Daily Rhythms of Blood Pressure, Heart Rate, and Body Temperature in Fed and Fasted Male Dogs

G. Piccione1, G. Caola1 and R. Refinetti2,3

Addresses of authors: 1Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Messina, 98168 Messina, Italy; 2Circadian Rhythm Laboratory, University of South Carolina, Walterboro, SC, USA; 3Corresponding author: Tel.: +1 843 549 6314; fax: +1 843 549 1317; E-mail: refinetti@sc.edu

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Summary
Daily or circadian rhythmicity in physiological processes has been described in a large number of species of birds and mammals. However, in dogs, most studies have either failed to detect rhythmicity or have found that rhythmicity reflects merely an acute exogenous effect of feeding rather than an autonomous rhythmic process. In the present study, we investigated the rhythmicity of body temperature, blood pressure, and heart rate in dogs fed daily as well as in dogs deprived of food for 60 h. Our results document clear rhythmicity in all three parameters and demonstrate that the rhythmicity is independent of the feeding schedule. The failure of various previous investigations to document daily rhythmicity in dogs is probably due to lack of experimental rigour rather than to weakness of daily rhythmicity in dogs.

Introduction
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, 2005). Extensive research has established that, in mammals, a circadian pacemaker located in the suprachiasmatic nucleus of the hypothalamus generates daily rhythmicity, which is modulated by environmental cycles of light and darkness, food availability, ambient temperature, and other factors (Van Esseveldt et al., 2000; Helfrich-Förster, 2004). To the extent that daily rhythmicity is a fundamental characteristic of animal physiology, thorough understanding of circadian rhythms is a necessity for effective veterinary practice (Piccione and Caola, 2002; Piccione and Refinetti, 2003).

Although studies conducted on small laboratory rodents and large farm animals have yielded highly reproducible results, studies on dogs have been rather inconsistent. Numerous investigators failed to detect daily rhythmicity in body temperature (Hawking et al., 1971; Marvin and Reese, 1986) or in the secretion of various hormones, including prolactin (Gobello et al., 2001), cortisol (Koyama et al., 2003), and growth hormone (Gobello et al., 2002). In other instances, rhythmicity was detected in body temperature (Rawson et al., 1965; Miyazaki et al., 2002), cardiovascular parameters (Mishina et al., 1999; Matsunaga et al., 2001; Miyazaki et al., 2002), and other functions (Corea et al., 1996; Liesegang et al., 1999) but the rhythmicity seemed to reflect merely an exogenous effect of feeding rather than an endogenous rhythmic process. While it is possible that canine physiology is unique in its lack of endogenous rhythmicity, it is also possible that studies conducted on dogs have lacked the experimental rigour necessary for the identification of rhythmicity. In a previous study, we showed that daily rhythmicity of body temperature can be reliably detected in Beagle dogs, even though the robustness of the rhythm was weaker than in laboratory rodents and farm animals (Refinetti and Piccione, 2003). Although feeding did not seem to be the cause of the rhythmic pattern, we did not address the issue of feeding-induced hyperthermia experimentally. In the present study, we investigated the rhythmicity of body temperature, blood pressure, and heart rate in dogs fed daily as well as in dogs deprived of food for 60 h.

Materials and Methods
The study was carried out on 10 1-year-old male dogs (purebred Beagle, mean body mass = 11 kg). The animals had been in our facilities for 2 months prior to the beginning of the study. They were visually inspected daily and were examined by a veterinarian monthly.

The animals were housed in individual pens (140 × 200 cm) lined with wood shavings. The pens were separated by concrete walls but had screen doors, which allowed the dogs to hear and smell each other but not to see or contact each other. Light timers were set to maintain a light–dark cycle with 12 h of light and 12 h of darkness each day (lights on at 08:00 hours). Ambient temperature was thermostatically maintained at 21 ± 2°C. Relative humidity was kept in the range of 50–60%.

The dogs were divided into two groups of five animals for 48 consecutive hours of recording. One group of dogs received normal feeding (270 g of a certified dog diet provided at
10:00 hours each day), whereas the other group was fasted starting 12 h prior to the first measurement. Animals in both groups had free access to water at all times. The two groups were housed in adjacent rooms connected by an open door. During the 2 days of the study, only the experimenters entered the rooms.

Measurements of rectal temperature, heart rate, and mean arterial blood pressure were conducted at 3-h intervals. Rectal temperature (3 cm deep) was recorded with an electronic thermometer (Model HI-92740; Hanna Instruments, Bedforshire, UK). Heart rate and blood pressure were measured with an oscillometric apparatus (Argus TM-7; Schiller, Barr, Switzerland). The blood pressure cuff was placed either on the forelimbs or the tail. The cuff was secured snugly using a Velcro self-adhesive cloth sleeve while the dog was minimally restrained in a standing position. Mean arterial blood pressure was obtained directly from the oscilometric apparatus.

Four rhythmic parameters were determined for each of the three variables in each dog: mean level, amplitude, acrophase, and robustness (strength of rhythmicity). For each animal, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (16 data points). The amplitude of a rhythm was calculated as half the range of oscillation, which on its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosine or procedure (Nelson et al., 1979). Rhythm robustness was computed as a percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity (Refinetti, 2004). Robustness greater than 20% is above noise level and indicates statistically significant rhythmicity. The significance of differences between means of acrophases and between means of rhythm robustness was tested by analysis of variance (ANOVA) followed by post-hoc pairwise comparisons by Tukey’s HSD test (Kirk, 1995). For comparisons of mean levels and amplitudes of each of the three rhythms between the two groups of dogs, unpaired t-tests were used. Differences were considered statistically significant if the probability of type I error was less than 0.01.

Results
Representative records of one dog that received normal feeding are shown in Fig. 1. All three variables exhibited clear daily rhythmicity. The rhythm of heart rate peaked in the middle of the light phase, the rhythm of blood pressure peaked early in the light phase, and the rectal temperature rhythm peaked in the transition between the light and dark phases. Except for rectal temperature, the rhythms started to ascend prior to feeding each day, thus suggesting that feeding-induced activity was not responsible for the observed rhythmicity. Food restriction for 60 h had very little effect on the recorded rhythms. Figure 2 shows the mean values for the three variables in each dog, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (16 data points). The amplitude of a rhythm was calculated as half the range of oscillation, which on its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosine or procedure (Nelson et al., 1979). Rhythm robustness was computed as a percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity (Refinetti, 2004). Robustness greater than 20% is above noise level and indicates statistically significant rhythmicity. The significance of differences between means of acrophases and between means of rhythm robustness was tested by analysis of variance (ANOVA) followed by post-hoc pairwise comparisons by Tukey’s HSD test (Kirk, 1995). For comparisons of mean levels and amplitudes of each of the three rhythms between the two groups of dogs, unpaired t-tests were used. Differences were considered statistically significant if the probability of type I error was less than 0.01.

ANOVA of the acrophase data indicated a significant effect of variable ($F_{2,16} = 166.6, P < 0.0001$), but no significant effect of feeding ($F_{1,8} = 2.171, P = 0.177$) or of the interaction between the two factors ($F_{2,16} = 2.498, P = 0.112$). In both fed and fasted dogs, the rhythm of blood pressure peaked earlier in the day than the rhythm of heart rate, and the rhythm of heart rate peaked earlier in the day than the rhythm of rectal temperature. Rhythm robustness was also not affected by feeding condition. ANOVA of the robustness data indicated a significant effect of variable ($F_{2,16} = 11.458, P = 0.001$), but no significant effect of feeding ($F_{1,8} = 3.21, P = 0.109$) or of the interaction between the two factors ($F_{2,16} = 0.033, P = 0.968$). The blood pressure rhythm was significantly more robust than the rhythms of heart rate and rectal temperature regardless of the feeding condition.

Discussion
Our results document clear rhythmicity of heart rate, blood pressure, and rectal temperature in dogs and demonstrate that the rhythmicity is independent of the feeding schedule. These results are in contrast with those of numerous studies that failed to detect daily rhythmicity in dogs (Hawking et al., 1971; Marvin and Reese, 1986; Gobello et al., 2001, 2002; Koyama et al., 2003) or failed to demonstrate rhythmicity in the absence of an experimenter-imposed cycle of feeding (Rawson et al., 1965; Corea et al., 1996; Liesegang et al., 1999; Mishina et al., 1999; Matsunaga et al., 2001; Miyazaki et al., 2002). On the other hand, our results are in agreement with a
limited number of studies on dogs in which daily rhythmicity was unequivocally demonstrated in heart rate (Ashkar, 1979; Olsson et al., 2003), locomotor activity (Nishino et al., 1997; Siwak et al., 2003), and urinary excretion (Uechi et al., 1994).

The failure of various previous investigations to document daily rhythmicity in dogs is likely due to lack of experimental rigour rather than to weakness of daily rhythmicity in dogs. Uncontrolled variations in ambient temperature or illumination can induce physiological responses that affect body temperature and cardiovascular parameters and obscure rhythmic patterns. Group-housed animals are likely to be disturbed by interactions with neighbours, and individually housed animals kept in a busy animal colony with frequent visits by animal caretakers are likely to be disturbed as well. The magnitude of rhythm robustness that we measured under controlled conditions in this study (62–85%, depending on variable and condition) is not smaller than that previously measured in other species. As a matter of fact, as shown in Fig. 4, the robustness of the body temperature rhythm of the dog measured in this study lies on the mean of the robustness of 10 other mammalian species previously studied under similar experimental conditions.

A regimen of daily feeding could affect circadian rhythms in two ways: it could ‘entrain’ (synchronize) the rhythms (Edmonds and Adler, 1977; Rusak et al., 1988; Jilge, 1991; Kennedy et al., 1991; Challet et al., 1996; Sharma et al., 2000) or it could ‘mask’ the rhythms through the mechanism of diet-induced thermogenesis (Wang, 1980; Welle et al., 1981; Rothwell and Stock, 1983; Diamond et al., 1985; Poehlman et al., 1988; Romom et al., 1993). Entrainment by the feeding regimen is unlikely to be of great significance in animals already entrained by a light–dark cycle and should have only a small effect on rhythm robustness. On the other hand, thermogenesis associated with feeding could have a significant effect at least on the rhythm of rectal temperature. The only effect of feeding condition that we observed in this study was a reduction in the mean level of the rectal temperature rhythm in fasted dogs. The reduction in mean level was not accompanied by a reduction in rhythm robustness, which indicates that cyclic presentation of food was not necessary for the generation of the rhythms of heart rate, blood pressure, or rectal temperature. A reduction in body temperature resulting from food restriction or food deprivation has been previously described in various mammalian species (Himms-Hagen, 1985; Wallace and Pfeiffer, 1992; Severinsen and Munch, 1999; Piccione et al., 2002a) and is considered to be an adaptive mechanism of energy conservation.

Finally, we should point out that, although our study demonstrates that daily rhythmicity of body temperature and cardiovascular function is not dependent on an environmental cycle of food availability, our animals were maintained under an environmental light–dark cycle. To demonstrate that an
organism exhibits truly endogenous rhythmicity, rhythms must be recorded while the organism is housed in total darkness and the organism must exhibit a free-running period slightly but significantly different from 24 h (Dunlap et al., 2004; Refinetti, 2005). Although studies under such strict conditions have been repeatedly conducted on rodents and birds, they have not yet been attempted in dogs. Therefore, although we can state that daily rhythmicity in dogs is not dependent on the feeding regimen, we can only suggest that the rhythms of body temperature and cardiovascular function in dogs are truly endogenously generated.

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References


Fig. 3. Analysis of four rhythmic parameters of 48-h records of heart rate, mean arterial blood pressure, and rectal temperature of fed and fasted dogs. Each bar corresponds to the mean (±SE) of five dogs. In each graph, means bearing different letters (a, b, c) are significantly different from each other (P < 0.01).

Fig. 4. Robustness of the daily rhythm of body temperature as a function of body size for 11 mammalian species. The dashed horizontal line indicates the 0.01 level of significance (i.e. robustness above noise level). The solid horizontal line indicates the mean robustness for the 11 species: 1, Siberian hamster (Phodopus sungorus); 2, Mongolian gerbil (Meriones unguiculatus); 3, Syrian hamster (Mesocricetus auratus); 4, tree shrew (Tupaia belangeri); 5, Laboratory rat (Rattus norvegicus); 6, Richardson’s ground squirrel (Spermophilus richardsonii); 7, Domestic dog (Canis familiaris); 8, Goat (Capra hircus); 9, sheep (Ovis aries); 10, horse (Equus caballus); 11, cattle (Bos taurus). Data from Refinetti (1998); Piccione et al. (2002a,b, 2003, and this study).