The Effect of Dietary Energy Source on Blood Metabolites in Standardbred Horses During Exercise

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Introduction

The basic driving force behind all types of equine exercise is the conversion of chemically bound energy into mechanical energy for muscular movement. The only source of energy that can be used directly to produce mechanical work in muscular activity is the dephosphorylation of adenosine triphosphate (ATP). In order to sustain high rates of energy generation (and work), ATP must be resynthesized at a rate equal to its hydrolysis in muscle. Two fundamental reactions can be employed for this resynthesis: 1) Glycolysis, which is the anaerobic metabolism of glucose or glycogen to lactate. 2) Oxidative phosphorylation, which involves the reduction of substrates (carbohydrates, fats, and proteins) and their oxidation via the tricarboxylic acid cycle and electron transport chain within mitochondria. The choice of substrate for energy generation during exercise is dependent on a number of factors including degree of fitness, intensity of exercise, duration of exercise and diet. Several studies with humans (Christensen and Hansen, 1939; Martin et al., 1978; and Jansson, 1980) have shown that the relative proportions of fat and carbohydrates in the diet influences the relative oxidation of these substrates in working muscle.

The following study was conducted to determine whether changes in dietary energy source (carbohydrate, protein and fat) influence substrate selection by equine muscle during different exercise intensities. The effect of these dietary manipulations on cardiovascular response and muscle and liver glycogen storage and utilization have already been reported (Pagan et al., 1986) This paper will concentrate on the effect of dietary energy source on blood metabolites in horses during exercise.

Materials and Methods

Three Standardbred horses were used in a 3 X 3 Latin square design trial to determine the effects of feeding diets containing different levels of carbohydrate, fat and protein to exercised horses. During each one month period the horses were fed a diet high in either carbohydrate (CHO), protein or fat along with a 50% dry matter rye grass haylage. During the first two weeks of each period the horses were lightly trained on a treamill two or three times per week. During week 3 of each period the horses performed a high speed exercise test on a motor driven treadmill. During week 4 of each period the horses performed a long slow exercise test on the treamill.

Experimental diets. A commercial horse feed (Fibergi, Sollebolagen AB) containing 12% crude protein (CP)(as fed basis) was used as the high CHO control. The other two experimental diets were a feed containing 20% CP (high protein diet) and a feed containing 15% soybean oil (high fat diet). The dry matter, crude protein, crude fat,

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neutral detergent fiber, and starch concentrations in each diet are shown in table 1 along with the nutrient concentrations contained in the haylage (Horsehage, Sollebolagen AB) which was fed along with each diet as a roughage source.

Feeding regime. During each period the experimental diets were fed along with the haylage twice daily in amounts estimated to provide a daily digestible energy (DE) intake of 130% maintenance (Pagan and Hintz, 1986), with the haylage supplying about 30% of the total DE. Daily intakes during each period are shown in table 2. The high protein diet provided an average of 138% as much protein, 70% as much fat and 54% as much starch as the control diet (CHO). The high fat diet provided 75% as much protein, 336% as much fat, and 60% as much starch as the control diet.

High speed exercise test. The high speed exercise test was performed at the fastest speed at which each animal could maintain its natural gait (2 horses trotted and 1 paced) on a flat treadmill for an extended period of time. Two horses (#1, trotter and #2, pacer) ran at 10 m/sec while #3 (trotter) was only able to maintain a speed of9 m/sec. During the first period, horses 2 and 3 ran for 14 min, while horse 1 was stopped after 10 min of exercise. However, during the second and third periods all of the horses ran for 14 min. Biopsies of the gluteus medius muscle were taken from the horses before and immediately after exercise, using the technique of Lindholm and Piehl (1974), and venous blood samples were taken at rest, every 2 min during exercise, immediately after exercise and 2 and 5 min after exercise.

Long slow exercise test. Near the end of week 4 of each period the horses were trotted at 5 m/sec for 105 min. The horses were stopped after 15 min (3 min rest), 30 min (3 min rest), 60 min (10 min rest) and 90 min (3 min rest). Before exercise, 5 incisions were made through the skin and fascia covering the right gluteus medius muscle of each horse. Muscle biopsies were taken through these incisions before exercise,when the horses were stopped at 15, 30 and 60 min of exercise and immediately after exercise. Liver biopsies were taken the day before the long runs and about an hour after exercise. A face mask was placed on the horses near the end of each rest period and respiratory quotient (RQ) was measured 3 min after the horses had resumed exercise. Blood samples were taken from a jugular catheter at rest and 15, 30, 60, 90, and 105 min of exercise and 2, 5, and 10 min after exercise.

Blood analysis. Blood lactate concentration was determined enzymatically on aliquots of venous blood deproteinized at the time of collection. Blood glucose was determined on whole blood using an enzymatic fluorometric technique (Lowry and Passonneau, 1973), and FFA concentration was determined fluorometrically on blood plasma.

Results

High speed exercise test. Muscle glycogen utilization during the high speed exercise test averaged 12.2 mmol/kg dry wt/min when the horses were fed the control diet, 5.6 when fed the high protein diet and 7.3 when fed the high fat diet. An average of 4 mmol/liter of blood lactate was reached in the control after 6 minutes of exercise (table 3), but this concentration of blood lactate was not reached until 12 min of exercise in the high fat and high protein groups. Blood lactate accumulation was positively correlated with muscle glycogen utilization (r=.84). Blood glucose (figure 1) dropped sharply

after the onset of exercise and was significantly lower ($p<.05$) than at rest in all treatment groups through 10 min of exercise. In the high protein group, glucose levels remained significantly lower ($p<.05$) than resting levels until 10 min after exercise. The last 4 min of exercise and post exercise glucose levels were not significantly different from rest in the high fat group. There was no significant difference between treatments in blood glucose before, during, or after exercise. Plasma FFA concentration (figure 2) was not significantly different from rest either during or after exercise in any of the treatment groups. There was also no significant difference in FFA between treatment groups before, during or after exercise.

Long slow exercise test. Blood lactate concentration remained low (around 1 mmol/l) throughout the exercise period in each treatment group. Thus, the vast majority of the energy generated was from aerobic pathways. Resting muscle glycogen (Pagan et al., 1986) was significantly higher ($p<.10$) in the control group than in the high protein and high fat groups. The control group also had higher muscle glycogen ($p<.05$) than the high fat group after 15, 30, 60 and 105 min of exercise and higher muscle glycogen than the high protein group after 60 min of exercise ($p<.10$) and 105 min of exercise ($p<.05$). Resting RQs (Pagan et al., 1986) were significantly higher in the control group than in either the high protein or high fat groups ($p<.05$). After 18 min of exercise, RQs were higher than resting values in the high fat and high protein groups. At this time, control group RQs were not significantly different from the high protein group values. However, after 33 min of exercise, the control group RQs were significantly higher than both the high protein ($p<.10$) and high fat ($p<.05$) fed horses. Control group RQs remained significantly higher than the high protein and high fat groups ($p<.05$) at 63 and 93 min of exercise. Blood glucose during the long slow exercise test is shown in figure 3. Glucose levels were significantly decreased from resting levels at 15 min of exercise in each treatment group, and these levels remained depressed throughout exercise. At 30 min of exercise, blood glucose in the high protein group was significantly lower ($p<.05$) than in the control group. FFA concentration during the long slow run is shown in figure 4. At 60 min of exercise the high fat group had significantly higher ($p<.05$) FFA levels in the blood than the control group.

Discussion

Diet affected the substrates selected by the horses during both the fast and slow exercise tests. During the fast exercise (about 75% VO2max) horses fed the control (high CHO) diet appeared to depend more heavily on anaerobic glycolysis for energy generation than when the high protein or high fat diets were fed. Plasma FFA did not appear to contribute significantly to energy generation in any of the treatment groups during the fast exercise, since plasma FFA levels were not elevated either during exercise or when exercise ceased (and energy demands dropped substantially). It is possible, however, that the horses fed the high protein and high fat diets generated a good deal of energy from the oxidation of intramuscular fat without any release of FFA into the blood.

In all treatment groups there was a sharp decline in blood glucose after the onset of exercise, but a large part of this drop may have

been due to dilution from the mobilization of red blood cells from the spleen, since whole blood glucose was measured. However, after 8 min of exercise, blood glucose tended to be lower in the high protein group than in the other two groups. Perhaps the high protein-low carbohydrate diet stimulated glucose production and utilization via gluconeogenesis in the liver.

Regardless of the mechanism, it appears that either high fat or high protein-low carbohydrate diets resulted in a sparing of muscle glycogen utilization during exercise at around 75% VO2max. It remains to be determined whether this sparing effect would contine to exist with the greatly increased demands for energy generation seen at racing speeds.

After 18 minutes of exercise during the long slow test, RQs indicated that an average of 64%, 54%, and 40% of the heat produced by the horses was from carbohydrate oxidation when the horses were fed the control, high protein , and high fat diets, respectively. The amount of energy generated from carbohydrate metabolism dropped as exercise continued, accounting for only 58%, 20%, and 8% of the heat produced after 63 min of exercise in the control, high protein, and high fat diets. After 93 min of exercise, only 44%, 12% and 16% of the heat produced was from carbohydrate oxidation. Thus, as exercise progressed, there was a shift of substrate utilization from carbohydrate oxidation to fat oxidation in each group. This shift coincided with an increase in FFA concentration with the greatest increase occurring in the high fat fed group. During early exercise, the high protein fed group appeared to rely heavily on blood glucose for energy generation as evidenced by a significantly lower blood glucose level after 30 min and significantly greater liver glycogen utilization than the control group (Pagan et al., 1986).

The long delay in mobilization and utilization of fat by these horses may have been the result of a lack of conditioning since the horses were not heavily trained during the experimental period. Further studies are needed to determine what other factors affect substrate selection in horses during different intensities and durations of exercise.

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Table 1. Diet Composition.

	Haylage	Control	High Protein	High Fat
Dry Matter %	48.7	85.3	83.1	86.6
Crude Protein %[a]	8.6	14.6	24.6	13.0
Crude Fat %[a]	2.8	3.1	2.0	18.2
Neutral Detergent Fiber %[a]	35.7	5.9	7.2	5.5
Starch %[a]	2.4	39.9	24.2	30.8

[a]dry matter basis

Table 2. Nutrient Intakes.

	Horse 1			Horse 2			Horse 3		
DIET	control	protein	fat	control	protein	fat	control	protein	fat
Period #	1	3	2	2	1	3	3	2	1
Body Weight (kg)	440	441	444	475	465	472	538	530	515
Experimental Diet (g DM/day)	4253	3756	3211	4477	3952	3379	4910	4334	3706
haylage (g DM/day)	2070	2070	2070	2270	2270	2270	2503	2503	2503
Crude Protein (g/day)	799	1102	595	849	1167	634	932	1281	697
Crude Fat (g/day)	180	133	642	202	143	679	222	157	745
Starch (g/day)	1747	959	1039	1841	956	1095	2019	1109	1202

Table 3. Blood Lactate Concentration During the High Speed Exercise Test (mmol/liter)

time	Control (CHO)	High Protein	High Fat
Rest	.93±.07[a]	.93±.03	.83±.03
2 min	2.87±.23	2.00±.20	2.00±.31
4 min	3.60±.70	2.33±.20	2.40±.20
6 min	4.10±.85	2.37±.12	2.80±.31
8 min	4.77±1.08	2.73±.15	3.10±.38
10 min	5.57±1.03	3.87±.35	3.90±.50
12 min	7.70[b]	5.00±.45	5.43±.59
14 min	9.90[b]	7.17±.67	6.70±.38

[a]mean±SE
[b]2 horses

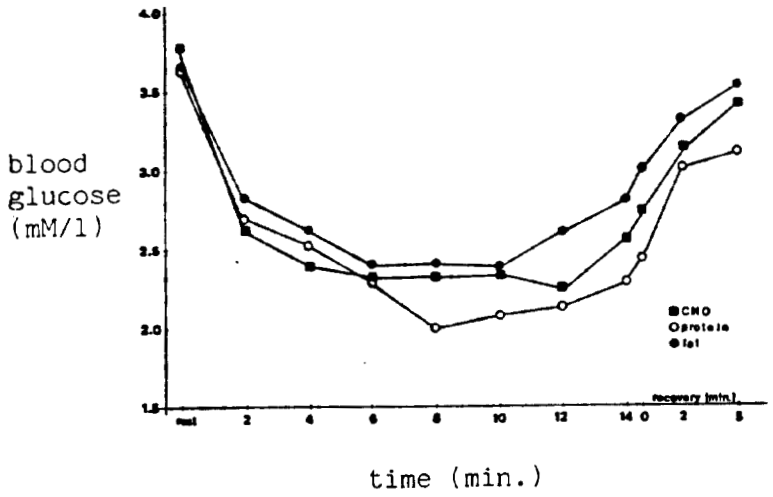

figure 1. Blood glucose during high speed exercise test

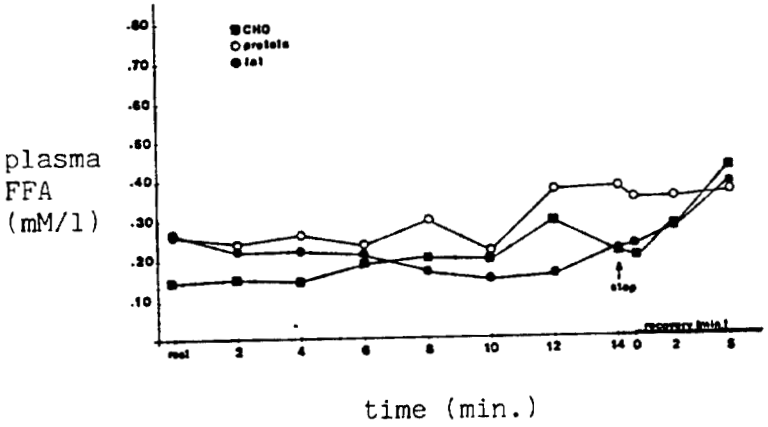

figure 2. Plasma FFA during high speed exercise test

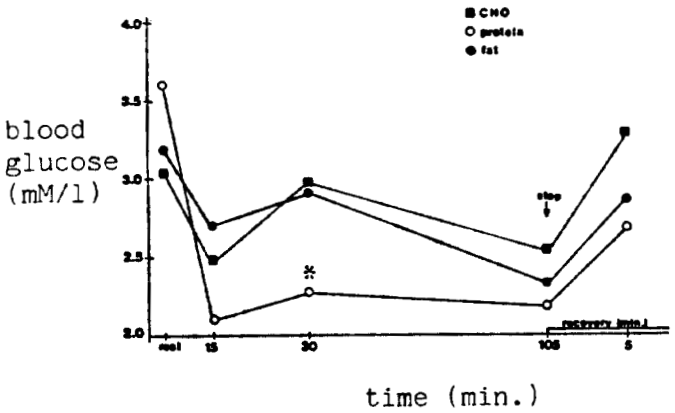

figure 3. Blood glucose during long slow exercise test

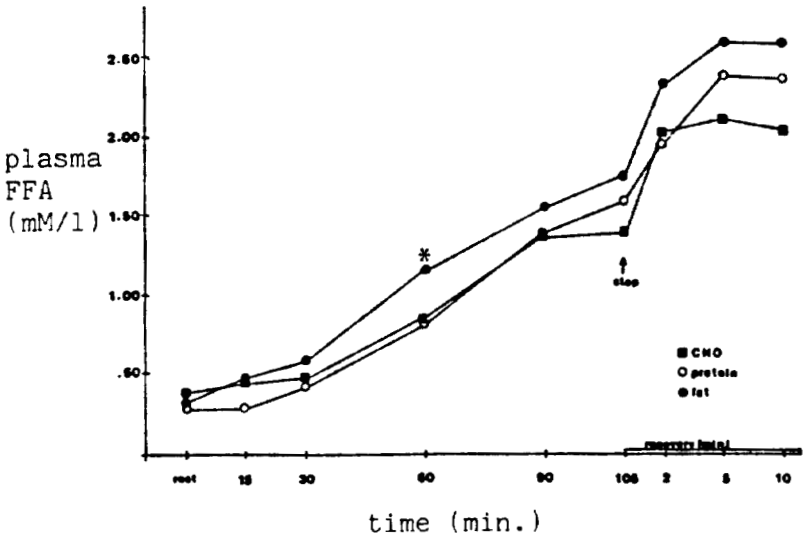

figure 4. Plasma FFA during long slow exercise test