# Effects of exercise intensity and duration on plasma β-endorphin concentrations in horses

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**Objective**—To determine the relationship between plasma β-endorphin (EN) concentrations and exercise intensity and duration in horses.

**Animals**—8 mares with a mean age of 6 years (range, 3 to 13 years) and mean body weight of 450 kg.

**Procedure**—Horses were exercised for 20 minutes at 60% of maximal oxygen consumption ($\dot{V}O_2$max) and to fatigue at 95% $\dot{V}O_2$max. Plasma EN concentrations were determined before exercise, after a 10-minute warmup period, after 5, 10, 15, and 20 minutes at 60% $\dot{V}O_2$max or at the point of fatigue (95% $\dot{V}O_2$max), and at regular intervals after exercise. Glucose concentrations were determined at the same times EN concentrations were measured. Plasma lactate concentration was measured 5 minutes after exercise.

**Results**—Maximum EN values were recorded 0 to 45 minutes after horses completed each test. Significant time and intensity effects on EN concentrations were detected. Concentrations were significantly higher following exercise at 95% $\dot{V}O_2$max, compared with those after 20 minutes of exercise at 60% $\dot{V}O_2$max (605.2 ± 140.6 vs 312.3 ± 53.1 pg/ml). Plasma EN concentration was not related to lactate concentration and was significantly but weakly correlated with glucose concentration for exercise at both intensities ($r$ = 0.21 and 0.30 for 60 and 95% $\dot{V}O_2$max, respectively).

**Conclusions and Clinical Relevance**—A critical exercise threshold exists for EN concentration in horses, which is 60% $\dot{V}O_2$max or less and is related to exercise intensity and duration. Even under conditions of controlled exercise there may be considerable differences in EN concentrations between horses. This makes the value of comparing horses on the basis of their EN concentration questionable. (*Am J Vet Res* 2000;61:969–973)

A number of neurotransmitters are released when nerves associated with sensations of pain or pleasure are stimulated. Among these neurotransmitters are 3 families of endogenous opioid peptides of pituitary origin: **endorphins (EN)**, enkephalins, and dynorphins.[1] These substances play a role in the response to physical and psychologic stress. Exercise is regarded as stressful, and the magnitude of stress depends on the intensity and duration of exercise and on the subject's level of fitness.[2] Frioli et al[3] found that physical stress causes release of EN into circulating blood in humans, and plasma concentrations of endogenous opioids have been reported to increase in horses following exercise.[4-6] Consequently, monitoring circulating EN concentrations may provide a means of assessing the stress associated with exercise in horses.

A reasonable amount of information exists regarding the relationship between exercise intensity and duration, and the concentration of circulating EN in humans,[7] but studies in horses have been limited in number and scope. One study of humans revealed that circulating EN concentrations are altered in an intensity- and duration-dependent manner, and that a critical threshold intensity of approximately 70% of **maximal oxygen consumption ($\dot{V}O_2$max)** is needed to increase plasma EN concentration.[8] It has also been reported by Kjaer[9] that plasma EN concentrations increased when intensity and duration of exercise were increased, yet an intensity of 55 to 60% of $\dot{V}O_2$max was required to observe any significant increase in plasma EN concentrations. Although it is presumed that a critical threshold intensity also exists in exercising horses, to our knowledge, it has yet to be identified. In humans, EN concentrations increase a maximum of 5-fold in response to exercise, whereas in horses, they have been reported to increase to 15 times the pre-exercise concentration in response to an incremental exercise test.[4] The respective effects of exercise intensity and duration on EN response to exercise in horses are not clear. Therefore, before any assessment can be made regarding the usefulness of measuring plasma EN concentration as a means of gauging stress in exercising horses, effects of exercise duration and intensity on EN concentration need clarification. The purpose of the study reported here was to investigate the roles of exercise intensity and duration on plasma concentrations of EN in horses exercising submaximally for a prolonged period, and in association with near-maximal treadmill exercise to fatigue.

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## Materials and Methods

**Horses**—Eight mares (5 Thoroughbreds and 3 Quarter Horses) ranging in age from 3 to 13 years (mean ± SEM, 5.8 ± 2.2 years) and weighing 394 to 520 kg (mean ± SEM, 449.7 ± 6.3 kg) were used. Horses were housed in outside pens, fed alfalfa hay twice daily, and provided water ad libitum. To the extent possible, we ensured that fitness of all horses was similar. Prior to the study, all horses were moderately conditioned with the same program of trotting and cantering on the treadmill for 20 minutes every other day for 1 month.

**Instrumentation**—Horses were fed as usual on the morning of the experiment, but water was withheld for 2 hours prior to exercise (from the onset of instrumentation). Local anesthesia was administered (2% lidocaine HCl,[a] SC), and a 14-gauge, 13.3-cm catheter[b] was inserted into the left jugular vein for collection of blood samples. Horses were then led on to the treadmill. Prior to exercise, a loosely fitting facemask for collection of expired gases was placed on each horse.

**Exercise protocol**—During the week prior to the start of the study, $\dot{V}O_2$max was determined by use of a standard protocol,[10] and regression equations linking oxygen consumption and treadmill speed were determined for each horse. All exercise was performed in a temperature-controlled room (21 to 23 C) on a treadmill inclined at 10%. Each horse was studied 2 times, using a crossover design, at running speeds calculated to require 60 and 95% of $\dot{V}O_2$max, respectively. After a 10-minute warmup period (5 minutes walking at 1.7 m/s followed by 5 minutes trotting at 4 m/s), horses were exercised for 20 minutes at the speed calculated to have an oxygen requirement that was 60% of $\dot{V}O_2$max, or at 95% of $\dot{V}O_2$max to fatigue. Fatigue was defined as the point at which horses could no longer match the speed of the treadmill belt, despite verbal encouragement. There were at least 10 days between each of the 2 exercise tests.

**Sample collection and analyses—Oxygen consumption ($\dot{V}O_2$)** was determined by analysis of expired gases collected via an open circuit flow-through system.[11] For the study at 60% $\dot{V}O_2$max, $\dot{V}O_2$ was determined, and blood samples were collected before exercise, after 5, 10, 15, 20, 25, and 30 minutes of exercise (including the warmup period), and 5, 10, 20, 30, 45, 60, 90, and 120 minutes following exercise. For the study at 95% $\dot{V}O_2$max, sampling times were identical except during the exercise period when blood was collected after 5 and 10 minutes (ie, warmup period) and at the end (approx 5 minutes) of exercise (ie, point of fatigue). Pre-exercise blood samples were collected with the horses standing quietly on the treadmill.

Blood was collected into dry plastic syringes and transferred to chilled vacutainer tubes[c] containing sodium fluoride and potassium oxalate for determination of plasma glucose[d] and lactate[e] concentrations (5-minute postexercise sample only) or to tubes containing 50 µl of bacitracin/ml and 1,000 units of trasylol as peptidase inhibitors for subsequent assay of EN concentrations. Plasma samples were obtained and stored at –70 C after blood was centrifuged at 1,500 × g for 10 minutes. Plasma from pre-exercise blood samples was also frozen and subsequently assayed for progesterone concentrations to assess estrous cycle status.[f]

Plasma EN concentrations were measured by use of **radioimmunoassay (RIA)** after extraction with 6 volumes of methanol; the RIA protocol has been described elsewhere.[12] Antiserum γ-10[g] was used as primary antibody. Antirabbit gammaglobulin and bovine EN were used as a second antibody and the standard, respectively. Antiserum γ-10 cross-reacts 100% with EN and 15 to 20% on a molar basis with ovine β-lipotropin. The minimum amount of EN detected was 5 to 8 pg/tube. Horse pooled plasma extracts, when run in the EN assay in different dilutions, had good parallelism between immunoreactivities in the pooled plasma extracts and EN standards (data not shown), suggesting that the assay detects EN immunoreactivity in horse plasma samples. The intra- and interassay coefficients of variation were 7 and 14%, respectively. All samples from each horse were measured together.

**Statistical analyses**—All results were expressed as mean ± SEM. Data generated before exercise, in association with the warmup period, at fatigue or after 20 minutes at 60% $\dot{V}O_2$max, and during recovery from exercise at 60% and 95% $\dot{V}O_2$max were analyzed with 2-way repeated-measures ANOVA, by use of a commercially available software program,[h] to test for effects of exercise duration and intensity. Data for all samples collected in conjunction with exercise at 60% $\dot{V}O_2$max were also analyzed by use of a 1-way repeated-measures ANOVA to further test for effects of exercise duration. Relationships between EN and glucose concentrations and EN concentrations and postexercise lactate concentrations were evaluated by use of linear regression analysis. Values of $P < 0.05$ were considered significant.

## Results

Mean $\dot{V}O_2$max was 130.3 ± 6.5 ml/kg/min. The 95% $\dot{V}O_2$max was obtained at a mean treadmill speed of 9.9 ± 0.5 m/s; time to fatigue for exercise at this intensity ranged from 3.5 to 6.4 minutes. Actual intensity of this exercise trial was 93.0 ± 1.7% $\dot{V}O_2$max. Mean treadmill speed at which 60% $\dot{V}O_2$max tests (actual intensity, 61.9 ± 2.8% $\dot{V}O_2$max) were performed was 5.6 ± 0.2 m/s. Mean EN concentration at the end of the warmup period was 158.9 ± 13.0 pg/ml; this was not significantly different from the pre-exercise mean (137.9 ± 13.4 pg/ml).

Results of progesterone assays indicated that 1 horse was in estrus. The EN concentrations in this mare were close to the mean and median values for each sampling time. Therefore, results were included with data from the other horses, rather than analyzing multiple groups of data for each exercise test.

**Tests at 95% $\dot{V}O_2$max**—Analysis of plasma EN concentrations indicated that EN concentration increased during exercise at 95% $\dot{V}O_2$max, and in some instances, into the recovery period. Pre-exercise plasma EN concentrations ranged from 93.1 to 270.1 pg/ml, whereas peak postexercise concentrations varied from 242.2 to 1,265.2 pg/ml after cessation of exercise to 10 minutes into the recovery period. In 1 horse, peak EN concentration was detected 20 minutes after exercise. Results 0 to 20 minutes after exercise were significantly greater than pre-exercise values and those recorded 45 to 120 minutes after cessation of exercise. Mean peak EN concentration was 605.7 ± 140.6 pg/ml, which represented a 4-fold increase over mean pre-exercise EN concentration **(Fig 1)**. Increases for individual horses ranged from 2 to 15.5 times their pre-exercise values. Plasma

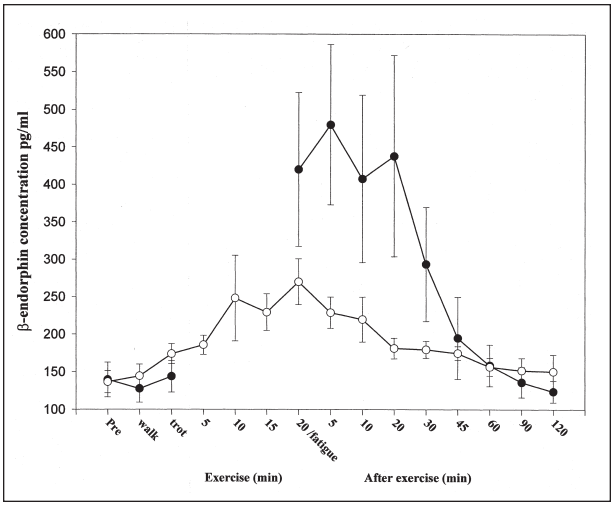


Figure 1—Plasma β-endorphin concentrations (pg/ml) measured before, during, and after exercise in 8 horses at 60% (○) and 95% (●) $\dot{V}O_2$max. Bars indicate SEM.

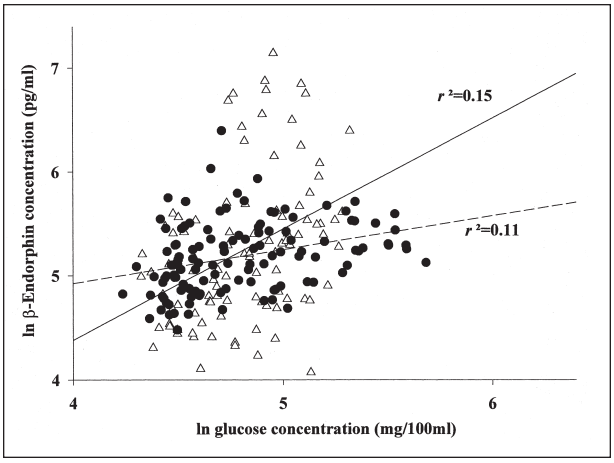


Figure 2—Correlation between plasma β-endorphin and glucose concentrations during and after exercise in 8 horses at 60% (△) and 95% (●) $\dot{V}O_2$max. The regression equation for data at 95% $\dot{V}O_2$max is lnEN = 0.097 + 1.07(lnGlucose) and is reflected by the solid line. For 60% $\dot{V}O_2$max, the regression equation is lnEN = 3.616 + 0.33(lnGlucose) and is represented by the dashed line.

EN concentrations returned to pre-exercise values 30 to 90 minutes after cessation of exercise. Plasma lactate concentration was 18.3 ± 1.8 mmol/L 5 minutes after cessation of exercise.

Pre-exercise plasma glucose concentrations ranged from 75.8 to 137.2 mg/dl. Peak concentrations ranged from 142 to 197 mg/dl and were reached after 5 to 30 minutes of recovery. These times generally coincided with peak EN concentrations. The increase from pre-exercise to peak plasma glucose concentration ranged from 1.5- to 2.6-fold for individual horses. Glucose concentrations returned to pre-exercise values more slowly (90 minutes to > 2 hours) than EN concentrations. Plasma glucose concentrations were significantly ($P$ = 0.003) but weakly correlated ($r$ = 0.30) with EN concentrations (**Fig 2**). Peak EN concentrations were not correlated with age ($r$ = 0.05) or breed.

**Tests at 60% $\dot{V}O_2$max**—Pre-exercise EN concentrations ranged from 87.9 to 223.9 pg/ml. At this exercise intensity, the highest EN concentrations ranged from 157.4 to 599.5 pg/ml and were measured during the first 10 minutes of the recovery period. Mean peak EN concentration was 312.3 ± 53.1 pg/ml. This was approximately 2.3 times greater than mean pre-exercise concentration (136.4 ± 15.7 pg/ml). Individual increases ranged from 1.5- to 4-fold. The EN concentration returned to pre-exercise values 30 to 120 minutes after cessation of exercise. Mean plasma EN concentration increased progressively during exercise. When compared with concentrations of EN measured in association with exercise at 95% $\dot{V}O_2$max, results generated at 60% $\dot{V}O_2$max did not differ significantly during exercise but were significantly lower 5 and 20 minutes after exercise. After 5 minutes of exercise at 60% $\dot{V}O_2$max, EN concentration was significantly greater than that at the end of the warmup period. Concentrations after 15 and 20 minutes were significantly higher than those recorded after 5 minutes at this intensity (Fig 1).

For tests at 60% $\dot{V}O_2$max, pre-exercise plasma glucose concentrations ranged from 89.2 to 130.5 mg/dl and peak concentrations ranged from 112.1 to 293.3 mg/dl. Overall, exercise at this intensity resulted in a near doubling of mean glucose concentration (104.6 ± 4.2 vs 172.8 ± 21.7 mg/dl). Glucose and EN concentrations were again significantly ($P$ = 0.004) related, but the correlation was also weak ($r$ = 0.21; Fig 2). Plasma glucose concentrations returned to pre-exercise values between 30 and 120 minutes after cessation of exercise.

Mean postexercise lactate concentration was 5.5 ± 1.6 mmol/L. This was significantly lower than the postexercise value for the test at 95% $\dot{V}O_2$max. A correlation between peak EN concentrations and postexercise lactate concentrations was not detected when values for each exercise test were pooled ($r$ = 0.12; $P$ = 0.64).

## Discussion

The primary objective of the study reported here was to evaluate the effects of duration and intensity of exercise on EN concentration. We found that horses working at greater relative intensities had higher plasma EN concentrations after exercise. Plasma EN concentrations increased progressively from pre-exercise values through the warmup period to the end of exercise at both 60% $\dot{V}O_2$max and 95% $\dot{V}O_2$max. Similarly, in humans, EN concentrations also increase more rapidly with exercise at higher intensities.[7] Furthermore, Art et al[4] reported that horses that reached $\dot{V}O_2$max while performing standardized incremental exercise tests had greater increases in EN concentration, compared with those that did not reach $\dot{V}O_2$max. Although relative exercise intensities were not stated, this finding also indicated that EN concentration is related to intensity, because the horses that reached $\dot{V}O_2$max would have been working harder than those horses that did not do so at the same speeds. McCarthy et al[2] demonstrated that EN concentrations at a given treadmill speed decreased in response to training, presumably because relative intensity of work at each speed also decreased as horses became more fit.

Intensity appeared to have a greater impact on EN concentration than exercise duration. However, we could not assess whether there would be any difference at the point of fatigue following exercise at different intensities, because horses did not gallop to the point of fatigue at 60% $\dot{V}O_2$max. Other work has indicated that fatigue at this intensity develops in 25 to 40 minutes.[13,14] Constant work at 60% $\dot{V}O_2$max resulted in EN concentrations that were significantly higher after 5 minutes of exercise than EN concentrations at the end of the warmup period. One study assessing EN concentrations in humans in relation to duration of exercise found EN concentrations did not increase at 60% $\dot{V}O_2$max exercise intensity. It was reported that a minimum, or threshold, intensity of 70% $\dot{V}O_2$max was required before a significant elevation in plasma EN concentration could be detected when humans exercised on a cycle ergometer for 10 minutes.[8] Furthermore, this threshold varies according to the type of exercise performed, as well as intensity and duration. In horses, although type of exercise does not vary much, it is apparent from our study that a critical intensity also exists for horses, and that it is also dependent on duration and intensity of exercise. Clearly, exercise at 95% $\dot{V}O_2$max exceeded the critical threshold in our horses. Had the horses exercised for < 5 minutes at 60% $\dot{V}O_2$max, it may have been surmised that no threshold was reached. However, we concluded that in horses exercising for 5 minutes or longer, critical threshold intensity for significant elevations in EN concentration is ≤ 60% $\dot{V}O_2$max.

It has been reported that EN concentrations may be an indicator of the stress associated with veterinary procedures in which horses are or are not restrained (eg, ear and upper lip twitching and placement of nasogastric tubes). Application of an upper lip twitch plus placement of a nasogastric tube, with the latter procedure followed by gastric infusion with saline solution, each resulted in significant increases in EN concentration.[15] Another study monitored EN concentration correlated with the use of a lip twitch, traveling in a horse trailer, and air transport. Mean resting EN concentration was 77.5 ± 9.7 pg/ml. Use of the lip twitch resulted in doubling of the resting EN concentration within 1 minute and a 5-fold increase within 5 minutes. Traveling in a horse trailer was not associated with an increase in EN concentration. In comparison, air travel induced a moderate and sustained increase in EN concentration.[6]

Individual EN concentrations varied widely in the horses in our study, particularly at 95% $\dot{V}O_2$max. It was not possible to determine if this large variation in values indicated different degrees of stress in each horse, because other specific or recognized indicators of stress were not assessed. Individual differences in other measured variables, such as heart rate or lactate concentrations that may have corroborated the impression that individual differences in EN concentration reflected different degrees of stress, were not detected. Similarly, the correlation between peak EN concentrations and postexercise lactate concentrations was weak and not significant.

It has been reported that estrus status influences EN concentrations in female rats.[12] However, change in EN concentration is primarily observed during the preovulatory period. Although 1 horse in our study had a plasma progesterone concentration that indicated it was in estrus, this had no apparent effect on EN concentration, because the EN concentrations in this horse were close to the median value for all 8 horses and were above and below results for other horses that were in anestrus or diestrus, respectively.

Catecholamines and other stress hormones (particularly cortisol) play a role in mobilization of glucose from hepatic glycogen stores and peripheral tissues. Although glucose concentrations increased in association with both exercise tests, there was little difference in concentrations measured at the 2 time periods, and correlations between glucose and EN were weak, even though they were significant. This suggests that very little of the observed hyperglycemia was directly related to increases in EN concentration, and that measurement of plasma or serum glucose concentration would be of little or no value as a means of differentiating between the stress levels of an exercise bout per se and the normal adrenergic effects of exercise.

A critical exercise threshold intensity exists for EN concentrations in horses, and this is related to exercise duration. Sizable individual differences in EN concentration were detected in the horses of this study, although horses were undergoing controlled exercise of the same relative intensities. Therefore, the basis for these considerable individual differences could not be determined.

[a]Lidocaine HCl, Elkins-Sinn, Cherry Hill, NJ.
[b]Teflon catheter, Angiocath, Becton Dickinson Deseret Medical, Sandy, Utah.
[c]Vacutainer Systems, Becton Dickinson, Rutherford, NJ.
[d]Procedure #635, Sigma Diagnostics, St Louis, Mo.
[e]YSI 2300, Yellow Springs Instruments, Yellow Springs, Ohio.
[f]Coat-A-Count Progesterone Diagnostic Products Corp, Los Angeles, Calif.
[g]Antiserum γ-10 provided by Dr SSC Yen, University of California, San Diego, Calif.
[h]Jandel Scientific Software, San Rafael, Calif.

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