Short communication

Daily rhythmicity in nutrient content of asinine milk

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Received 21 November 2007; received in revised form 10 January 2008; accepted 13 January 2008

Abstract

Asinine (donkey) milk has been proposed as an alternative to bovine (cow) milk for consumption by human infants who are allergic to bovine milk proteins. To expand the current knowledge of asinine lactation, we investigated daily rhythmicity in the concentration of major constituents of asinine milk. We observed no daily rhythmicity in somatic cell count and pH but observed robust rhythmicity in lipid, lactose, and protein content (robustness greater than 70%, \( p < 0.01 \)). Lipid and lactose content peaked during the night, whereas protein content peaked during the day. Lipid content of asinine milk was lower than that of human milk, and asinine milk resembled human milk better than bovine milk regarding sugar and protein content.

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Keywords: Circadian rhythm; Equus asinus; Lactation; Milk

1. Introduction

Dairy products have been a part of human diet for more than 7000 years (Copley et al., 2003; Cordain et al., 2005). Yet, 2 to 8% of infants exhibit an immunological reaction to cow milk proteins, and the majority of adults of certain ethnic groups exhibit intolerance to milk sugar (lactose) (Vandenplas et al., 2007). Milk allergy in infants is of particular clinical relevance because of milk’s sole or predominant role in infant nutrition.

In the United States, 34% of mothers do not breast feed their infants (Ryan et al., 2006), and alternatives to cow milk are needed if their infants develop cow milk allergy. Hydrolyzed cow milk, vegetable formulas derived from soy, and milk from nonbovine mammals are some of the alternatives that have been explored (Muraro et al., 2002). Particularly promising is asinine (donkey) milk, whose composition resembles the composition of human milk much more than does bovine milk (Guo et al., 2007; Salimei et al., 2004).

Daily oscillation in the levels of physiological variables 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, 2006), and it is to be expected that milk composition will also exhibit daily variation. Indeed, daily variation in milk composition has been documented in humans (Lubetzky et al., 2006; Stafford et al., 1994) as well as in ruminants (Nielsen et al., 2003; Ploszaj et al., 1997).

In the present study, we investigated daily rhythmicity of milk composition in donkey mares with the purpose of expanding the knowledge of lactation in this species.
Expansion of the current knowledge on lactation in donkey mares is particularly important in view of the potential use of asinine milk as a hypoallergenic substitute for bovine milk in human infant nutrition (Iacono et al., 1992).

2. Materials and methods

2.1. Animals

The animals used in the study were 5 multiparous Ragusana donkey mares (Equus asinus), 6 to 8 years old, 320 ± 15 kg body mass. Mares were housed in individual indoor stalls under natural summer conditions in Sicily: a natural 24-hour photoperiod (sunrise at 05:00, sunset at 20:00) with an ambient temperature of 19–26 °C and relative humidity of 40–50%. Hay was available ad libitum and was supplemented with 3 kg of concentrate (47% oats, 30% corn, 20% barley, and 3% vitamins and minerals), dispensed once daily at 09:00. The animals ate all 3 kg of the concentrate (88% dry matter, 11% crude protein, 4% ether extract, 10% crude fiber, 19% neutral detergent fiber) and 5 to 6 kg of hay (88% dry matter, 10% crude protein, 2% ether extract, 28% crude fiber, 53% neutral detergent fiber) each day. Water was available ad libitum. Protocols of animal husbandry and experimentation followed applicable regulations in Sicily and South Carolina.

2.2. Procedures

As a general procedure, the donkey mares were milked manually every 3 h (5 to 9 min per milking). All milk was collected (approximately 900 mL per mare per milking), and 50 mL samples were used for laboratory testing. Measurements of pH were conducted immediately after milk collection with a pH meter (model HI8424, Hanna Instruments, Bedfordshire, UK). Samples were stored at 4 °C until determination of concentrations of lipids, protein, and lactose, as well as somatic cell count, sequentially with a milk analyzer (Milkoscan, FOSS, Hillerød, Denmark).

2.2.1. Phase 1

In a first phase, the mares were milked every 3 h for two full consecutive days, starting at 08:00 on the first day and ending at 08:00 on the third day. The foals were physically (but not visually and olfactorily) separated from the mares during this time.

2.2.2. Phase 2

To control for the sequential effect of milking, the study was repeated with a slightly different protocol. Instead of conducting 9 sequential milk collections at 3-hour intervals each day (from 08:00 on one day to 08:00 on the next day), milk collections were conducted in random sequence with one collection per day for 9 days (e.g., 20:00 on the first day, 02:00 on the second day, 11:00 on the third day, etc.). During this phase, the foals were separated from the mares 3 h before milk collection and returned to the mares after the collection each day.

2.2.3. Phase 3

Because milk composition might vary with the interval between milking episodes, foals were separated from the mares for 24 h before milking in this phase. Milking was conducted once a day for 18 days while the time of milking varied in 3-hour intervals (for two full “days” of data sampled over 18 days).

2.2.4. Phase 4

To verify that the daily variation in milk composition identified in the first three phases was true at various stages of lactation, 24-hour milk collection (at 3-hour intervals) was conducted in the 5 mares at 1, 3, 5, and 7 months postpartum.

2.3. Data analysis

Time series consisting of 9 or 18 data points were analyzed by cosinor rhythmometry (Nelson et al., 1979). 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 (Nelson et al., 1979). Comparisons of group means were conducted by ANOVA with Tukey's HSD post hoc tests (Kirk, 1995).

3. Results

The mean results for the five milk constituents in the five mares in Phases 1 and 2 (sequential and random milking) are shown in Fig. 1. Cosinor analysis of the mean values revealed no daily (24.0-hour) rhythmicity in somatic cell count and pH but revealed robust rhythmicity in lipid, lactose, and protein content (robustness greater than 70%, $p<0.01$). Lipid and lactose content peaked during the night, whereas protein content peaked during the day. The results from sequential milking were not significantly different from the results from random milking, as determined by the absence of a significant effect of collection procedure (sequential vs. random) in repeated-measures factorial ANOVAs ($p>0.10$).

In Phase 3 (foals separated from the mares for 24 h before milking), there was again no significant daily rhythmicity in somatic cell count or pH but there was robust rhythmicity in lipid, lactose, and protein content (mean robustness = 76%, $p<0.001$). The mean values for protein content are shown in Fig. 2. Protein content peaked in the middle of the light phase of the light–dark cycle.

The mean results from Phase 4 (obtained over the course of lactation) are shown in Fig. 3. The mean level of the various milk constituents did not change significantly, except that lipid content gradually decreased as lactation proceeded ($F_{3, 12}=18.731$, $p<0.001$). Not shown in the figure are the results of cosinor analysis of the data. In each of the four stages of lactation, there was significant daily rhythmicity in lipid, lactose, and protein content but not significant rhythmicity in somatic cell count and pH. Except for the reduction in the mean level of lipid content, there was no significant change in rhythmic parameters (including rhythm robustness) through the four stages of lactation.

4. Discussion

The results consistently demonstrate the presence of daily rhythmicity in the concentrations of lipid, lactose, and protein (but not in somatic cell count and pH) of asinine milk. Sequential milking was not a confound because the trends were very similar when the data were collected through random sampling instead of through sequential sampling. Furthermore, the milking procedure itself did not affect rhythmicity because rhythmicity was still clear when milk was collected only once a...
day over 18 days while the foals were separated from the mares for many hours before milking. Except for a gradual reduction in lipid content, there was no significant change in rhythmic parameters throughout the lactation period from 1 to 7 months postpartum. These results confirm and expand the results of previous studies documenting daily variation of milk composition in humans (Lubetzky et al., 2006; Stafford et al., 1994) and ruminants (Nielsen et al., 2003; Ploszaj et al., 1997).

The daily rhythms of lipid and lactose content peaked during the night, whereas the rhythm of protein content peaked during the day. Although the processes responsible for this internal phase relationship are not yet fully understood, the alignment of internal rhythms with the outside world is known to be affected by the time of feeding as well as by the light–dark cycle (Stephan, 2002). Because we did not manipulate the time of feeding or the light–dark cycle, however, we are unable to make inferences about causal determination of the phase relationships observed in this study. Regardless of peak time, the rhythms of lipid, lactose, and protein content were quite robust. With a mean robustness of 76%, the daily rhythm of milk constituents revealed itself as more robust than the daily rhythms of 14 out of 21 variables previously recorded in horse mares (Equus caballus) (Piccione et al., 2005).

The finding that milk protein content is consistently higher during the day implies that industrial milking aimed at the production of hypoallergenic milk for human infant consumption should perhaps be concentrated at night, when protein content is lower. However, because the magnitude of the daily oscillation is modest (coefficient of variation = 2%), the clinical significance of daily variation in protein content requires further investigation.

The gross nutrient contents of human, bovine, and asinine milks are compared in Table 1. Asinine milk has less fat than human milk but resembles human milk better than does bovine milk regarding sugar and protein. The lower fat content may contribute to better cardiovascular health (Parikh et al., 2005). Although we did not investigate the protein profile in detail in the present study, others have previously demonstrated that donkey milk has a low protein allergenic content (Guo et al., 2007; Salimei et al., 2004), thus making asinine milk a favorable candidate for the replacement of bovine milk in the diet of milk-allergic human infants.

Acknowledgements

The research reported here was partially supported by the National Science Foundation Grant IBN-0343917 to R. Refinetti.

References


