate the duration of the light and dark phases of the prevailing light-dark cycle, respectively.](image)

![Fig. 3. Mean (+SE) rhythm robustness of puppies of three dog breeds during the first 2 months of life. The dashed horizontal line delineates statistical significance ($p < 0.05$).](image)
stable level by 6 weeks after birth. Similarly, we observed no daily rhythmicity during the first week after birth in calves in a previous study (Piccione et al., 2003). A dawn–dusk difference in body temperature was first noticeable on day 9 and increased gradually until a stable daily rhythm was attained approximately 8 weeks after birth. In lambs and foals, rhythmicity matured more rapidly, a stable level being achieved 4 weeks after birth (Piccione et al., 2002). The reasons for the differences in timing among calves, dogs, lambs, and foals are not evident. These differences cannot be explained by differences in body size, as both foals and calves are larger than dogs but the former develop more rapidly than dogs whereas the latter develop more slowly. Other possible explanations are differences in the nursing behavior of the mothers (which we have not studied) or differences in the thermoregulatory physiology of the various species. Different species of domestic animals exhibit different parameters of body temperature rhythmicity in adulthood (Piccione and Refinetti, 2003), and it would be reasonable to expect that differences also exist in the ontogenetic development of rhythmicity.

4.2. Canine rhythm of body temperature

There were significant changes in mesor, amplitude, and acrophase of the body temperature rhythm as the puppies matured, which was expected. We did not find significant differences due to sex, however. Differences in breed had no significant effect on the acrophase, had a small effect on the amplitude of the rhythm, and had a large effect on the mesor (with smaller breeds having higher mesors, as discussed in greater detail below). The intra-breed variance in mesor at 2 months of age was rather small (inter-individual range \(+0.6\) °C), and the mean breed mesors for the three breeds at 2 months of age were
within a narrow range of 0.4 °C (despite the strong, statistically significant effect of breed on the mesor). For Basset Hound dogs, the robustness of the body temperature rhythm was approximately 60%, the mesor was 39.2 °C, the amplitude was 0.3 °C, and the acrophase occurred at 19:00 (the time of lights off). These values are very similar to those we have previously found in Beagle dogs of similar body size (Refinetti and Piccione, 2003; Piccione et al., 2005). Compared with other mammalian species (see Refinetti, 2006), the temperature rhythm of the dog has a relatively high mesor, small amplitude, and average robustness.

4.3. Body size and core temperature

Daily rhythmicity of body temperature matured over the first 6 weeks of life of our puppies, and the animals gained considerable weight over the same period of time, but body size did not seem to be an important element in the development of rhythmicity because the development was similar in the three breeds that differed greatly in body size (see Fig. 3). On the other hand, the difference in body size associated with the different breeds had a strong impact on the absolute level of body temperature (i.e., on the mesor of the temperature rhythm) regardless of age (Fig. 4). As a matter of fact, we found a strong inverse correlation between temperature and body size among the puppies and dams of the three breeds (Fig. 5) and among 115 adult dogs from 19 different breeds (Fig. 6).

Interspecies studies of the relationship between body temperature and body size based on literature surveys have either failed to identify a significant correlation (Morrison and Ryser, 1952; Lovegrove, 2003) or identified a weak positive correlation (Aschoff, 1982; White and Seymour, 2003; Mortola and Lanthier, 2004; Refinetti, 2006), which contradicts our intraspecies finding in dogs. On the other hand, an intraspecies study on human subjects identified a significant inverse correlation between body temperature and body mass (Adam, 1989), which is in agreement with our finding in dogs. Furthermore, in a recent literature review, Clarke and Rothery (2008) found no significant relationship between temperature and body mass in mammals overall but did find significant relationships for particular subgroups. For instance, a positive scaling relationship was found in bats, whereas a negative scaling relationship was found in artiodactyls (Clarke and Rothery, 2008).

Why smaller dogs should have higher temperatures is not evident. Smaller animals have higher specific metabolic rates, but they also have greater rates of dry heat loss—and body temperature is the result of a delicate balance between processes of heat production and heat loss (Cossins and Bowler, 1987; Blumberg, 2002). Allometric equations describing the relationship between metabolic rate and body size are usually power functions with exponents between 0.67 and 0.75—or exponents between −0.25 and −0.33 if metabolic rate is expressed per unit of body mass (Schmidt-Nielsen, 1997). The relationship between temperature and body size in our sample of 115 dogs had an exponent of −0.24 (when adjusted for zero offset), which closely approximates the exponent for metabolic rate. It is possible, therefore, that smaller dogs have higher body temperatures because their specific metabolic rates (and, therefore, endogenous heat production) are greater, whereas their conductances are presumably independent of body size. Whether this is true or not, Clarke and Rothery’s (2008) finding that the scaling of body temperature is positive in some phylogenetic groups but negative in others implies that the causes of the scaling must be found in ecological factors that affected the evolution of different phylogenetic groups differently.

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