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-HT2) 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 ≥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 O₂ Consumption

Incremental exercise tests were done 2 wk before the study was begun to determine the maximal rate of O₂ consumption (V˙O₂max) and the treadmill speed representing 50% of V˙O₂max of each horse. O₂ 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 until 9 m/s, then the treadmill grade was increased to 7% and speed was increased by 1 m/s every 1.5 min until O₂ consumption failed to increase with further increases in speed (V˙O₂max). After the V˙O₂max test, horses were rested for 30 min, and O₂ consumption was measured while each horse ran on a 2% grade at the speed calculated to elicit 40% of V˙O₂max. After 5 min at this pace, speed was increased 0.25 m/s every 5 min until O₂ consumption was 50% of V˙O₂max. 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 V˙O₂max 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-
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°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 ice-chilled 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 at 15 min at 2°C). After centrifugation, aliquots of plasma were distributed among plastic cryogenic storage tubes, rapidly frozen on dry ice, and stored at −80°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 µl of the internal standard, i.e., methionine sulfone (0.4 mM in 0.1 M HCl), and 350 µl of arterial plasma were transferred into a polypropylene tube and quickly vortexed. Then 350 µl of this mixture were transferred into a microcentrifuge capsule (10,000-mol wt cutoff) and centrifuged at 4°C for 20 min to obtain protein-free filtrate. The resultant filtrate was immediately frozen on dry ice and stored at −80°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 needed O2 consumption.

Fig. 1. Plasma free tryptophan (fTrp) concentration (mean ± 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 O2 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 µl of standard, sample, or saline (blank) were transferred into appropriate wells. Then 50 µl of reagent A were distributed into the wells, and the plate was gently agitated for mixing purposes. The microplate was then incubated for 10 min at 37°C and 100% relative humidity. The microplate was removed, 100 µl 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

\[ \dot{\text{V}}_{\text{O}_2}\text{max} \] and Speed at 50% of \[ \dot{\text{V}}_{\text{O}_2}\text{max} \]

Individual \[ \dot{\text{V}}_{\text{O}_2}\text{max} \] values ranged from 107 to 138 ml·kg\(^{-1}\)·min\(^{-1}\), and treadmill speed eliciting 50% of \[ \dot{\text{V}}_{\text{O}_2}\text{max} \] 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 \[ \dot{\text{V}}_{\text{O}_2}\text{max} \] for the P & G, P & P, T & G, and T & P trials were 122.4 ± 11.9, 102.3 ± 10.3, 87.1 ± 6.8, and 86.1 ± 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 ± 9 kg before exercise to 380 ± 10 kg in all exercise trials. There was no significant difference in weight loss between treatments. Heart rate at rest was 30 ± 2 beats/min, increased (P < 0.05) to 131 ± 5 beats/min at 5 min of exercise, and was 139 ± 4 beats/min at fatigue. Heart rate was not significantly different between trials. Hematocrit at rest was 31 ± 2%, and increased (P < 0.05) to 45 ± 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
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).
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 ± 3.5 and 16.1 ± 3.1 ng/ml, respectively) than in the P & G and P & P trials (4.4 ± 0.6 and 3.14 ± 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

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Isoleucine</th>
<th>Leucine</th>
<th>Valine</th>
<th>Free Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>P</td>
<td>G</td>
<td>P</td>
</tr>
<tr>
<td>0</td>
<td>105 ± 7</td>
<td>118 ± 8</td>
<td>133 ± 15</td>
<td>142 ± 15</td>
</tr>
<tr>
<td>15</td>
<td>114 ± 10</td>
<td>114 ± 6</td>
<td>137 ± 7</td>
<td>131 ± 9</td>
</tr>
<tr>
<td>30</td>
<td>110 ± 10</td>
<td>123 ± 12</td>
<td>145 ± 23</td>
<td>139 ± 9</td>
</tr>
<tr>
<td>F - 24</td>
<td>106 ± 11</td>
<td>114 ± 13</td>
<td>147 ± 28</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>F - 9</td>
<td>96 ± 14</td>
<td>103 ± 9</td>
<td>136 ± 21</td>
<td>123 ± 8</td>
</tr>
</tbody>
</table>

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.
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&G and T&P 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&G and T&P 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 Newsholme 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 that 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 Newsholme 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 treadmill exercise.
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. We acknowledge the assistance of Dr. D.L. Palmquist with amino acid analysis. Funding was provided by the Ohio Standardbred and Thoroughbred Research Funds and the Gatorade Sports Science Institute (Barrington, IL).

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