

Short paper

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## Daily rhythm of circulating fat soluble vitamin concentration (A, D, E and K) in the horse

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Published: 15 July 2004

Received: 13 May 2004

*Journal of Circadian Rhythms* 2004, 2:3 doi:10.1186/1740-3391-2-3

Accepted: 15 July 2004

This article is available from: <http://www.jcircadianrhythms.com/content/2/1/3>

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### Abstract

**Background:** Many physiological processes of mammalian species exhibit daily rhythmicity. An intrinsic relationship exists between fat soluble vitamins (A, D, E and K) and several body functions. Few investigations on the rhythmic pattern of vitamins in domestic animals have been carried out. The present study evaluated the circadian rhythmicity of fat soluble vitamins in the horse.

**Methods:** Blood samples from 5 Thoroughbred mares were collected at four-hour intervals over a 48-hour period (starting at 8:00 hours on day 1 and finishing at 4:00 on day 2) via an intravenous cannula inserted into the jugular vein. Fat soluble vitamin concentration in the serum (A, D, E and K) was measured by HPLC. One-way repeated measures analysis of variance (ANOVA) was used to determine significant differences.  $p$  values  $< 0.05$  were considered statistically significant.

**Results:** ANOVA showed a highly significant effect of time in all the horses for the vitamins studied ( $p < 0.0001$ ). The application of the periodic model and the statistical analysis of the "Cosinor" enabled us to define the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring: all the studied vitamins showed diurnal acrophases with values between 15:16 and 18:08 hours.

**Conclusion:** Fat soluble vitamins exhibit daily rhythmicity with diurnal peak. Further investigations could help optimize the use of these substances according to their circadian (or other) rhythms.

### Background

Daily or circadian rhythmicity is a ubiquitous property of mammalian physiology [1,2]. Vitamins are organic compounds required in trace amounts to promote a multitude of body functions. Given the intrinsic relationship of these substances to the relative physiological processes (mechanisms of vision, mineral homeostasis, oxidative stress, blood clotting), several studies have been carried out on the temporal changes of fat soluble vitamins in humans, revealing the existence of a circannual [3,4] and

a circadian pattern [5,6], while also in laboratory animals a daily rhythmicity has been observed [7,8]. Other studies have been conducted on the effect of vitamin D administration on the circadian mineral rhythms of humans [9,10]. In previous studies, some authors have reported the concentration-time curve, by taking serial blood samples within 24 hours, in order to calculate some indices, such as the peak, the time to the peak and the area subtended by the increments [11]. Given the lack of experimental studies on the rhythmic pattern of vitamins in

domestic animals based on organism's physiological status, we investigated the existence of circadian rhythmicity of fat soluble vitamins (A, D, E and K) in the horse.

### Methods

The subjects were 5 Thoroughbred mares, 8 years old. They were housed in individual stalls under a natural spring photoperiod (sunrise at 06:06, sunset at 18:49) and natural indoor temperature (19–21°C). Food (hay and concentrate) was provided three times daily, with water ad libitum. The study was carried out in April. Blood samples were collected at four-hour intervals over a 48-hour period (starting at 8:00 hours on day 1 and finishing at 4:00 on day 2) via an intravenous cannula inserted into the jugular vein. Blood samples were transferred into Vacutainer tubes containing no additive. The tubes were clotted at room temperature for 1 hour and subsequently centrifuged at 2500 g for 10 min. The resulting serum was stored at -80°C until analysis. Serum vitamin concentration (A, D, E and K) was measured by high performance liquid chromatography (HPLC), using fluorimetric detection for A and K and UV spectrophotometry for D and E vitamins. All the results were expressed as mean  $\pm$  SD. One-way repeated measures analysis of variance (ANOVA) was used to determine significant differences.  $p$  values  $< 0.05$  were considered statistically significant. Data were analyzed using the software STATISTICA 5.5 (StatSoft Inc., USA). For each measurement we applied a trigonometric statistical model to the average values of each time series, so as to describe the periodic phenomenon analytically, by individuating the main characteristic parameters: Mesor (Midline Estimating Statistic of Rhythm), expressed in the same conventional unit of the relative parameter, with the confidence interval (C.I.) at 95%, Amplitude (A), expressed in the same unit as the relative Mesor, and Acrophase ( $\phi$ ), obtained with the single Cosinor method [12] and expressed in hours, with the C.I. at 95%.

### Results and discussion

The results obtained during the experimental period indicate the existence of a daily rhythm of circulating fat soluble vitamin concentration in the horse, as shown in Figure 1. ANOVA showed a highly significant effect of time in all the horses for the vitamins studied, as follows: vitamin A,  $F_{(11,44)} = 22.89$ ,  $p < 0.0001$ ; vitamin D<sub>2</sub>,  $F_{(11,44)} = 68.07$ ,  $p < 0.0001$ ; vitamin E,  $F_{(11,44)} = 11.50$ ,  $p < 0.0001$ ; vitamin K,  $F_{(11,44)} = 12.27$ ,  $p < 0.0001$ . The application of the periodic model and the statistical analysis of the "Cosinor" enabled us to define the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring. All the vitamins studied showed diurnal acrophases, as follows: vitamin A, at 15:20 both for the 1<sup>st</sup> and the 2<sup>nd</sup> day; vitamin D<sub>2</sub>, at 14:16 (1<sup>st</sup> day) and at 15:12 (2<sup>nd</sup> day); vitamin E, at 15:48 (1<sup>st</sup> day) and at 16:00 (2<sup>nd</sup>

day); vitamin K, at 17:00 (1<sup>st</sup> day) and at 18:08 (2<sup>nd</sup> day). It seems reasonable to hypothesise that the presence of rhythmic diurnal variations in the activity of vitamins could be linked to some physiological or clinical events of the organism. Little is known about the temporal variability in serum vitamin concentration and its probable relationship to the biological processes, despite the important role of these substances in maintaining the body's physiological status.

### Conclusion

It is concluded that a daily rhythmicity exists in serum concentration of vitamins A, D, E and K in the horse, with diurnal acrophases. The observation of acrophases included between 15:16 and 18:08 during the experimental period for all the vitamins studied could suggest the existence of exogenous and/or endogenous synchronizers, as observed for other hematochemical parameters. Further studies involving experimental manipulation of feeding time and ration quality can produce the necessary knowledge for the optimization of the use of these substances according to their temporal pattern. Our research could be useful for its clinical implications, for example, in the choosing of the time of day at which vitamin administration reveals its greatest effect.

### Authors' contributions

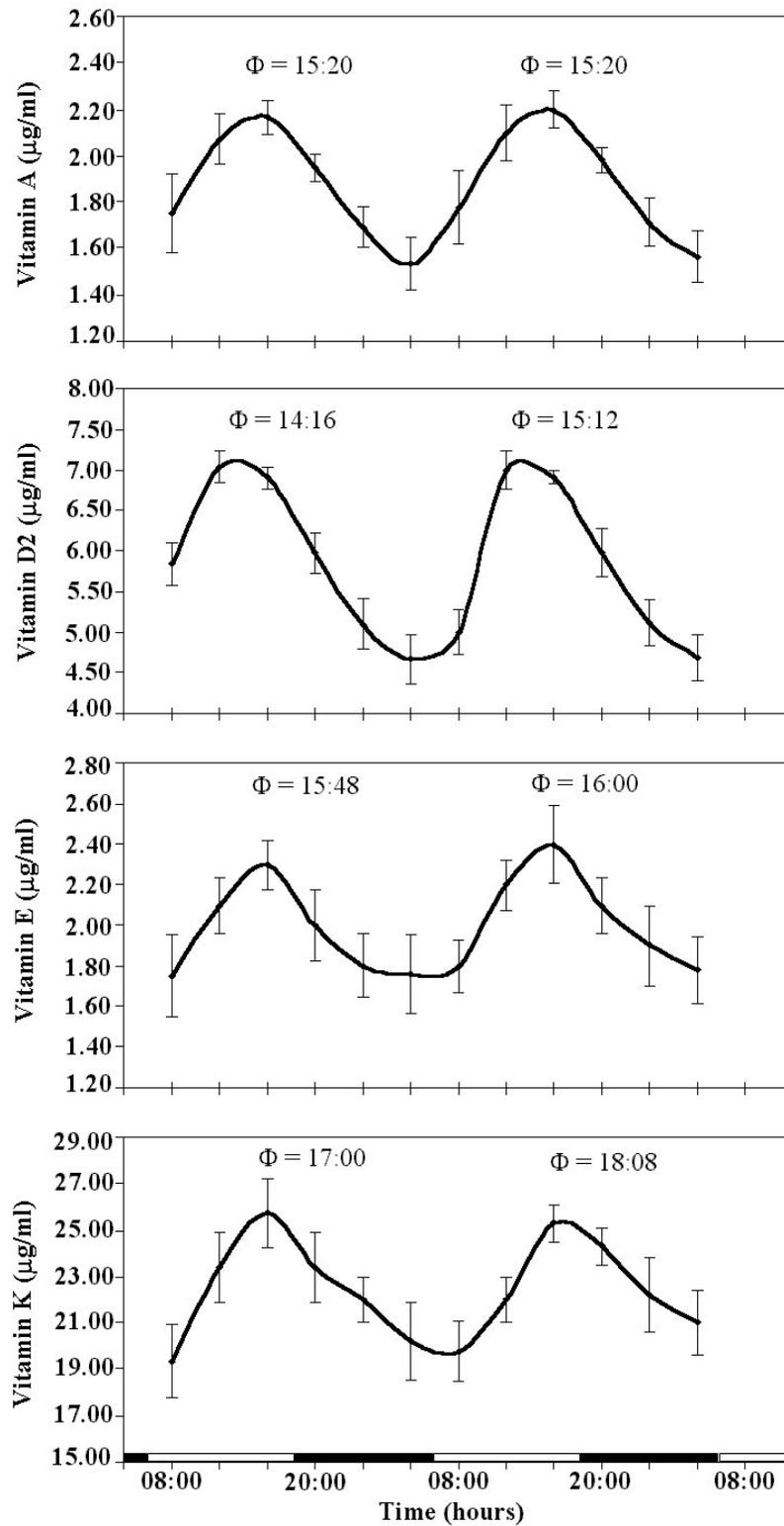
GP-Designed the study and evaluated the data statistically

AA-Carried out the data collection procedures

FG-Carried out the data collection procedures

GC-Supervised the data collection procedures and conducted bibliographic research.

All authors read and approved the final manuscript.



**Figure 1**  
**Daily rhythm of serum fat soluble vitamin concentration in the horse.** Each point represents mean ± SD. Φ indicates the acrophases. Black and white bars at the bottom of the figure indicate the duration of the dark and light phases of the natural light-dark cycle.

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