Caloric deprivation decreases sympathetic nervous activity, which results in decreases in HR, blood pressure, and metabolic rate in rodents. Horses are different from most other mammals in that they have an extremely high parasympathetic tone, which dominates their autonomic nervous activity under resting conditions. It has been reported that HRs up to 110 beats/min are controlled primarily by parasympathetic activity in horses, determined on the basis of the response of HR to increased or decreased systemic blood pressure caused by injection of various pharmacological agents. Response of the autonomic nervous system to changes in the environment or to stressors may be different in horses versus other animals because of this difference in resting parasympathetic tone. Because food is typically withheld from horses prior to undergoing surgery, the effects of withholding of food on cardiac function are of clinical interest. Reduced parasympathetic tone after surgery may contribute to stasis of the gastrointestinal tract (ileus) and susceptibility to colic in horses.

Autonomic nervous activity is thought to affect HRV in a characteristic manner in many species of mammals. Results of analysis of HRV indices derived from the cardiovascular system in horses are of clinical relevance because they may serve as indicators of the autonomic nervous system's response to stressors. This study was designed to determine whether withholding of food affects autonomic nervous system balance by analysis of heart rate (HR), HR variability (HRV), and frequency of second-degree atrioventricular block in horses.

Objective—To determine whether withholding of food affects autonomic nervous system balance by analysis of heart rate (HR), HR variability (HRV), and frequency of second-degree atrioventricular block in horses.

Animals—5 healthy Thoroughbreds.

Procedures—For two 24-hour periods in a crossover study, food was withheld from horses or horses were maintained on their regular feeding schedule (control conditions) in their stalls and Holter monitor ECG recordings were obtained. The ECGs were analyzed by use of fast-Fourier transformation, and power spectrum densities were calculated for low-frequency (0.01 to 0.07 Hz) and high-frequency (0.07 to 0.6 Hz) variations in HR. Serum cortisol and plasma ACTH, norepinephrine, and glucose concentrations were measured at predetermined time points.

Results—Withholding of food resulted in significantly lower HR and more frequent second-degree atrioventricular block (the frequency of which was inversely related to the HR), compared with findings for control conditions. Circadian rhythms were similar during food-withholding and control conditions; peak HR was detected from 7:00 to 8:00 PM, and the lowest HR was detected in the early morning. During food-withholding conditions, the low-frequency-to-high-frequency ratio was lower than during control conditions. Serum cortisol concentration was higher and plasma glucose concentration was lower at 6:00 PM in horses when food was withheld, compared with findings during control conditions.

Conclusions and Clinical Relevance—Indices of HRV seemed to be sensitive to changes in autonomic nervous activity and may be useful as clinical indices of the neuroendocrine response to stressors in horses.
from Fourier analysis of ECG recordings suggest that parasympathetic activity is associated with HF and LF variation in HR and sympathetic activity is associated with LF variation in HR in horses. Calculation of HRV indices is a noninvasive method to determine autonomic system activity.14–16

The term stress (in its modern biological context) has been defined as “the non-specific response of the body to any demand for change” (ie, there is a single uniform response to stressors).17 This statement has been discounted by other authors.18 The neuroendocrine components of stress are thought to comprise an immediate sympathetic nervous response and longer-lasting endocrine responses (eg, cortisol and ACTH release).19

Transportation of horses that exposes them to environmental stressors causes pronounced changes in HR and HRV indices; these effects were readily detected in 1 study that investigated effects of transportation in a small (n = 3) group of horses. These effects are part of a complex group of responses, commonly termed transport stress, that are linked to transport-associated respiratory tract disease (shipping fever).16,17

The objective of the study reported here was to determine whether withholding of food for 24 hours causes changes in autonomic nervous activity as indexed by use of measurement of HR and calculation of HRV indices and whether transportation and withholding of food cause similar changes in autonomic nervous activity in horses. We hypothesized that withholding of food from horses would alter autonomic nervous activity primarily because of changes in parasympathetic tone. The hypothesis was tested by evaluating changes in HR, LF power, HF power, and the LF:HF ratio in horses from which food was withheld for 24 hours; results were compared with findings for those same horses that were fed their usual diet under otherwise identical conditions. Additionally, assays were performed to determine serum cortisol and plasma ACTH, norepinephrine, epinephrine, and glucose concentrations, which are factors associated with the neuroendocrine response to stressors.

Materials and Methods

Horses—Five healthy Thoroughbreds (4 geldings and 1 female; mean ± SE age, 7.8 ± 1.5 years; mean ± SE weight, 542 ± 14 kg) were included in the study. The horses were obtained from the University of California-Davis School of Veterinary Medicine Center for Equine Health’s research herd of horses that had been purchased or donated for use in research and teaching programs. Horses were housed in individual stalls (6 × 5 m) with shaved wood bedding and had access to pastures. For each horse, all experimental procedures were performed in its allocated stall. During the study, all horses developed occasional second-degree AVB that resolved when they were trotted on a lead for 30 seconds.

Experimental protocol—The protocol for the study was approved by the University of California-Davis Animal Use and Care Committee. Horses were acclimated to the ECG equipment for 5 days (8 h/d) prior to the experiments. Horses wore an elastic surcingle around their girth that held adhesive ECG electrodes in position. A lead electrode and the ground electrode were positioned dorsally near the epaxial muscles, and another lead electrode was positioned ventrally near the sternum; all electrodes were placed on the left side of the horses. The afternoon preceding an experiment (day 0), a 14-gauge polypropylene catheter was inserted by use of aseptic technique into a jugular vein after local anesthetization of skin with 1 mL of lidocaine.

Horses were randomly assigned to 1 of 2 initial experimental conditions: typical feeding schedule (control conditions) or withholding of food (food-withholding conditions). During control conditions, horses were fed hay at 7:00 AM and at 4:00 PM. During food-withholding conditions, food was withheld from horses from 6:00 PM on day 0 (at which time all hay was removed from stalls) until 7:00 AM on day 2. The ECGs were recorded and blood samples were collected during a 24-hour period (from 7:00 AM on day 1 to 7:00 AM on day 2). One week later, horses were assigned to undergo the other experimental condition (crossover design), and the experiment was repeated.

A blood sample (20 mL) was obtained from each horse at 5 time points during the experiments (day 1, 7:00 AM, 12:00 PM, and 6:00 PM; day 2, 12:00 AM and 6:00 AM). During collection of each blood sample, an investigator entered the stall, cleaned the catheter port with alcohol, and connected syringes to the catheter via extension set tubing; this was intended to minimize investigator contact with the horse during collection of blood samples. The investigator quietly waited for 10 minutes in the stall before each blood sample was collected; this was intended to avoid collection of blood samples immediately after stimulation of horses by the investigator, which would potentially have increased release of catecholamines. Blood (20 to 30 mL) was withdrawn through the catheter and catheter tubing into a syringe and discarded before the blood samples were obtained.

Blood samples were transferred into tubes containing EDTA and serum collection tubes that had been kept in ice (0°C to 4°C). Blood samples in EDTA-containing tubes were centrifuged (1,300 × g at 4°C for 10 minutes) immediately, and plasma was collected. Blood samples in serum collection tubes were kept at 23°C for 30 minutes until the blood clotted; samples were centrifuged (1,300 × g at 23°C for 10 minutes), and serum was collected. Plasma and serum samples were stored at −70°C until analysis.

The ECG recordings were obtained by use of a Holter-type ECG.2 The Holter monitor and surcingle were attached to each horse during the morning of day 1. During the experiment, ECGs were recorded continuously for 24 hours (from 7:00 AM on day 1 to 7:00 AM on day 2) for each horse.

Blood sample analysis—Serum cortisol and plasma ACTH, norepinephrine, and epinephrine concentrations were assayed by use of enzyme immunoassay kits.3,4 Plasma glucose concentration was determined by use of a lactate and glucose analyzer.1

HRV analysis—The ECGs were analyzed by use of an ECG processor analyzing system5 as previously described.7 The software identified R waves and calcu-
lated the R-R interval tachogram. Noise that the software identified as R waves was eliminated manually by use of visual inspection and examination of values that were outside the interval of 75% to 125% of the mean value. If the frequency of second-degree AVB was >10% of the total frequency of the HR, HRV was not analyzed for that interval. A spline curve was fit to the tachogram from which data sets of 512 points were sampled at 200-millisecond intervals with the sampling window advanced 20 seconds for each data set. These values were selected as a compromise to balance the need for a large time series for accuracy versus the need for reasonably short recording periods. Each data set was applied to a Hamming window, and a fast-Fourier transformation was performed to obtain the power spectrum density of the fluctuation. The LF power and HF power were calculated as the areas under the curve within their frequency ranges for each hour of the experiment. Frequency of the LF power was set at 0.01 to 0.07 Hz and frequency of the HF power was set at 0.07 to 0.6 Hz, as previously determined for horses. Heart rate, HRV indices (LF power and HF power), and the LF-HF ratio were determined from each recording.

**Statistical analysis**—Values were expressed as mean ± SE. Statistical comparisons for HR and HRV indices were performed by use of 2-way repeated-measures ANOVA; the factors were feeding conditions (food-withholding vs control) and time at which data were collected. Data that were not normally distributed were transformed by use of a Box-Cox power transformation. This yielded normally distributed data, except for frequency of second-degree AVB, which were extremely kurtotic and could not be normalized. Therefore, the effect of withholding of food on the frequency of second-degree AVB was evaluated by use of a nonparametric Mann-Whitney U test, and the effect of time on the frequency of second-degree AVB during food-withholding and control conditions was evaluated by use of a nonparametric Friedman repeated-measures ANOVA on ranks. Data were logarithmically transformed as needed for homoscedasticity. Least squares regression analysis was used to evaluate the relationship between HR and the frequency of second-degree AVB, and an ANCOVA was used to determine whether the slopes of the least squares linear regression lines were different. The F statistic was calculated by use of the ratio of the mean square error of the pooled model to the sum of the mean square error of the separate regressions with appropriate df.

Values of resting HR and HRV indices were compared by use of a 2-way ANOVA. Data collected during food-withholding and control conditions in the present study were compared; data from the present study were also compared with data from another study in which horses were exposed to a presumably more intense stressor (transport) than were horses in the present study. Data collected during control conditions in the present study were compared with data collected during control conditions (stall rest and normal feeding schedule) in the other study; data collected during food-withholding conditions in the present study were compared with data collected during transport in the other study.

Concentrations of plasma and serum humoral factors were compared by use of a 2-way repeated-measures ANOVA (pooled data for all time points) and a Holm-Sidak multiple comparisons procedure (data at each time point). The norepinephrine concentration in 1 plasma sample from 1 horse was estimated by use of a general linear model because the sample was lost; appropriate corrections were made to the df and sums of squares for that ANOVA. Statistical analyses were performed by use of statistical software. A value of P < 0.05 was considered significant.

**Results**

The only arrhythmia detected in the ECGs was second-degree AVB, which was identified in all horses. In all horses, second-degree AVB was mild and resolved after trotting, which was performed at times other than when data were being recorded.

The HR and frequency of second-degree AVB in horses at each time point during food-withholding and control conditions were summarized (Figure 1). Heart rate was lower during food-withholding conditions than during control conditions in horses at every (24/24) time point. The HR was significantly (P = 0.016) lower during food-withholding conditions (31.7 ± 0.5 beats/min) than during control conditions (38.2 ± 0.6 beats/min). Time was a significant (P < 0.001) factor for HR. The interaction of withholding of food and time was not significant (P = 0.526). The highest HRs were detected during the afternoon and evening, and the lowest HRs were detected during the morning.

The frequency of second-degree AVB was higher during food-withholding conditions than during control conditions in horses at 23 of 24 time points (Figure 1). The frequency of second-degree AVB was significantly (P < 0.001) higher during food-withholding conditions (76.8 ± 8.6 AVBs/h) than during control conditions (24.1 ± 2.7 AVBs/h). Time significantly affected the frequency of second-degree AVB during control conditions (P = 0.007) and during food-withholding conditions (P = 0.020). The highest frequencies of second-degree AVB were detected in the morning, and
the lowest frequencies were detected in the late afternoon and early evening (ie, diurnal variation in the frequency of second-degree AVB was inversely related to diurnal variation in HR).

For data collected during control conditions, the model predicting second-degree AVB as a function of HR ($R^2 = 0.286; P = 0.007$) had the following form: frequency of second-degree AVB (AVBs/h) = (−2.33 × HR [beats/min]) + 113 (Figure 2). For data collected during food-withholding conditions, the model predicting second-degree AVB as a function of HR ($R^2 = 0.439; P < 0.001$) had the following form: frequency of second-degree AVB (AVBs/h) = (−10.8 × HR [beats/min]) + 420. The slopes of the 2 regressions were significantly ($P < 0.001$) different. For pooled (control and food-withholding conditions) data, the model predicting frequency of second-degree AVB as a function of HR ($R^2 = 0.582; P < 0.001$) had the following form: frequency of second-degree AVB = (−7.22 × HR [beats/min]) + 303). The F statistic was not significant ($P > 0.300$), which indicated that the regression calculated from the pooled data was no worse than the regressions that were calculated separately.

Because 2 horses had second-degree AVB for > 10% of their total heart beats between 7:00 AM and 12:00 PM and between 9:00 AM and 6:00 AM, meaningful HRV indices could not be calculated for those time points. Therefore, HRV indices were compared for data collected from 1:00 PM through 8:00 PM on day 1 (Figure 3). For data determined from 1:00 PM through 8:00 PM, HR was significantly ($P < 0.001$) lower during food-withholding conditions (45.8 ± 0.1 beats/min) than during control conditions in the other study 16 (411 ± 0.6 beats/min). The LF power was significantly ($P = 0.008$) lower during control conditions in the present study (1,148 ± 23 milliseconds$^2$) than during control conditions in the other study 16 (1,567 ± 125 milliseconds$^2$). The HF power was significantly ($P = 0.006$) higher during control conditions in the present study (572 ± 11 milliseconds$^2$) than during control conditions in the other study 16 (38.8 ± 0.6 beats/min). The LF:HF ratio was significantly ($P = 0.026$) lower during control conditions in the present study (1.78 ± 0.10) than during control conditions in the other study 16 (4.73 ± 0.19).

Because data at the 1 PM time point for the transport group in the other study 16 were collected during the first hour of transport and appeared to be outliers, only data that were collected from 2:00 PM through 8:00 PM were used in comparisons of data collected during

Figure 2—Relationship between the frequency of second-degree AVB and the mean HR in the 5 horses in Figure 1 during control conditions (black circles) and during food-withholding conditions (white circles). Dashed lines represent regressions calculated for data from horses during control conditions (short dashes) and food-withholding conditions (long dashes). The solid line represents the least squares regression fit to the pooled data.

Figure 3—Mean ± SE HRV indices for LF power (A), HF power (B), and LF:HF ratio (C) from 1 PM through 8 PM in the 5 horses in Figure 1 during control conditions (black circles) and during food-withholding conditions (white circles).
Epinephrine concentration data were not determined because the assay did not function properly. For pooled data (all time points), there were no significant differences in serum cortisol and plasma ACTH, norepinephrine, and glucose concentrations in blood samples collected during food-withholding conditions versus control conditions in the present study. However, values in samples collected during food-withholding conditions and control conditions differed, albeit not significantly (plasma glucose concentration, 4.66 ± 0.13 mM/L vs 4.94 ± 0.13 mM/L, respectively [P = 0.061]; serum cortisol concentration, 4.24 ± 0.26 μg/dL vs 3.41 ± 0.27 μg/dL, respectively [P = 0.070]; and plasma norepinephrine concentration, 6.39 ± 0.76 ng/mL vs 7.02 ± 0.72 ng/mL, respectively [P = 0.089]). The interaction of withholding of food and time of sample collection was significant (P = 0.003) for plasma glucose concentration. Plasma ACTH concentration was not significantly (P = 0.637) different in blood samples collected during food-withholding conditions (8.62 ± 1.02 pg/mL) and control conditions (8.32 ± 0.69 pg/mL). Analysis of pairwise comparisons of values at each time point revealed significantly higher serum cortisol concentration and significantly lower plasma glucose concentration in samples collected during food-withholding conditions versus control conditions at 6:00 PM (Figure 4).

Discussion

The present study was conducted to determine whether withholding of food affects autonomic nervous activity in horses by use of power spectral analysis of HRV and determination of the frequency of second-degree AVB. It has been suggested that power spectral analysis of HRV is a noninvasive index of autonomic nervous activity.11,13 Studies11,13 in which blockade of the autonomic nervous system and Fourier analysis of HR power spectra were used revealed that the power spectrum of HRV in the HF power domain is primarily attributable to parasympathetic nervous activity. Sympathetic and parasympathetic nervous activities contribute to the LF power spectrum.11,12 It has been suggested that the LF:HF ratio is an index of cardiac sympathovagal balance.12,19

The diurnal pattern in HR was similar in horses during food-withholding and control conditions in the present study and during control conditions in another study.16 In the present study and in that other study,16 peak daily HR was detected between 7:00 AM and 8:00 AM, and the lowest daily HR was detected in the early morning.

In the present study, HR was significantly lower and both LF power and HF power were higher in horses during food-withholding conditions versus control conditions. These results suggest that parasympathetic nervous activity may increase and exert a more pronounced effect on cardiac function in horses during food-withholding conditions than during control conditions. The finding that the frequency of second-degree AVB was significantly higher during food-withholding conditions versus control conditions also supports this interpretation because second-degree AVB is associated with increased parasympathetic nervous activity.
activity in horses. Activity during withholding of food.

The lower LF:HF ratio during food-withholding conditions, compared with that during control conditions, may be attributable to a decrease in sympathetic nervous activity, an increase in parasympathetic nervous activity (as suggested by the higher HF power during food-withholding conditions vs control conditions), or both. Results of other studies in which sympathetic nervous activity was determined on the basis of the turnover rate of norepinephrine or the detection of sympathetic nerve activity suggest that caloric deprivation decreases sympathetic nervous activity. It is difficult to compare results of the present study with results of those other studies because different methods were used. However, there was no evidence in the present study that sympathetic nervous activity increased during withholding of food (on the basis of HR or HRV), and there was no difference in circulating concentrations of norepinephrine during food-withholding conditions versus control conditions. These results suggested that the neuroendocrine response to control conditions was stronger than the neuroendocrine response to withholding of food during the 24-hour experiment. These results may indicate that excitement associated with feeding elicited a stronger sympathetic nervous response than is generated during withholding of food or that sympathetic nervous activity may have been tempered by higher parasympathetic nervous activity during withholding of food.

Heart rate correlates with cardiac output and oxygen consumption according to the Fick principle. A positive correlation between HR and energy expenditure has been reported for several mammalian species. In the present study, HR in horses during withholding of food was significantly lower than during control conditions. This result may indicate that energy expenditure decreases during withholding of food in the horses. It has been reported that withholding of food is associated with decreases in HR and oxygen consumption and an increase in the SD of the interval between heartbeats in rats. In the present study, increased parasympathetic nervous activity may have contributed to a decreased metabolic rate in horses from which food was withheld.

It has been reported that hypoglycemia (blood glucose concentration, 2.7mM) stimulates both sympathetic and parasympathetic nervous activity in humans because hypoglycemia induced by insulin infusion increases plasma adrenaline concentration and HF power. In the present study, plasma glucose concentrations in blood samples collected from horses during food-withholding and control conditions did not differ significantly. The lowest glucose concentration (4.36 ± 0.26mM) was detected in blood samples collected during food-withholding conditions at 6 pm on day 1. Therefore, it is unlikely that autonomic nervous activity was affected by hypoglycemia in the horses, because the glucose concentrations were not less than the lower limit of the reference range (4.1mM). In horses, exposure to environmental stressors (eg, transportation) increases circulating concentrations of endocrine markers of stress, such as cortisol and ACTH. Because excitement causes release of catecholamines in horses, blood samples in the present study were obtained after a 10-minute delay during which the investigator stood quietly next to the horse. This was intended to allow catecholamine concentrations that may have transiently increased because of stimulation of the horse by the investigator to subside before collection of blood samples. This delay should have been sufficient to allow clearance of elevated circulating catecholamines because the half-lives of these catecholamines are short (<30 seconds) in horses. However, the 10-minute delay may have had a more modest effect on serum cortisol and plasma ACTH concentrations because their half-lives are longer (30 to 70 minutes) than the half-life of catecholamines in horses.

In the present study, serum cortisol concentration was significantly higher at 6:00 pm on day 1 during food-withholding conditions versus the value at the corresponding time point during control conditions; plasma glucose concentration was significantly lower during food-withholding conditions than during control conditions. The value of the LF power and the LF:HF ratio were higher at 6:00 pm than they were at 5:00 pm and were higher at 7:00 pm than they were at 6:00 pm in horses during food-withholding conditions; however, the value of the HF power was stable. This pattern is consistent with increasing sympathetic nervous activity at that time point, which may have been associated with increased circulating cortisol concentration. During food-withholding conditions, the horses might have had modest excitement and stress at that time because the duration of food withholding was sufficiently long for these horses to have become hungry. Excitement and stress may have been exacerbated in these horses when caretakers moved through the barn to feed the other horses. There was no evidence that plasma ACTH or norepinephrine concentration was affected by withholding of food.

Significant decreases in HRV indices have been reported for horses that are exposed to environmental stressors during transport. In contrast, both LF power and HF power were consistently higher during food-withholding than during control conditions in the present study. The difference in results between the present study and the other study may have caused repeated excitement in the horses in the other study, which resulted in a different pattern of HRV changes than that detected in the present study. The possibility that the ages of the horses in the present study and in the other study may have influenced the patterns of their responses cannot be ruled out. All horses in the other study were 2 years old, whereas horses in the present study ranged from 3 to 11 years old; therefore, caution is warranted in interpretation of the differences in results. Although there were many similarities in data collected during control conditions in the present study and in the other study, there was 1 notable difference. The 2 horses that had the highest frequencies of second-degree AVB in the present study were the 2 oldest horses, and all horses had some second-degree AVB. However, only 1 horse had second-degree AVB (and only during a 3-hour period) in the previous study.
In the horses in the present study, HR was significantly lower and the frequency of second-degree AVB was significantly higher during 24 hours of withholding of food, compared with findings during control conditions. An inverse relationship was detected between HR and the frequency of second-degree AVB. The LF power and the HF power were higher and the LF:HF ratio was lower in horses during food-withholding conditions versus control conditions. These patterns are consistent with an increase in parasympathetic nervous activity during withholding of food in horses. The pattern of changes detected in the HRV indices during withholding of food in the present study was different from that observed in another study in which the environmental stres sor for horses was transport.

References