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Daily Rhythmicity of Glycemia in Four Species of Domestic Animals under Various Feeding Regimes

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Abstract: Daily rhythmicity of physiological processes has been described for numerous variables in numerous species. A major source of this rhythmicity is a circadian pacemaker located in the mammalian hypothalamus, but very little is known about how the pacemaker generates the multiplicity of bodily rhythms. Research on rats has shown that the rhythm of blood glucose concentration is not a mere consequence of the rhythm of food ingestion, but is rather generated directly by the pacemaker. In this study, we investigated the rhythm of blood glucose concentration in four different species of domestic animals under four different feeding regimes. Our results suggest that, as in rats, the rhythm of blood glucose concentration is not a mere consequence of the rhythm of food ingestion in sheep and cattle. In dogs and horses, however, the rhythmicity of blood glucose concentration seems to be contingent on the presence of a feeding regime.

Key words: cattle, circadian rhythm, dog, glucose, horse, sheep.

A regular pattern of 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 [1, 2]. 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 [3, 4]. Very little is known, however, about how the hypothalamic pacemaker controls the multitude of daily rhythms in the body. It is very unlikely that the central clock generates each and every rhythm individually, but are most rhythms simply derived from a few clock-controlled rhythms?

Glucose concentration in the blood (glycemia) is an important physiological variable that has been shown to exhibit daily rhythmicity in animals fed on a regular schedule or ad libitum [5–9]. Because carbohydrates are a substantial part of the diet of most mammals, one would expect the daily rhythm of glycemia to be a direct result of the behavioral rhythm of food ingestion. Yet, a study in laboratory rats showed that the rhythm of blood glucose concentration persists in animals fasted for 36 h as well as in animals fed multiple isocaloric temporally equidistant meals [8]. Further investigation provided strong evidence that the hypothalamic clock controls the daily rhythm of glycemia by direct action on the liver through the sympathetic nervous system [10]. To ascertain whether the daily rhythm of blood glucose concentration is independent of the rhythm of food ingestion in species other than the laboratory rat, we studied the daily oscillation of glycemia in dog, sheep, horse, and cattle under different feeding regimes. Use of these four species is valuable not only for comparative purposes in mammalian species varying greatly in size from 15 kg (dog) to 700 kg (cattle), but also for commercial purposes, because two of the species (dog and horse) are often exploited as athletes for human entertainment, and the other two (sheep and cattle) are exploited as sources of meat for human consumption.

METHODS

Animals. The subjects, which were all female, were 5 six-year-old bitches (*Canis familiaris*, Beagle breed), 5 three-year-old ewes (*Ovis aries*, Comisana breed), 5 seven-year-old mares (*Equus caballus*, English Thoroughbred breed), and 5 four-year-old cows (*Bos taurus*, Italian Brown breed).

The bitches were housed in individual indoor pens (140 × 200 cm) lined with wood shavings. 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 07:00). Ambient tem-

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perature was thermostatically maintained at 21 ± 2°C. Unless otherwise indicated, approximately 270 g of a certified dog diet (Teklad 2021 Global Dog, Harlan Laboratory, Udine, Italy) was provided to each animal daily at 09:00. Water was freely available at all times.

Ewes were housed in individual covered stalls under natural autumn conditions in Sicily (38°11′30″ north, 15°33′12″ east, 120 m above sea level), namely, a natural 24 h photoperiod (sunrise at 07:00, sunset at 19:00) with an ambient temperature of 17–24°C and relative humidity of 40–50%. Unless otherwise indicated, each animal was fed 200 g of concentrate (23% oats, 36% corn, 38% barley, and 3% minerals and vitamins) per day and had free access to alfalfa hay and water.

Mares were housed in individual indoor stalls with windows under natural autumn conditions (natural sunlight from 07:00 to 19:00, ambient temperature in the 17–24°C range, and relative humidity of 40–50%). Unless otherwise indicated, each mare was fed 3.5 kg of a mix of cereals (50% oats and 50% barley) per day and had free access to alfalfa hay and water.

Cows were housed in individual covered stalls under natural autumn conditions (natural sunlight from 07:00 to 19:00, ambient temperature in the 17–24°C range, and relative humidity of 40–50%). Unless otherwise indicated, each cow was fed 5 kg of concentrate (23% oats, 36% corn, 38% barley, and 3% minerals and vitamins) per day and had free access to organic grains such as corn, flax meal, wheat, soy meal, and rice bran, and also to silage, alfalfa hay, and water.

Procedures. The protocols of animal husbandry and experimentation followed applicable regulations in Sicily and South Carolina. Each of the five animals from each of the four species was studied for two consecutive days under each of four conditions: fasting, one meal per day at 09:00, two meals per day at 09:00 and 16:00, and food available ad libitum. The conditions were spaced a week apart from each other. In the fasting condition, dogs were fasted for 24 h prior to and during the 2-day recording period. Similarly, horses were fasted for 48 h, and sheep and cattle were fasted for 72 h prior to the 2-day recording period.

Blood samples were collected through jugular intravenous catheters (FEP G20 1 × 32 mm) every 4 h for 48 consecutive hours. Dim red light (<3 lx, 15 W Safelight lamp filter 1A, Kodak Spa, Milano, Italy) was used for blood collection during the dark phase. The samples were centrifuged at 1,500 ×*g* for 30 min and were immediately tested for glucose concentration by an enzymatic colorimetric test (Glucose PAP fluid monoreagent, Centronic GmbH, Wartenberg, Germany). This test operates by oxidation of glucose by glucose oxidase in the presence of oxygen, which forms gluconolactone and hydrogen peroxide. Hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to produce a red-violet quinoneimine dye as indicator. The color intensity of the red dye is directly proportional to the glucose concentration.

Data analysis. Determinations of serum glucose concentration from each animal produced time series consisting of 12 equally spaced data points, which were analyzed by cosinor rhythmometry [11, 12]. 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). 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 [11]. Comparisons of group means were conducted by factorial ANOVA [13].

RESULTS

The raw data from a representative dog are shown in Fig. 1. Daily rhythmicity of glycemia was not observed in the fasting condition (cosinor amplitude test, $F_{2,9} = 1.118$, $p = 0.370$), whereas robust rhythmicity was observed when the animal was fed once a day ($F_{2,9} = 15.541$, $p = 0.002$), twice a day ($F_{2,9} = 77.50$, $p < 0.001$), or ad libitum ($F_{2,9} = 27.936$, $p < 0.001$).

The results were consistent within each species, but there were large interspecies differences in some of the rhythmic parameters. In particular, cattle and sheep exhibited robust daily rhythmicity under the fasting condition as well as under the other conditions, as exemplified for one sheep in Fig. 2. Also noteworthy in Figs. 1 and 2 is the absence of postprandial hyperglycemia. Elevated blood glucose levels after a meal are not to be expected in ruminants (sheep and cattle), but can occur in simple-stomached animals (dog and horse) for 2 to 3 h postprandially. Our measurements taken in 4 h intervals evidently prevented the detection of acute postprandial hyperglycemia.

The mean results of the analysis of the four parameters (mesor, amplitude, acrophase, and robustness) in the four species (horse, dog, cattle, and sheep) under the four conditions (fasting, one meal a day, two meals a day, and ad libitum) are shown in Fig. 3. The mesor (mean level of serum glucose concentration) was significantly higher in the nonruminant species (horse and dog) than in the ruminant species (cattle and sheep), as confirmed by a significant effect of species in a factorial mixed-model ANOVA ($F_{3,16} = 58.978$, $p < 0.001$). Regardless of species, the mesor tended to be slightly but significantly lower under the fasting condition, as indicated by a significant effect of condition ($F_{3,48} = 74.77$, $p < 0.001$) but no significant interaction of the two factors ($F_{9,48} = 0.887$, $p = 0.54$).

There was no consistent interspecies difference in rhythm amplitude ($F_{3,16} = 0.483$, $p = 0.70$), but the amplitude was significantly smaller under the fasting condition than under the other conditions ($F_{3,48} = 11.249$, $p < 0.001$).

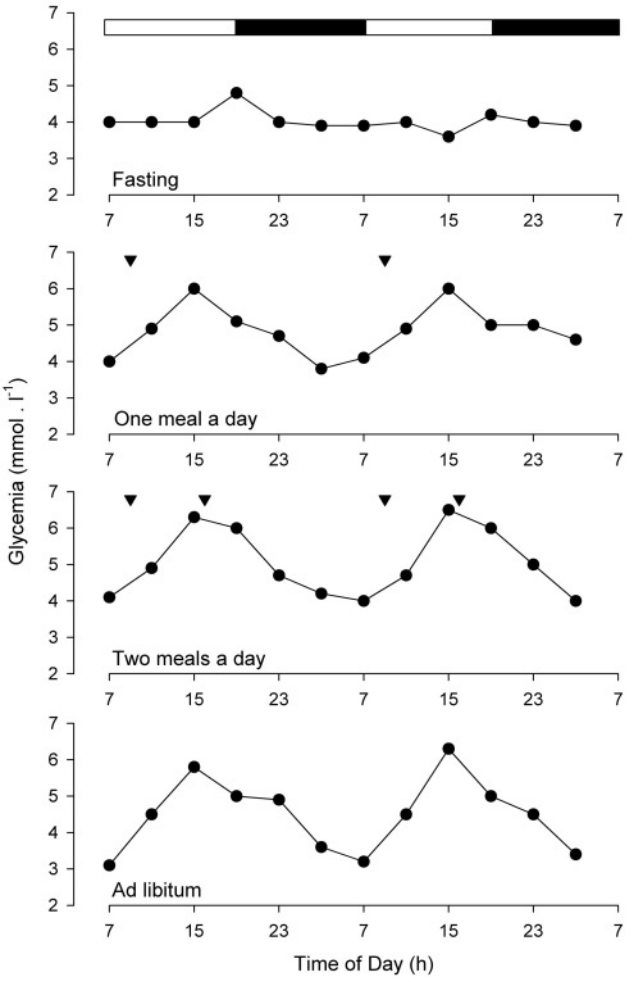

Fig. 1. Measurements of serum glucose concentration of a representative dog carried out every 4 h for 48 consecutive hours. Each panel corresponds to one of four feeding regimes, as indicated. The inverted triangles denote meal times. The horizontal white and black bars at the top of the figure indicate the duration of the light and dark phases of the light-dark cycle, respectively.

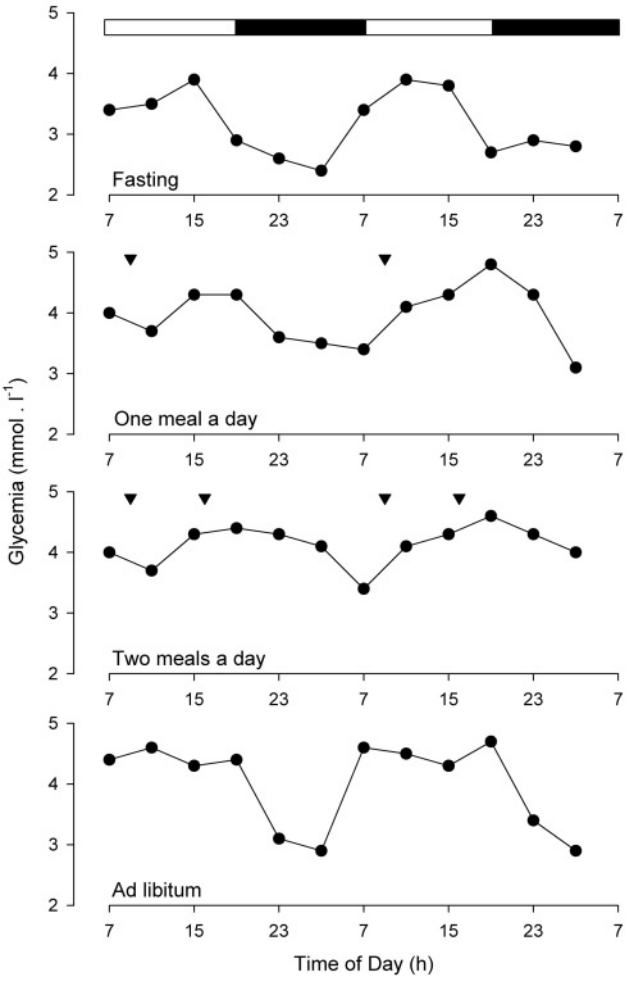

Fig. 2. Measurements of serum glucose concentration of a representative sheep carried out every 4 h for 48 consecutive hours. Conventions same as in Fig. 1.

Although rhythm amplitude was visibly smaller in horses and dogs than in cattle and sheep, the effect of the interaction between species and condition did not reach statistical significance ($F_{9,48} = 1.454$, $p = 0.19$).

The acrophase (time of the daily peak) was significantly earlier in dogs (14:24) than in cattle (18:06), with the other two species in between (horses: 15:18, sheep: 16:25) ($F_{3,16} = 4.175$, $p = 0.022$). There was a significant effect of condition ($F_{3,48} = 10.569$, $p < 0.001$), which was particularly consistent in the form of earlier acrophases in the ad libitum condition than in the one-meal-a-day or two-meals-a-day conditions. There was no significant interaction between species and condition ($F_{9,48} = 0.302$, $p = 0.97$).

Lastly, rhythm robustness did not significantly differ from species to species ($F_{3,16} = 0.161$, $p = 0.92$), but it was significantly affected by feeding condition ($F_{3,48} = 8.061$, $p < 0.001$). Robustness was minimal under the fasting condition in the nonruminant species (horse and dog), but was normal in the ruminant species (cattle and sheep), as reflected in the significant interaction effect ($F_{9,48} = 3.391$, $p = 0.003$).

DISCUSSION

Our results fully support previous observations of daily rhythmicity of blood glucose concentration in animals fed ad libitum [5–9]. In the four species used in this study, rhythm robustness was approximately 50%, which is substantially higher than the cutoff for statistical significance.

Because the acrophase of the rhythm of glycemia was consistently earlier in the ad libitum condition than in

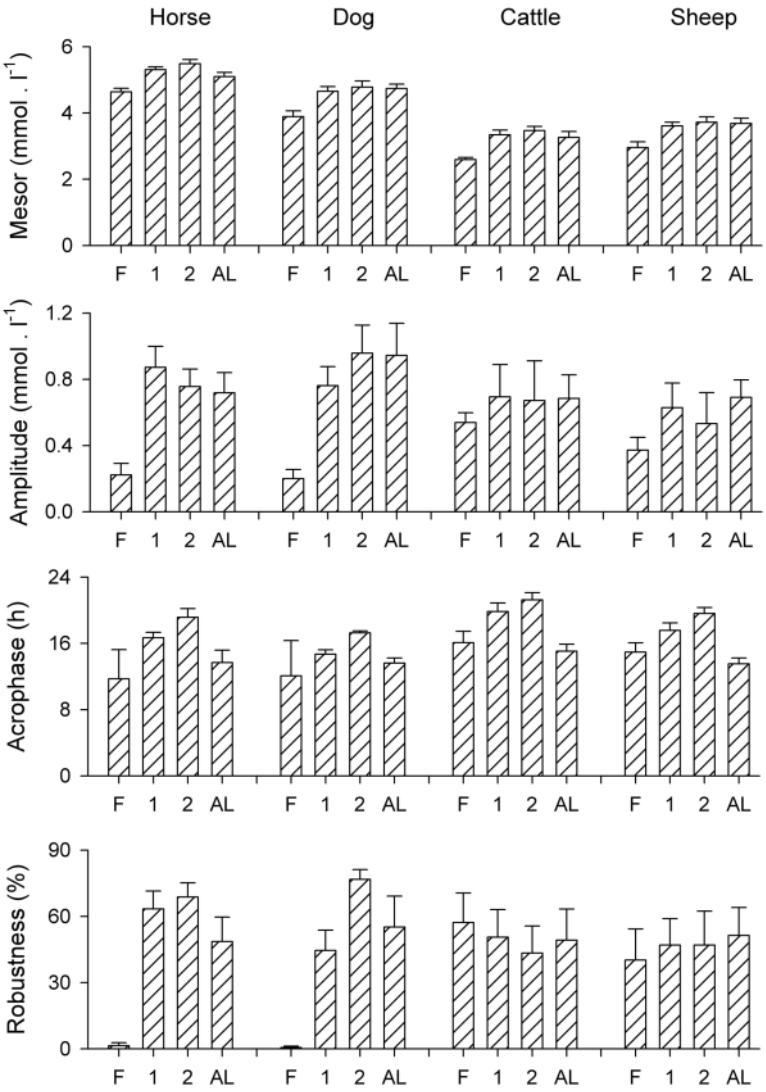

Fig. 3. Mean values of rhythmic parameters computed by the cosinor procedure. Each bar corresponds to the mean (± SEM) of five animals. Abbreviations: F for "fasting," 1 for "one meal a day," 2 for "two meals a day," and AL for "ad libitum." Rhythm robustness greater than 32% (in the lower panel) indicates the presence of statistically significant rhythmicity ($p < 0.05$).

the one-meal-a-day or two-meals-a-day conditions in all four species, it seems reasonable to infer that the phase of the rhythm can be modulated by the time of feeding. Whether rhythmicity was only modulated by the time of feeding, rather than created by it, varied with the species. During fasting, rhythmicity was abolished in horses and dogs but not in cattle and sheep. The fact that glucose in the blood must derive either from the diet or from endogenous storage/production (primarily hepatic gluconeogenesis) implies that rhythmicity of glycemia during fasting is endogenously generated (or generated by a feeding-independent exogenous process). Considering that fasting for sheep and cattle was started 72 h prior to the 2-day interval of data collection, it is unlikely that incomplete digestion was responsible for the preservation of rhythmicity in these two ruminant species. Yet, the disappearance of rhythmicity in dogs and horses (fasted for 24 and 48 h prior to data collection, respectively) suggests that in these species the normal rhythm of glycemia is dependent on dietary glucose and that gluconeogenesis does not exhibit autonomous daily rhythmicity.

The loss of rhythmicity that we observed in fasted dogs and horses cannot be a general characteristic of nonruminants because the glycemia rhythm was previously found to persist in fasted rats [8, 10]. On the other hand, there is no clear explanation for why the glycemia rhythm persisted in fasted sheep and cattle but not in fasted dogs and horses. Two previous studies on dogs do not help clarify the matter. One of them indicated that the glycemia rhythm persists in fasted animals [14], whereas the other failed to identify rhythmicity in fasted or fed animals [15]. We have previously demonstrated the persistence of the daily rhythms of body temperature, blood pressure, and heart rate in fasted dogs [16], so that a general lack of feeding-independent rhythmicity in dogs cannot be brought up as an explanation for the vanishing rhythm of glycemia.

Our results indicate that the effects of fasting on gly-

cemia are not the same in all species. Whereas the hypothalamic clock seems to specifically originate the rhythm of glycemia in rats, sheep, and cattle, the existence of the rhythm seems to derive from the rhythm of feeding in dogs and horses. The causes of this interspecies difference remain to be determined, and further studies on a larger number of species will hopefully provide clues for the solution of this enigma.

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