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# Effect of tryptophan and of glucose on exercise capacity of horses

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**Farris, James W., Kenneth W. Hinchcliff, Kenneth H. McKeever, David R. Lamb, and Donald L. Thompson.**

Effect of tryptophan and of glucose on exercise capacity of horses. *J. Appl. Physiol.* 85(3): 807–816, 1998.—We hypothesized that central fatigue may have a role in limiting the endurance capacity of horses. Therefore, we tested the effect of infusing tryptophan and/or glucose on endurance time and plasma concentrations of free tryptophan and other substrates thought to affect tryptophan uptake into the brain of seven mares (3–4 yr of age, 353–435 kg) that ran on a treadmill at 50% of maximal O<sub>2</sub> consumption to fatigue. With use of a counterbalanced crossover design, the horses were infused with tryptophan (100 mg/kg in saline solution) or a similar volume of saline solution (placebo) before exercise. During exercise, horses received infusions of glucose (2 g/min, 50% wt/vol) or a similar volume of saline. Thus the treatments were 1) tryptophan and glucose (T & G), 2) tryptophan and placebo (T & P), 3) placebo and glucose (P & G), and 4) placebo and placebo (P & P). Mean heart rate, hematocrit, and concentration of plasma total solids before and during exercise were similar for all trials. Mean time to exhaustion was reduced ( $P < 0.05$ ) for T & P and T & G compared with P & P [86.1 ± 6.9 and 87.1 ± 6.8 vs. 102.3 ± 10.3 (SE) min], whereas endurance for P & G (122.4 ± 11.9 min) was greater than for all other trials ( $P < 0.05$ ). Compared with nontryptophan trials, during the tryptophan trials plasma prolactin increased ( $P < 0.05$ ) nearly threefold before exercise and almost twofold early in exercise. Muscle glycogen concentrations were reduced ( $P < 0.05$ ) below preexercise values in the P & G and P & P trials only. However, glucose infusions (P & G) did not affect ( $P > 0.05$ ) concentrations of plasma free fatty acids or ratios of branched-chain amino acids to free tryptophan. In conclusion, tryptophan infusion reduced endurance time, which was consistent with the central fatigue hypothesis. The failure of glucose infusion to alleviate the effects of tryptophan and the absence of significant muscle glycogen reduction in the tryptophan trials suggest that the early onset of fatigue in the tryptophan trials is not due to a lack of readily available substrate.

central fatigue; oxygen consumption; serotonin; prolactin; branched-chain amino acids

FATIGUE DURING PROLONGED exercise may be of central origin and involve an increase in brain serotonin. According to Newsholme et al. (34), the events leading to an exercise-induced increase in brain serotonin concentration are as follows: exercise produces an increase in plasma nonesterified fatty acid (NEFA) concentration; the increased NEFA displace tryptophan molecules from their binding sites on plasma albumin (12), resulting in an increase in the plasma concentration of unbound or free tryptophan (fTrp);

during exercise, muscle uptake of branched-chain amino acids (BCAA) lowers plasma BCAA concentrations; the increase in fTrp coupled with the decrease in BCAA leads to enhanced transport of tryptophan across the blood-brain barrier, because fTrp and BCAA compete for the same brain transport mechanism; the increased availability of brain tryptophan provides substrate for increased synthesis and possible release of serotonin; increased serotonergic activity in the brain may reduce motor drive, thus contributing to overall fatigue. In humans, glucose supplementation late in exercise delays the increase of NEFA and fTrp and increases endurance (13). We previously demonstrated that infusion of glucose during submaximal treadmill exercise delays the onset of fatigue in Standardbred mares (17). Thus we hypothesized that central fatigue may limit endurance capacity in horses and that by manipulating fTrp and BCAA we might predictably alter equine exercise capacity.

We tested this hypothesis by infusing tryptophan and/or glucose before and/or during exercise as a means of altering plasma fTrp and BCAA and measured the resulting changes in exercise time to fatigue and in plasma concentrations of free amino acids, NEFA, glucose, and prolactin. Plasma prolactin concentration was used as a minimally invasive indirect marker of altered central serotonin/dopamine concentrations (25, 26, 42). In humans an increased concentration of plasma fTrp, when given intravenously (6, 22), when consumed orally with water (50), or when induced by prolonged exercise (43), is associated with an increased plasma prolactin concentration. Increases in plasma fTrp during strenuous bicycle ergometer exercise are also positively correlated with plasma prolactin (20). Furthermore, ketanserin, a 5-hydroxytryptamine receptor (5-HT<sub>2</sub>) antagonist, reduces the prolactin response after graded maximal cycle ergometer tests (15). Also, prolactin is released in a dose-response manner after treatment with serotonin, 5-hydroxytryptophan, fluoxetine (serotonin reuptake inhibitor), and 1-(*m*-chlorophenyl)piperazine, a serotonin 1c-specific receptor agonist (25, 26, 48). Therefore, plasma prolactin concentrations appeared to be an appropriate marker of central serotonin activity.

In horses the response of prolactin to tryptophan infusion was previously undetermined, but plasma prolactin concentrations are increased in horses subjected to exercise or stress (9). Thus we hypothesized that tryptophan infusions would increase fTrp and the fTrp-to-BCAA ratio in plasma and would decrease treadmill endurance in horses, whereas we predicted

that glucose infusions would decrease NEFA, fTrp, and the fTrp-to-BCAA ratio in plasma and would improve endurance.

## MATERIALS AND METHODS

### *Experimental Design*

The effects of combinations of tryptophan and glucose infusions were examined in a four-way crossover study. Seven clinically normal mares ranging in age from 3 to 4 yr and in body weight from 361 to 448 kg were used. Left carotid arteries of all the horses were surgically relocated to a subcutaneous position  $\geq 1$  yr before the study. The horses were accustomed to running on the treadmill but were otherwise untrained. Each horse was fasted for 24 h on five separate occasions, four of which were experimental exercise trials and one of which was a nonexercise control trial. The order of treatment in exercise trials was randomized and counterbalanced. This experiment was approved by the Laboratory Animal Care and Use Committee of The Ohio State University.

### *Determination of Maximal and Relative $O_2$ Consumption*

Incremental exercise tests were done 2 wk before the study was begun to determine the maximal rate of  $O_2$  consumption ( $\dot{V}O_{2\max}$ ) and the treadmill speed representing 50% of  $\dot{V}O_{2\max}$  of each horse.  $O_2$  uptake was measured using an open-circuit calorimetry system (Oxymax X-L, Columbus Instruments, Columbus, OH) that was calibrated before each use with certified gases (24). After a 10-min control period during which the animal stood quietly on a treadmill, each horse underwent the maximal exercise test. The horse ran on the treadmill up a 2% grade at an initial speed of 3 m/s; the speed increased 2 m/s every 1.5 min up to 9 m/s, then the treadmill grade was increased to 7% and speed was increased by 1 m/s every 1.5 min until  $O_2$  consumption failed to increase with further increases in speed ( $\dot{V}O_{2\max}$ ). After the  $\dot{V}O_{2\max}$  test, horses were rested for 30 min, and  $O_2$  consumption was measured while each horse ran on a 2% grade at the speed calculated to elicit 40% of  $\dot{V}O_{2\max}$ . After 5 min at this pace, speed was increased 0.25 m/s every 5 min until  $O_2$  consumption was 50% of  $\dot{V}O_{2\max}$ . This treadmill speed was recorded and used for all subsequent experimental trials.

### *Exercise and Diet Control*

Exercise and diet were controlled for 3 days and 1 day, respectively, before each exercise trial in an attempt to minimize intertrial variability in hepatic and intramuscular carbohydrate stores. Horses ran at 50% of  $\dot{V}O_{2\max}$  for 20 min 3 and 2 days before each experimental trial. After each 20-min run, horses were given 0.9 kg of feed corn and returned to pasture. Food and water were provided normally until 24 h before the experimental trial, when horses were placed in stalls and given free access to water but were not fed until after the trial. Horses were weighed to the nearest 0.1 kg before and after each trial.

### *Instrumentation*

Before each trial an indwelling catheter (14-gauge, 6.5-in., Angiocath, Deseret, Sandy, UT) was placed percutaneously into the left jugular vein and another into the right jugular vein. Venous blood samples were drawn from an extension set attached to the catheter in the right jugular vein. Arterial blood samples were drawn from a catheter (18-gauge, Angiocath) placed percutaneously into the subcutaneously relo-

cated left carotid artery. Heart rate was obtained from the arterial pressure waveform generated via a pressure transducer (model P23, Gould, Cleveland, OH) connected to a fluid-filled line attached to this same arterial catheter. The waveform was recorded for 30 s at the end of each sampling interval on a hemodynamic recorder (model VR-12, PPG Biomedical Instruments, Pittsburgh, PA).

### *Treatment Infusions*

In each trial the horses received two infusions: one before exercise and another during exercise. Combinations of these two infusions, as described below, were the treatment conditions for the experimental trials. Before exercise, 4.0 liters of a sterile electrolyte solution containing 100 mg/kg body wt of tryptophan (catalog no. BP395-100, Fisher Scientific, Pittsburgh, PA) (36) or the vehicle solution only (placebo) were infused into the catheter in the right jugular vein through an intravenous drip apparatus pressurized by a peristaltic pump. Preexercise infusions were delivered in 15–20 min. During exercise, glucose (50% wt/vol) or normal saline (placebo) was continuously infused from the onset of exercise at a rate of 4.0 ml/min until fatigue. Each horse participated in four exercise trials and randomly received each of the following treatment combinations: 1) tryptophan (100 mg/kg body wt) before exercise plus placebo while running (T & P), 2) placebo before exercise plus glucose (2 g/min in 50% wt/vol solution) while running (P & G), 3) tryptophan before exercise plus glucose while running (T & G), and 4) placebo before exercise plus placebo while running (P & P). The total volume of infusate delivered during exercise depended on the duration of exercise.

### *Exercise and Control Trials*

During the four experimental trials, horses underwent treadmill exercise to fatigue at 50% of  $\dot{V}O_{2\max}$ . Each horse was subjected to four experimental trials, with treatments as described above, and one parallel control trial in which no treatment was given and horses stood on the treadmill for 2–2.5 h. All experiments were performed in a climate-controlled room in which the temperature was maintained at  $\sim 23^\circ\text{C}$  and the relative humidity at  $\sim 68\%$ . Fans were located in front of and on each side of the horse during exercise to optimize convective and evaporative cooling. Horses were assigned morning or afternoon starting times for all trials. Morning trials began between 1000 and 1100, and afternoon trials began between 1300 and 1400. Each horse started at the same time of day for each of the trials.

Horses ran at 50% of  $\dot{V}O_{2\max}$ , determined as described above, at a 2% grade until fatigue. Fatigue was defined as the time at which the horse could no longer maintain the required pace on the treadmill, despite vigorous humane encouragement. The times to fatigue for all experimental trials were determined by the same individual, who was blinded to the treatment.

### *Muscle Biopsies and Muscle Glycogen Analysis*

Samples of the medial gluteal muscle were obtained before exercise from the left side and taken after exercise from the right side according to previously published methods (27). Briefly, under sterile conditions and local anesthesia, an incision was made through the skin over the muscle with a scalpel blade. The muscle fascia was nicked with the scalpel blade, the biopsy needle was inserted through the opening, and under suction from a 60-ml syringe the biopsy sample of  $\sim 50$  mg was obtained. The incision was then sutured closed, and antibiotic ointment was applied to the wound.

Muscle samples were prepared and analyzed according to the methods of Fink and Costill (19). Briefly, the samples were frozen in liquid nitrogen rapidly after collection and stored at  $-80^{\circ}\text{C}$  until analysis. Samples were analyzed for glycogen using acid incubation of the muscle followed by neutralization and subsequent analysis of glucose residues using the hexokinase/glucose-6-phosphate dehydrogenase reaction and fluorometric measurement of the NADPH produced (28).

*Blood Sample Collection and Analysis*

Venous (17 ml) and arterial (28 ml) blood samples were taken at the following times: after instrumentation but before the preexercise infusion (rest), after the preexercise infusion when the horse had been standing quietly on the treadmill for 10 min (preexercise), after 5 min of exercise, after 15 min of exercise and every 15 min thereafter until fatigue, and at fatigue. Venous blood was collected in a plastic syringe and immediately placed into ice-chilled evacuated glass tubes (Vacutainer; Becton Dickinson, Parsippany, NJ) containing the anticoagulant EDTA. Venous samples were used for measurement of plasma concentrations of prolactin and total solids and for determination of hematocrit. Arterial blood was collected in a plastic syringe and immediately placed into evacuated tubes as follows: for glucose analysis, a tube containing sodium fluoride and potassium oxalate (Vacutainer), and for analysis of NEFA and amino acids, a tube containing EDTA (Vacutainer). All tubes from each collection time were centrifuged together in a refrigerated centrifuge (1,600 *g* for 15 min at  $2^{\circ}\text{C}$ ). After centrifugation, aliquots of plasma were distributed among plastic cryogenic storage tubes, rapidly frozen on dry ice, and stored at  $-80^{\circ}\text{C}$  until analysis.

*Hematocrit and total protein in venous plasma.* Hematocrit of venous blood was determined in triplicate using a microhematocrit centrifuge. Plasma total protein concentrations were estimated by refractometry (Cambridge Instruments).

*Plasma volume change.* The percent change in plasma volume during exercise was calculated using the change in plasma total protein concentration from a "reference" sample collected at 5 min of exercise (32). Plasma total protein concentrations were measured by refractometer using the EDTA-anticoagulated plasma. All blood chemistry data, except for the preexercise data, were corrected for percent changes in plasma volume.

*Arterial plasma glucose.* Plasma glucose concentration was determined in duplicate using a glucose analyzer (model 23L, Yellow Springs Instruments, Yellow Springs, OH). The analyzer was calibrated before each use, and calibration was checked against known standards before each sample was measured. All samples from a given horse were measured on the same day using the same batch of stock buffers and standards. The intra- and interassay coefficients of variation for this method were  $<1\%$ .

*Free amino acid concentrations in arterial plasma.* Additional preparation of arterial plasma was necessary for amino acid analysis. With use of a calibrated pipette, 350  $\mu\text{l}$  of the internal standard, i.e., methionine sulfone (0.4 mM in 0.1 M HCl), and 350  $\mu\text{l}$  of arterial plasma were transferred into a polypropylene tube and quickly vortexed. Then 350  $\mu\text{l}$  of this mixture were transferred into a microcentrifuge capsule (10,000-mol wt cutoff) and centrifuged at  $4^{\circ}\text{C}$  for 20 min to obtain protein-free filtrate. The resultant filtrate was immediately frozen on dry ice and stored at  $-80^{\circ}\text{C}$  until analysis. Arterial plasma free amino acid concentrations were measured using the "PICO-TAG" method developed by Waters (8). Briefly, this method involves precolumn derivatization of the amino acids in the sample with phenylisothiocyanate followed by reverse-phase HPLC separation using a two-solvent gradient and fluorometric detection at a wavelength of 254 nm. The system components for derivatization and HPLC separation of fTrp were obtained from Waters (manual number 88140, rev. 3, Waters Chromatography Div., Millipore, Milford, MA). Amino acid peaks were computer integrated, and concentration was determined from the area under the response peak compared with the internal standard methionine sulfone. Plasma samples from all seven horses were analyzed for fTrp, leucine, isoleucine, and valine during the P & G and P & P trials only. Intra-assay ( $n = 10$ ) and interassay ( $n = 20$ ) coefficients of variation for fTrp, leucine, isoleucine, and valine were  $<2.6$ ,  $<6.6$ ,  $<7.6$ , and  $<2.6\%$ , respectively. Pilot experiments determined that there was a large prolonged increase in plasma fTrp concentration in three stationary and running horses in response to tryptophan infusion (Fig. 1). Therefore, amino acid concentrations were not measured during the T & P and T & G trials.

*Arterial plasma NEFA.* Plasma NEFA were determined using a commercial kit that utilizes an in vitro enzymatic colorimetric method (Wako Chemicals, Dallas, TX). The assay was modified for use with a microplate reader. All reagents

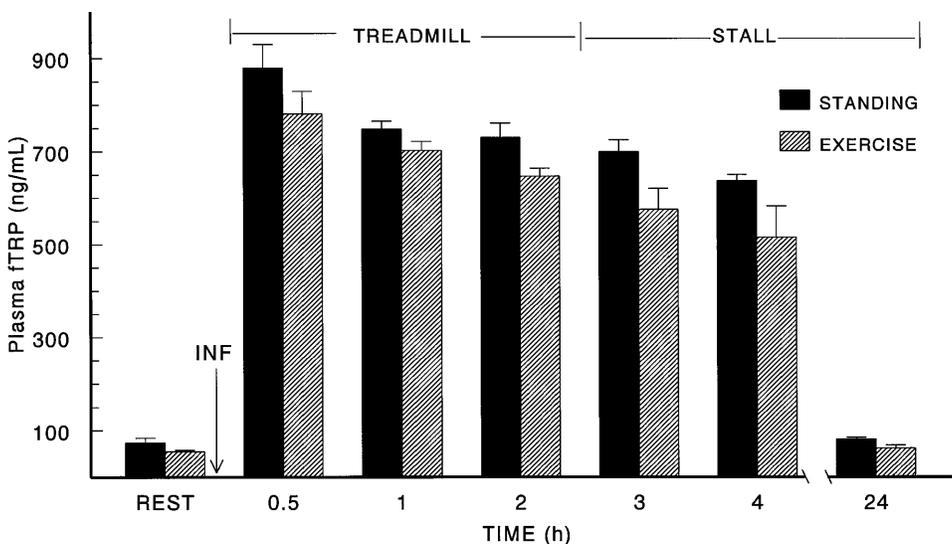


Fig. 1. Plasma free tryptophan (fTrp) concentration (mean  $\pm$  SE) before (Rest) and after a 30-min infusion of tryptophan (100 mg/kg body wt in 4 liters of lactated Ringer solution, Inf). Horses ( $n = 3$ ) initially stood or began running on a treadmill 30 min after infusion and were returned to their stalls 2.5 h after infusion, where subsequent samples were taken. In exercise trial, horses ran for 90 min at 50% of maximal  $\text{O}_2$  consumption.

were diluted exactly as specified in the kit. Working standards were made from the stock standard each day and used to make a standard curve for each plate. With use of a calibrated pipette, 10  $\mu$ l of standard, sample, or saline (blank) were transferred into appropriate wells. Then 50  $\mu$ l of reagent A were distributed into the wells, and the plate was lightly agitated for mixing purposes. The microplate was then incubated for 10 min at 37°C and 100% relative humidity. The microplate was removed, 100  $\mu$ l of reagent B were added, and the plate was gently agitated and then incubated for another 10 min at 37°C and 100% relative humidity. Then the plate was removed, allowed to cool for 2 min at room temperature, and immediately read at 550 nm on a microplate reader. The microplate wells were read against the "blank" wells, and unknowns were calculated using a linear regression based on absorbance of the known standards. Intra- and interassay coefficients of variation for this method were <3.8 and 5.4%, respectively. Parallelism for diluted samples was demonstrated ( $r = 0.97$ ).

**Prolactin in venous plasma.** Venous plasma concentrations of prolactin were determined using RIA methods (9, 46). All samples from individual horses were processed in the same batch. Intra- and interassay coefficients of variation for this method were <3%. Pilot experiments confirmed a marked plasma prolactin response to tryptophan infusion (Fig. 2).

#### Statistical Analysis

Treadmill endurance times were analyzed using a repeated-measures ANOVA with the factors being treatment, time, and order of treatment. Significant differences between treatment means ( $P < 0.05$ ) were located using Student-Newman-Keuls post hoc tests.

The data for all other dependent measures were analyzed using a two-way ANOVA for repeated measures with treatment and time as independent factors. Student-Newman-Keuls tests were used to locate significant differences between means ( $P < 0.05$ ). This analysis was confined to the data collection times from preexercise to 45 min of exercise and at fatigue, inasmuch as these times were common to treatment effects in all trials.

Student-Newman-Keuls tests were used to locate significant differences between means ( $P < 0.05$ ). Pearson product-moment correlation coefficients were calculated to determine

the strength of linear relationships between time to fatigue and muscle glycogen concentration.

## RESULTS

### $\dot{V}O_{2\max}$ and Speed at 50% of $\dot{V}O_{2\max}$

Individual  $\dot{V}O_{2\max}$  values ranged from 107 to 138  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , and treadmill speed eliciting 50% of  $\dot{V}O_{2\max}$  ranged from 4.8 to 6.7 m/s.

### Exercise Time to Fatigue

There was a significant effect of treatment on time to fatigue. Exercise durations at 50% of  $\dot{V}O_{2\max}$  for the P & G, P & P, T & G, and T & P trials were  $122.4 \pm 11.9$ ,  $102.3 \pm 10.3$ ,  $87.1 \pm 6.8$ , and  $86.1 \pm 6.9$  (SE) min, respectively. Exercise duration was significantly longer for the P & G trial than for the other three exercise trials. Also, horses ran significantly longer in the P & P trial than in the T & P or the T & G trial. Times to fatigue in the T & P and T & G trials were not significantly different.

### Body Weight, Heart Rate, Hematocrit, and Plasma Total Protein Concentration

Body weights significantly decreased from  $398 \pm 9$  kg before exercise to  $380 \pm 10$  kg in all exercise trials. There was no significant difference in weight loss between treatments. Heart rate at rest was  $30 \pm 2$  beats/min, increased ( $P < 0.05$ ) to  $131 \pm 5$  beats/min at 5 min of exercise, and was  $139 \pm 4$  beats/min at fatigue. Heart rate was not significantly different between trials. Hematocrit at rest was  $31 \pm 2\%$ , and increased ( $P < 0.05$ ) to  $45 \pm 2\%$  at 5 min of exercise, stabilized near 45% through 45 min of exercise, and then was further elevated ( $P < 0.05$ ) to just >48% at fatigue. Hematocrit was similar for all exercise trials. Plasma total protein concentration before exercise was 6.8 g/dl

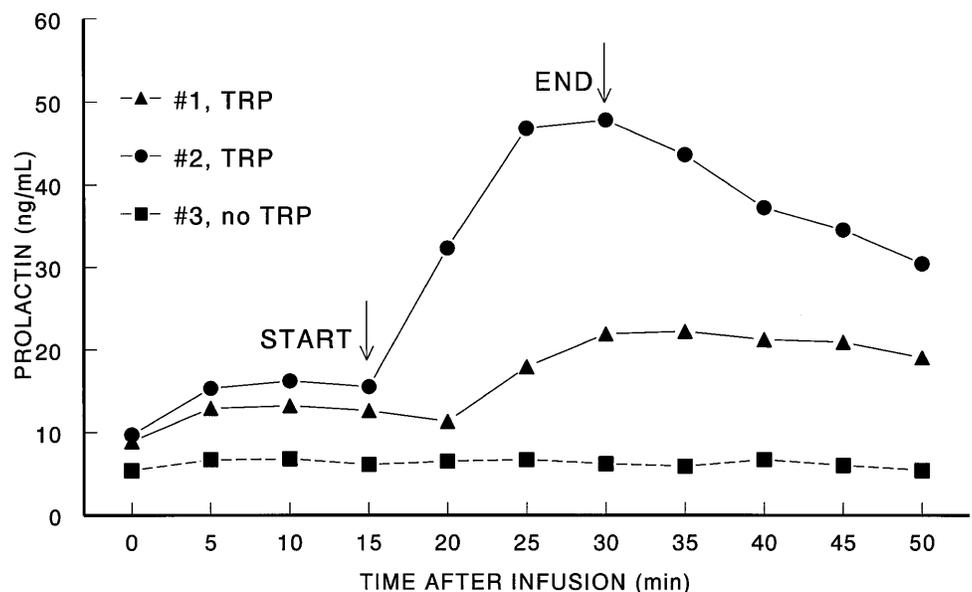


Fig. 2. Plasma prolactin concentration in 3 horses before, during, and after 15 min of exercise after infusion of tryptophan (TRP, 100 mg/kg in saline solution). First plasma sample was taken immediately after infusion. Exercise occurred between start and end arrows for all horses. Horses 1 and 2 received TRP; horse 3 received only vehicle solution.

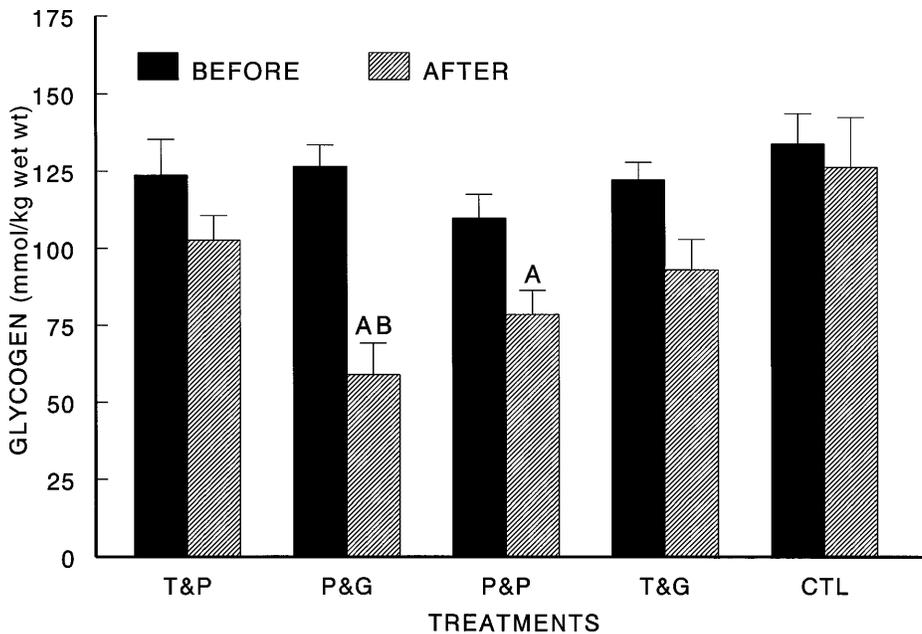


Fig. 3. Muscle glycogen in 6 horses before and after 4 exercise trials and a standing control trial. T & P, TRP infusion and placebo; P & G, placebo and glucose; P & P, placebo and placebo; T & G, TRP and glucose; CTL, control (horses stood on treadmill, with no exercise or infusion for duration equal to longest exercise trial). <sup>A</sup>Less than before exercise ( $P < 0.05$ ). <sup>B</sup>Different from corresponding value after exercise in T & P and T & G trials ( $P < 0.05$ ).

before exercise and 7.8 g/dl at fatigue. Plasma total protein concentration was similar for all trials.

*Muscle Glycogen*

Preexercise muscle glycogen concentrations were similar for all trials (Fig. 3). Postexercise muscle glycogen concentrations were not significantly decreased below preexercise values in the T & P and T & G trials. Although muscle glycogen concentrations decreased significantly in the P & G and P & P trials after exercise, the mean muscle glycogen concentration after exercise was significantly lower in the P & G trial than in the T & P and T & G trials. In the P & G and P & P trials, when tryptophan was not infused, postexercise muscle glycogen was correlated to exercise duration ( $r = -0.654, P = 0.01$ ). Additionally, there was a significant correlation ( $r = 0.451, P = 0.02$ ) between the exercise-induced changes in muscle glycogen concentration and exercise duration for all trials.

*Plasma Glucose*

At each timed collection period, plasma glucose concentrations for the P & G and T & G trials were similar to each other, as were concentrations for the T & P and P & P trials (Fig. 4). Plasma glucose concentrations before exercise were similar for all trials. Plasma glucose concentrations in the P & G and T & G trials remained elevated above those in the T & P and P & P trials from 30 min until fatigue. In the T & P and P & P trials, glucose concentrations were similar to preexercise levels throughout exercise.

*Plasma NEFA*

There was a significant main effect for treatment on plasma NEFA concentration; the average NEFA concentration was greater for the P & P trial than for the T & P and T & G trials (Fig. 4). The P & G trial was not different from the other three trials. There was also a

significant main effect for time, but no treatment-by-time interaction. In all exercise trials, NEFA concentrations fell from preexercise levels at 5 min, increased slightly by 30 min, and were significantly elevated above preexercise levels at fatigue (Fig. 4).

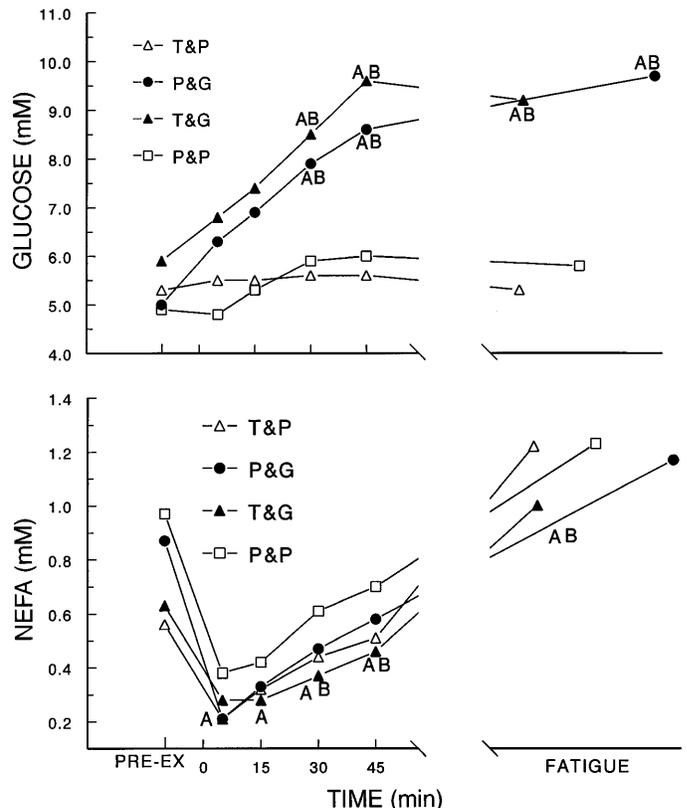


Fig. 4. Plasma glucose and nonesterified fatty acid (NEFA) concentrations in 6 horses. Pre-ex, before exercise. For glucose: <sup>A</sup>greater than Pre-ex ( $P < 0.05$ ); <sup>B</sup>greater than T & P and P & P trials ( $P < 0.05$ ). For NEFA: <sup>A</sup>all trials are lower than corresponding Pre-ex, except at fatigue, where values are greater than corresponding Pre-ex ( $P < 0.05$ ); <sup>B</sup>all trials greater than corresponding 5-min value ( $P < 0.05$ ).

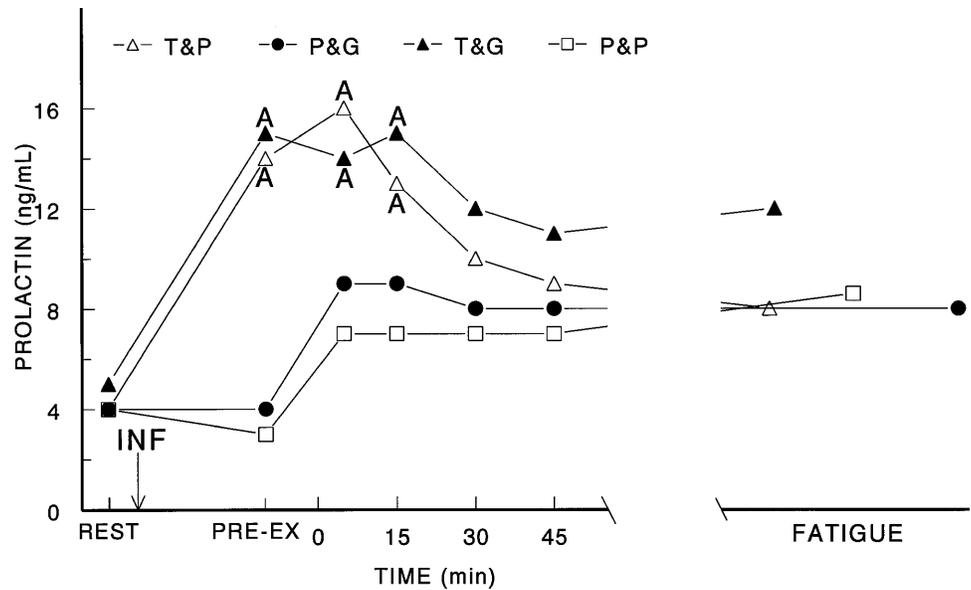


Fig. 5. Plasma prolactin concentration in 6 horses. <sup>A</sup>Greater than P & P and P & G trials at corresponding time points ( $P < 0.05$ ).

#### Prolactin Concentration in Venous Plasma

Plasma prolactin concentrations for the T & P and T & G trials were similar at all times, as were concentrations for the P & G and P & P trials (Fig. 5). Plasma prolactin concentrations before exercise were greater in the T & P and T & G trials ( $14.4 \pm 3.5$  and  $16.1 \pm 3.1$  ng/ml, respectively) than in the P & G and P & P trials ( $4.4 \pm 0.6$  and  $3.14 \pm 0.6$  ng/ml, respectively). This pattern was maintained during the first 15 min of exercise, but by 30 min prolactin concentrations converged among treatments and were not significantly different for the remainder of the trials.

#### Plasma Amino Acids

Exercise increased plasma fTrp concentration in the P & G and P & P trials (Table 1). There were no significant changes in the P & G and P & P trials for plasma concentrations of valine, leucine, or isoleucine during exercise (Table 1) but the fTrp-to-BCAA ratio was significantly increased above preexercise baseline by 15 min of exercise and remained elevated throughout exercise for P & G and P & P trials (Fig. 6). Also the concentrations of fTrp, valine, leucine, isoleucine, and the fTrp-to-BCAA ratio during the P & G and P & P

trials were not affected by the infusion of glucose compared with placebo during exercise.

#### DISCUSSION

The results from this study demonstrate that tryptophan infusion before exercise reduced exercise time to fatigue and confirm our previous findings that glucose infusions during prolonged submaximal treadmill running increase exercise duration in the horse (17). Additionally, the tryptophan-induced decrease in exercise duration occurred even though muscle glycogen stores were not significantly reduced at fatigue and blood glucose concentrations had not declined during exercise. However, glucose infusion did not attenuate increases in plasma NEFA, fTrp, or prolactin in response to exercise. Therefore, unlike the results observed in humans during submaximal cycle ergometer exercise (13), glucose supplementation in horses did not elicit the expected metabolic changes that would have been consistent with the central fatigue hypothesis.

The pilot studies demonstrated that the tryptophan infusion would maintain plasma fTrp at concentrations at least ninefold greater than resting levels for the time that horses ran during the exercise trials. Pilot work in

Table 1. Plasma isoleucine, leucine, valine, and free tryptophan before and after exercise and before fatigue in horses infused with glucose and with a saline solution

Time, min	Isoleucine		Leucine		Valine		Free Tryptophan	
	G	P	G	P	G	P	G	P
0	105 ± 7	118 ± 8	133 ± 15	142 ± 15	185 ± 9	195 ± 14	39 ± 4	41 ± 2
15	114 ± 10	114 ± 6	135 ± 7	131 ± 9	194 ± 9	193 ± 10	52 ± 5	49 ± 4
30	110 ± 10	123 ± 12	145 ± 23	139 ± 9	207 ± 22	209 ± 12	55 ± 7	56 ± 4
F - 24	106 ± 11	114 ± 13	147 ± 28	112 ± 4	187 ± 11	197 ± 12	53 ± 4	54 ± 5
F - 9	96 ± 14	103 ± 9	136 ± 21	123 ± 8	202 ± 30	200 ± 12	55 ± 6	52 ± 3

Values are means ± SE of 6 horses, expressed in nmol/l. Horses were infused with glucose (G, P & G trial) on one occasion and with a saline solution (P, P & P trial) on another occasion, and values were measured before (0) and after 15 and 30 min of exercise and at 24 (F - 24) and 9 (F - 9) min before fatigue.

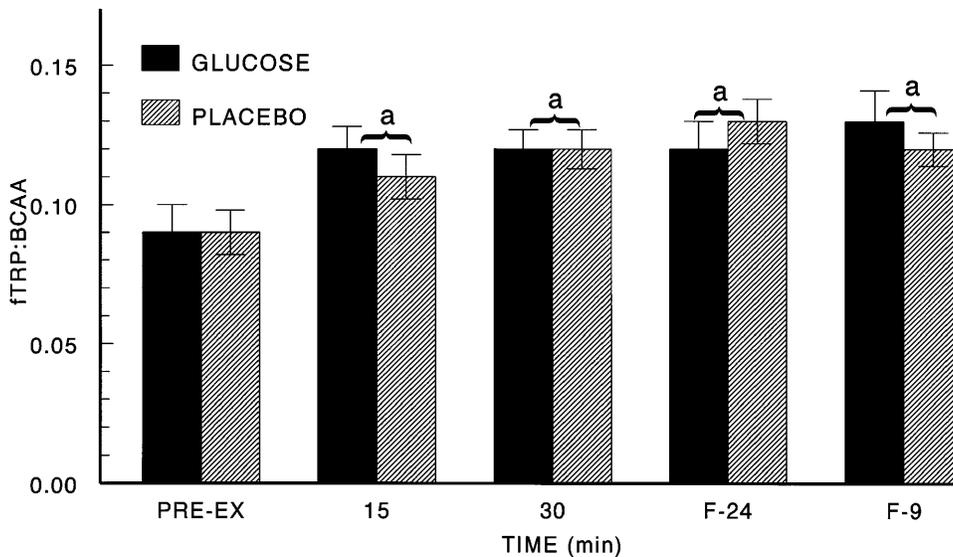


Fig. 6. Ratio of fTrp to branched-chain amino acids (BCAA) in 6 horses after P & G or P & P trial. F - 24 and F - 9, ~24 and 9 min before fatigue. <sup>a</sup>Both trials greater than corresponding Pre-ex.

two horses also demonstrated that tryptophan infusion increased plasma prolactin concentration by 30–60% before exercise began and by 100–400% after 30 min of exercise. Furthermore, during the actual experimental trials, plasma prolactin concentration increased by ~200% before and during the first 15 min of exercise after tryptophan infusion, whereas only a nonsignificant increase in prolactin concentration occurred throughout exercise when the vehicle solution was infused. The increase in prolactin concentration when tryptophan was infused is consistent with results from other species (6, 22, 43). Because increased tryptophan availability raises brain serotonin concentration (5) and a rise in brain serotonin is associated with prolactin release (25, 26, 48), it is tempting to postulate that the increased prolactin concentrations in the tryptophan trials (T & G and T & P) implicate serotonin as the link in the early onset of fatigue in these trials. However, the fact that prolactin concentrations were not significantly different among treatments from 30 min to fatigue could mean that brain serotonin concentrations were also similar in all groups after 30 min of exercise or that other factors such as vasoactive intestinal peptide (VIP), thyrotropin-releasing hormone (TRH), endogenous opiate peptides (33, 45), or dopamine were modulating prolactin release and inhibition. Even though there is much evidence relating serotonin activity to prolactin release, serotonin may act indirectly through VIP and TRH, both of which directly stimulate prolactin release from the anterior pituitary (7, 40), with VIP appearing to have more of a direct effect than TRH (49). However, VIP activity has been dissociated from prolactin release during exercise (37) and after consumption of meals with different macronutrient contents (23). In contrast to prolactin secretagogues, increased activities of central dopamine (7, 21) and dopamine agonists (14) inhibit prolactin secretion. Further work with serotonin and/or dopamine antagonists might clarify the relationship of tryptophan to fatigue in similar studies.

The muscle glycogen and plasma glucose results are consistent with the possibility of a central component of fatigue in this study. Preexercise muscle glycogen concentrations were similar in all trials, indicating that the exercise and diet controls effectively prevented intertrial variations that could have confounded exercise performance results (39). Even though fatigue occurred earliest in the T & P and T & G trials, plasma glucose concentrations during these trials were stable throughout exercise, and postexercise muscle glycogen concentration was not significantly reduced below preexercise levels in these trials. Therefore, it is unlikely that insufficient glucose or muscle glycogen constituted significant components of fatigue in the T & P and T & G trials. Conversely, for the longer-duration P & G and P & P trials when tryptophan was not infused, muscle glycogen availability was related to fatigue. While investigating the relationship of central serotonin alterations to the early onset of fatigue, Bailey et al. (3) demonstrated that muscle glycogen concentration was significantly greater after exhaustive treadmill running in rats given a serotonin agonist than in rats given the placebo or serotonin antagonist (3). Also, the rats given a serotonin antagonist and those given the placebo had similar muscle glycogen concentrations at exhaustion. Therefore, the muscle glycogen results in these horses are similar to those in the rats in the study by Bailey et al.

As indicated by the apparently greater utilization of glycogen during the P & G than during the P & P trial, the infusion of glucose during exercise did not seem to spare muscle glycogen utilization. This result is consistent with most data obtained from humans during prolonged cycle ergometer exercise to fatigue (10) and suggests that the additional plasma glucose available in the P & G trial allowed for greater carbohydrate oxidation to help meet the energy demands of exercise and delay the onset of fatigue (11). The absence of a decrease in blood glucose concentration in any treatment may be explained by the faster running speeds

(4.8–6.7 m/s) of these horses compared with the 3.5–4.5 m/s speeds and longer endurance times of horses in field studies that documented hypoglycemia in horses (29, 30, 38, 41).

The concentrations of plasma NEFA were not significantly different between the four treatments at any of the time points measured. If the hypothesis of News-holme et al. (34) regarding the metabolic control of exercise-induced fatigue were to be supported by our data in horses, a greater plasma NEFA concentration should have been observed late in exercise trials under placebo conditions than in exercise trials when glucose was infused. The lack of a glucose treatment effect on NEFA is difficult to explain. Compared with euglycemia, hyperglycemia in humans reduces NEFA and glycerol turnover by 30%, independent of concentrations of glucoregulatory hormones (4), and can prevent or delay the increase of plasma free fatty acids during cycle ergometer exercise (11, 13, 51). This decreased NEFA response is presumably mediated by the suppression of lipolysis that is related to increased plasma concentrations of insulin and glucose (4). However, when carbohydrate is infused into humans (47) or ingested by humans (51) during submaximal exercise that is preceded by a period of fasting, the NEFA response to exercise is similar to that under placebo conditions.

The present results and the results from two other studies of Standardbred horses in which carbohydrate-, fat-, or protein-supplemented diets were used before prolonged exercise (16, 35) suggest that the NEFA response to prolonged exercise is not altered by glucose infusion or diets differing in carbohydrate content in the horse. However, Stull and Rodiek (44) showed that when corn was fed 1 or 4 h before a 55-min submaximal exercise test of progressively increasing intensity, plasma NEFA concentration was reduced during exercise compared with when no feed was given. It is problematic to compare the findings of investigations differing in exercise protocols and substrate interventions, but it is possible that the horses in our study maintained sufficient plasma glucose concentrations to prevent differences in NEFA concentrations between the P & P and P & G treatments so that the expected metabolic changes related to central fatigue did not occur. It is also possible in these horses that the period of fasting before exercise somehow abolished the expected effect of glucose infusion on NEFA concentration during exercise.

The similarity of the prolactin data in the T & P and T & G trials shows that glucose did not alter the effect of infused tryptophan on plasma concentrations of prolactin. The inability of glucose to affect the prolactin response during this type of exercise in the horse was also demonstrated by the similar prolactin values in the P & G and P & P trials. These results are not consistent with the hypothesized reduction of fTrp and of prolactin in response to glucose infusion. They are thus inconsistent with the notion of central fatigue during exercise.

The plasma fTrp and fTrp-to-BCAA ratio responses of horses to elevated blood glucose are apparently different from those of humans (13). Davis et al. (13) reported that plasma NEFA concentration was reduced in a dose-response manner to increased carbohydrate consumption and that plasma fTrp was highly correlated ( $r = 0.86$ ) to changes in plasma NEFA concentrations. This difference may be a function of species differences in NEFA responses to elevated blood glucose. We are aware of only one previous report on plasma tryptophan concentrations during exercise (16). Essen-Gustavsson et al. (16) reported no change from baseline in total plasma tryptophan (not fTrp) concentrations during prolonged exercise in horses. Also, in the same report, BCAA concentrations increased during exercise, whereas we detected no such rise. This discrepancy may be related to differences in dietary control and exercise treatments. The liver releases BCAA during exercise in humans (18); if this occurs in horses, BCAA must have been removed by muscle in the present study, because plasma BCAA concentrations did not increase. In humans, MacLean et al. (31) also found no increase in plasma BCAA concentration and suggested that these amino acids were oxidized in muscle during exercise. The measurement of tissue release, uptake, and oxidation of BCAA during exercise will be necessary in future investigations to quantify the turnover of BCAA during submaximal exercise in the horse.

In conclusion, the most important finding of this study is a decrease in exercise time to fatigue compared with the placebo trial when tryptophan was infused (100 mg/kg body wt) before exercise. The tryptophan infusion results are consistent with the central fatigue hypothesis that an increased plasma fTrp concentration is related to the early onset of fatigue during prolonged exercise (34). It is plausible that the tryptophan infusions were related to central mechanisms of fatigue associated with increased central serotonin concentration (1, 2). However, the use of prolactin as a marker of central serotonin activity did not allow for these relationships to be clearly supported in this study. The fact that the early onset of fatigue when tryptophan was infused was not associated with a decreased muscle glycogen concentration also is consistent with fatigue mechanisms other than those associated with peripheral substrate availability. However, glucose infusion during exercise in the horse did not alter plasma NEFA, fTrp, or BCAA concentration responses to exercise. Because fTrp and the fTrp-to-BCAA ratio were increased at 15 min of exercise and remained unchanged thereafter and because the increase in NEFA concentration was not significantly correlated with plasma fTrp concentration, the hypothesis regarding metabolic control of central fatigue presented by News-holme and colleagues (34) was not supported in these horses when tryptophan was not infused before exercise. Also of importance is the finding that glucose infusion (P & G) prolonged exercise duration compared with the placebo condition (P & P). This confirms previous results in horses performing prolonged tread-

mill exercise (17). Future investigations should address the effects of serotonin agonists/antagonists and should characterize tissue release and utilization of amino acids in the horse during prolonged exercise.

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## REFERENCES

- Bailey, S. P., J. M. Davis, and E. N. Ahlborn. Effect of increased brain serotonergic activity on endurance performance in the rat. *Acta Physiol. Scand.* 145: 75–76, 1992.
- Bailey, S. P., J. M. Davis, and E. N. Ahlborn. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. *J. Appl. Physiol.* 74: 3006–3012, 1993.
- Bailey, S. P., J. M. Davis, and E. N. Ahlborn. Serotonergic agonists and antagonists affect endurance performance in the rat. *Int. J. Sports Med.* 14: 330–333, 1993.
- Carlson, M. G., W. L. Snead, J. O. Hill, N. Nurjhan, and P. J. Campbell. Glucose regulation of lipid metabolism in humans. *Am. J. Physiol.* 261 (Endocrinol. Metab. 24): E815–E820, 1991.
- Chaouloff, F., D. Laude, and J. L. Elghozi. Physical exercise: evidence for differential consequences of tryptophan on 5-HT synthesis and metabolism in central serotonergic cell bodies and terminals. *J. Neural Transm.* 78: 121–130, 1989.
- Charney, D. S., G. R. Heninger, J. F. Reinhard, Jr., D. E. Sternberg, and K. M. Hafstead. The effect of intravenous L-tryptophan on prolactin, growth hormone, and mood in healthy subjects. *Psychopharmacology (Berl.)* 78: 38–43, 1982.
- Clemens, J. A., C. J. Shaar, and E. B. Smalstig. Dopamine, PIF, and other regulators of prolactin secretion. *Federation Proc.* 39: 2907–2911, 1980.
- Cohen, S. A., M. Meys, and T. L. Tarvin. The PICO-TAG method. In: *A Manual of Advanced Techniques for Amino Acid Analysis*. Milford, MA: Waters Div., Millipore, 1990, WM02, rev. 1.
- Colborn, D. R., D. L. Thompson, Jr., T. L. Roth, J. S. Capehart, and K. L. White. Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings. *J. Anim. Sci.* 69: 2556–2562, 1991.
- Coyle, E. F., A. R. Coggan, M. K. Hemmert, and J. L. Ivy. Muscle glycogen utilization during prolonged exercise when fed carbohydrate. *J. Appl. Physiol.* 61: 165–172, 1986.
- Coyle, E. F., M. T. Hamilton, J. G. Alonso, S. J. Montain, and J. L. Ivy. Carbohydrate metabolism during intense exercise when hyperglycemic. *J. Appl. Physiol.* 70: 834–840, 1991.
- Curzon, G., J. Friedel, and P. J. Knott. The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature* 242: 198–200, 1973.
- Davis, J. M., S. P. Bailey, J. A. Woods, F. J. Galiano, M. T. Hamilton, and W. P. Bartoli. Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. *Eur. J. Appl. Physiol.* 65: 513–519, 1992.
- DeMeirleir, K., L. Baeyens, M. L'Hermite, M. L'Hermite-Baleriaux, J. Olbrecht, and W. Hollmann. Pergolide mesylate inhibits exercise-induced prolactin release in man. *Fertil. Steril.* 43: 628–631, 1985.
- DeMeirleir, K., M. L'Hermite-Baleriaux, M. L'Hermite, R. Rost, and W. Hollmann. Evidence for serotonergic control of exercise-induced prolactin secretion. *Horm. Metab. Res.* 17: 380–381, 1985.
- Essen-Gustavsson, B., E. Blomstrand, K. Karlstrom, A. Lindholm, and S. G. B. Persson. Influence of diet on substrate metabolism during exercise. *Equine Exerc. Physiol.* 3: 288–298, 1991.
- Farris, J. W., K. W. Hinchcliff, K. H. McKeever, and D. R. Lamb. Glucose infusions during prolonged exercise in standardbred horses. *Equine Vet. J. Suppl.* 18: 357–361, 1995.
- Felig, P., and J. Wahren. Amino acid metabolism in exercising man. *J. Clin. Invest.* 50: 2703–2714, 1971.
- Fink, W. J., and D. L. Costill. *Analytical Methods for the Measurement of Human Performance*. Muncie, IN: Human Performance Laboratory, Ball State University, 1990, p. 109–113.
- Fischer, H. G., W. Hollman, and K. DeMeirleir. Exercise changes in plasma tryptophan fractions and relationship with prolactin. *Int. J. Sports Med.* 12: 487–489, 1991.
- Flores, C. M., B. A. Hulihan-Giblin, P. J. Hornby, M. D. Lumpkin, and K. J. Kellar. Partial characterization of a neurotransmitter pathway regulating the in vivo release of prolactin. *Neuroendocrinology* 55: 519–528, 1992.
- Heuter, G., G. Hajak, A. Reimer, B. Poeggeler, M. Blomer, A. Rodenbeck, and E. Ruther. The metabolic fate of infused L-tryptophan in men: possible clinical implication of the accumulation of circulating tryptophan and tryptophan metabolites. *Psychopharmacology (Berl.)* 109: 422–432, 1992.
- Hill, P. J., H. Thijssen, L. Garbaczewski, H. P. Koppe-schaar, and F. De Waard. VIP and prolactin release in response to meals. *Scand. J. Gastroenterol.* 21: 958–960, 1986.
- Hinchcliff, K. W., K. H. McKeever, W. W. Muir, and R. Sams. Effect of furosemide and weight carriage on energetic responses of horses to incremental exertion. *Am. J. Vet. Res.* 54: 1500–1504, 1993.
- Jorgensen, H., U. Knigge, and J. Warberg. Involvement of 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub> receptors in the mediation of the prolactin response to serotonin and 5-hydroxytryptophan. *Neuroendocrinology* 55: 336–343, 1992.
- Kahn, R. S., and S. Wetzler. m-Chlorophenylpiperazine as a probe of serotonin function. *Biol. Psychiatry* 30: 1139–1166, 1991.
- Lindholm, A., and K. Piehl. Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of Standardbred horses. *Acta Vet. Scand.* 15: 287–309, 1974.
- Lowry, O. H., and J. V. Passonneau. *A Flexible System of Enzymatic Analysis*. New York: Academic, 1972, p. 174–177.
- Lucke, J. N., and G. M. Hall. Biochemical changes in horses during a 50-mile endurance ride. *Vet. Rec.* 102: 356–358, 1978.
- Lucke, J. N., and G. M. Hall. Long distance exercise in the horse: Golden Horseshoe Ride 1978. *Vet. Rec.* 106: 405–407, 1980.
- MacLean, D. A., L. L. Spriet, E. Hultman, and T. E. Graham. Plasma and muscle amino acid and ammonia responses during prolonged exercise in humans. *J. Appl. Physiol.* 70: 2095–2103, 1991.
- McKeever, K. H., K. W. Hinchcliff, S. M. Reed, and J. T. Robertson. Role of decreased plasma volume in hematocrit alterations during incremental treadmill exercise in horses. *Am. J. Physiol.* 265 (Regulatory Integrative Comp. Physiol. 34): R404–R408, 1993.
- Meites, J., J. F. Bruni, and D. A. Van Vugt. Effects of endogenous opiate peptides on release of anterior pituitary hormones. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu. New York: Raven, 1979, p. 36–55.
- Newsholme, E. A., I. N. Acworth, and E. Blomstrand. Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In: *Advances in Myochemistry*, edited by G. Benzi. London: John Libby Eurotext, 1987, p. 127–138.
- Pagan, J. D., B. Essen-Gustavsson, A. Lindholm, and J. Thornton. The effect of dietary energy source on blood metabolites in Standardbred horses during exercise. *Proc. 10th Equine Nutr. Physiol. Symp. Equine Nutr. Physiol. Soc., Colorado State University, 11–13 June 1987*, p. 420–431.
- Paradis, M. J., R. G. Breeze, W. M. Bayly, D. F. Counts, and W. W. Laegrid. Acute hemolytic anemia after oral administration of L-tryptophan in ponies. *Am. J. Vet. Res.* 52: 742–747, 1991.

37. **Rolandi, E., E. Reggiani, R. Franceschini, G. B. Arras, A. Cataldi, F. De Lucia, and T. Barreca.** Prolactin release induced by physical exercise is independent from peripheral vasoactive intestinal polypeptide secretion. *Ann. Clin. Res.* 20: 428–430, 1988.
38. **Rose, R. J., R. A. Purdue, and W. Hensley.** Plasma biochemistry alterations in horses during an endurance ride. *Equine Vet. J.* 9: 122–126, 1977.
39. **Sherman, W. M., D. L. Costill, W. J. Fink, and J. M. Miller.** Effect of exercise and diet manipulation on muscle glycogen and its subsequent utilization during performance. *Int. J. Sports Med.* 2: 114–118, 1981.
40. **Shimatsu, A., Y. Kato, H. Ohta, K. Tojo, Y. Kabayama, T. Inoue, N. Yanaiharu, and H. Imura.** Involvement of hypothalamic vasoactive intestinal polypeptide (VIP) in prolactin secretion induced by serotonin in rats. *Proc. Soc. Exp. Biol. Med.* 175: 414–416, 1984.
41. **Snow, D. H., M. G. Kerr, M. A. Nimmo, and E. M. Abbot.** Alterations in blood, sweat, urine and muscle composition during prolonged exercise in the horse. *Vet. Rec.* 110: 377–384, 1982.
42. **Stoff, D. M., A. P. Pasatiempo, J. H. Yeung, W. H. Bridger, and H. Rabinovich.** Test-retest reliability of the prolactin and cortisol responses to D,L-fenfluramine challenge in disruptive behavior disorders. *Psychiatry Res.* 42: 65–72, 1992.
43. **Struder, H. K., W. Hollmann, P. Platen, R. Wostman, A. Ferrauti, and K. Weber.** Effect of exercise intensity on free tryptophan to branched-chain amino acids ratio and plasma prolactin during endurance exercise. *Can. J. Appl. Physiol.* 22: 280–291, 1997.
44. **Stull, C. L., and A. V. Rodiek.** Effects of postprandial interval and feed composition on energy availability in exercising thoroughbreds. *Equine Vet. J. Suppl.* 18: 362–366, 1995.
45. **Tache, Y., G. Charpenet, M. Chretien, and R. Collu.** Role of serotonergic pathways in hormonal changes induced by opioid peptides. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu. New York: Raven, 1979, p. 110–131.
46. **Thompson, D. L., Jr., L. Johnson, R. L. St. George, and F. Garza.** Concentrations of prolactin, luteinizing hormone and follicle stimulating hormone in pituitary and serum of horses: effect of sex, season and reproductive state. *J. Anim. Sci.* 63: 854–860, 1986.
47. **Van Baak, M. A., and J. M. V. Mooij.** Effect of glucose infusion on endurance performance after  $\beta$ -adrenoceptor blocker administration. *J. Appl. Physiol.* 77: 641–646, 1994.
48. **Van de Kar, L. D.** Neuroendocrine pharmacology of serotonergic (5-HT) neurons. *Annu. Rev. Pharmacol. Toxicol.* 31: 289–320, 1991.
49. **Watanobe, H., and S. Sasaki.** Effect of thyroid status on the prolactin-releasing action of vasoactive intestinal peptide in humans: comparison with the action of thyrotropin-releasing hormone. *Neuroendocrinology* 61: 207–212, 1995.
50. **Wolf, P. D., and L. Lee.** Effect of serotonin precursor, tryptophan, on pituitary hormone secretion. *J. Clin. Endocrinol. Metab.* 45: 123–133, 1977.
51. **Wright, D. A., W. M. Sherman, and A. R. Dernbach.** Carbohydrate feedings before, during, or in combination improve cycling endurance performance. *J. Appl. Physiol.* 71: 1082–1088, 1991.

