

The Effect of Exercise and Diet on Muscle and Liver
Glycogen Repletion in Standardbred Horses

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Introduction

Muscle and liver glycogen are important substrates for energy generation in horses performing various intensities of exercise. In a review of energy considerations during exercise, Hodgson (1985) summarized a number of experiments that measured muscle glycogen utilization in horses performing a wide variety of exercises from endurance rides covering up to 160 km to Thoroughbreds racing at speeds greater than 950 m/min. In all of these exercises muscle glycogen was used to produce energy, with the amount of utilization increasing with work intensity.

During long distance exercise, muscle glycogen has been shown to be almost totally depleted in horses (Snow et al., 1982). Because of the potential for fatigue that accompanies muscle glycogen depletion, a number of experiments have been conducted with horses to manipulate muscle glycogen storage (Kline and Albert, 1981; Toppliff et al., 1983, Toppliff et al., 1985). In these studies rather extreme dietary manipulations were used in combination with intense exercise to deplete, then replete muscle glycogen. While these methods of glycogen "loading" have been generally successful at increasing glycogen storage in the muscle, they are considered too severe to be used under practical management situations. Therefore, the following studies were conducted to determine whether muscle and liver glycogen stores can be manipulated using more acceptable diets and exercise regimes.

Materials and Methods

Two experiments were conducted to determine the effect of exercise and diet on muscle and liver glycogen repletion in horses. In the first experiment, four Standardbreds were fed either a high carbohydrate (CHO) or high fat diet in a two period switchback type experiment. The dry matter, crude protein, crude fat, neutral detergent fiber, and starch concentrations in each diet are shown in table 1. During the first 12 days of each three week period, the horses were fed their respective diets along with timothy hay at an intake estimated to provide 110% of their maintenance digestible energy requirement (Pagan and Hintz, 1986) and they were allowed daily exercise in small paddocks. Pre exercise biopsies of the gluteus

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medius muscle and liver were taken, followed by a six day exercise period in which the horses were exercised on a flat treadmill at 6 m/s for 15 min on day 1, 20 min on day 2, 25 min of days 3,4, and 5, and 30 min on day 6. After 2 days of exercise, feed intake was increased to 120% of each horse's estimated maintenance DE requirement for the remainder of the period. During this time the horses ate an average of 2.66 kg hay and 3.78 kg grain per day on the high CHO diet and 2.72 kg hay and 3.16 kg grain per day on the high fat diet.

A post exercise muscle biopsy was taken immediately after the final 30 min exercise bout and muscle biopsies were taken at 24 hour intervals for the three following days. A liver biopsy was also taken after three days of rest.

In a separate experiment (experiment 2) described elsewhere in these proceedings (Pagan et al., 1987), 3 Standardbreds were fed the high CHO diet, the high fat diet and a high protein diet at 130% of their estimated maintenance DE requirement. Muscle biopsies were taken before and immediately after a strenuous bout of exercise (9 or 10 m/s for 14 min). The horses were then allowed to rest for 7 to 10 days during which they were either confined to their stalls or turned out for a few hours at a time in small paddocks. During this rest period the horses continued to receive their respective diets at a level of intake estimated to equal 130% of the maintenance DE requirement. At the end of the rest period the horses' gluteus medius muscle was again biopsied.

The muscle and liver biopsies were freeze-dried, dissected free of connective tissue and blood under a dissection microscope and weighed. Glycogen content was determined after acid hydrolysis as glucose residues using an enzymatic fluorometric technique (Lowry and Passonneau, 1973).

Results

Muscle and liver glycogen contents from the first experiment before and after 6 days of exercise are shown in table 2. Muscle glycogen content was significantly lower ($p < .05$) than at rest immediately after the 6th day of exercise, and 1 and 2 days post exercise in both the high CHO and high fat groups. Muscle glycogen content on the third day after exercise was not significantly different from resting values in either treatment group. When fed the high CHO diet, post exercise muscle glycogen averaged 79.8%, 82.4%, 83.0%, and 87.9% of pre exercise values immediately after exercise and 1,2, and 3 days post exercise, respectively. When fed the high fat diet, post exercise glycogen averaged 78.7%, 82.2%, 91.3%, and 93.3% of pre exercise values immediately after exercise, and 1,2 and 3 days post exercise, respectively. Muscle glycogen content was not significantly different between treatment groups at any sampling time.

Pre exercise liver glycogen averaged 1214 mmol/kg dry wt in the high CHO group and 1375 mmol/kg dry wt in the high fat group. Liver glycogen 3 days after the 6th day of exercise averaged 1501 mmol/kg dry wt and 1346 mmol/kg dry wt or 123.6% and 97.8% of pre exercise values in the high CHO and high fat diets, respectively.

Muscle glycogen before and immediately after the strenuous work test and after 7-10 days rest are shown in table 3 for the high CHO and

high fat diets. Pre exercise muscle glycogen averaged 555 mmol/kg dry wt and 503 mmol/kg dry wt in the high CHO and high fat diets, respectively. These levels were not significantly different. After the exercise bout, muscle glycogen was significantly lower ($p < .05$) in both treatment groups, averaging 71.0% and 79.5% of pre exercise values in the high CHO and high fat diets. After 7-10 days of rest, muscle glycogen increased significantly ($p < .10$) over pre exercise levels in both treatment groups, averaging 122.3% and 115.1% of pre exercise in the high CHO and high fat diets, respectively. At this time, muscle glycogen levels were significantly higher ($p < .10$) in the high CHO group than in the high fat group.

Discussion

In the first experiment, no glycogen "loading" occurred following exercise with either diet. In fact, muscle glycogen concentration tended to be slightly lower than pre resting values even after three days of rest. The horses did not fully restore their muscle glycogen reserves even though they continued to receive a level of energy intake in excess of their maintenance requirement. Liver glycogen, however, was fully restored in both treatments and even increased in the high CHO group. The endurance type exercise used in experiment 1 resulted in only about a 20% decrease in glycogen stores. It is likely that most of this glycogen came from slow twitch muscle fibers since type I fibers are depleted of glycogen first during endurance type exercise (Lindholm, et al., 1974; Snow et al., 1982; Hodgson et al., 1986). Perhaps these slow twitch muscle fibers are less sensitive to glycogen loading than the type IIA and type IIB fibers.

Toppliff et al. (1985) depleted muscle glycogen by 42% with the combination of a high fat, high protein, low carbohydrate diet and a 5 day period of sprinting and galloping exercise. After 3 days of rest with a high CHO diet, muscle glycogen increased to 122% of pre exercise levels. This was similar to the results found in experiment 2 with the high CHO diet, where muscle glycogen was depleted by 29% by a strenuous exercise bout and increased to 122% of the pre exercised values. It is likely that in both of these experiments a good deal of the glycogen depletion occurred in the type II fibers.

If glycogen "loading" is dependent on the muscle fiber type involved, then the exercise regime used to deplete the muscle of glycogen must be intensive enough to deplete the appropriate fibers. Unfortunately, the horses that are most likely to benefit from glycogen loading are the ones that perform endurance type exercise where muscle glycogen depletion may contribute to fatigue. These animals rarely undergo such intensive exercise programs.

There is still some question whether glycogen loading is beneficial at all for horses. In Toppliff's study, the horses had reduced work performance after glycogen loading and Pagan et al. (1986) reported higher heart rates and more anaerobic glycolysis during strenuous work when the horses were fed high CHO diets that resulted in greater levels of muscle glycogen.

Acute rhabdomyolysis ("tying-up") has been associated with high carbohydrate diets for many years. Carlstrom (as reported by McLean,

1973) proposed in the 1930s that high CHO diets result in increased storage of glycogen and on exercise there is an abnormally high rate of lactic acid production which causes degeneration of the muscle cells. During experiment 1 of the present study, one of the horses "tied-up" badly following the 6th day of exercise when fed the high CHO diet. This horse experienced a great deal of pain and had difficulty moving. A blood sample taken after exercise showed greatly elevated CPK levels (>200 ukat/l). This horse's pre and post exercise muscle glycogen concentrations were 645 mmol/kg dry wt and 552 mmol/kg dry wt when fed the high CHO diet. After switching to the high fat diet the horse's pre and post exercise muscle glycogen concentrations were 522 mmol/kg dry wt and 412 mmol/kg dry wt, and after exercise there were no signs of "tying-up". Obviously, there are factors other than diet involved in the etiology of this disease, but it appears likely that high muscle glycogen may be detrimental to horses already predisposed to "tying-up".

Muscle glycogen "loading" appears to be most successful when high CHO diets are fed to horses following intensive exercise bouts which deplete large amounts of glycogen from the muscle. The usefulness of this practice, however, is questionable.

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Table 1. Diet Composition.

	<u>High CHO</u>	<u>High Fat</u>
dry matter %	85.3	86.6
Crude Protein % ^a	14.6	13.0
Crude Fat % ^a	3.1	18.2
Neutral detergent fiber % ^a	5.9	5.5
Starch % ^a	39.9	30.8

^adry matter basis

Table 2. Muscle Glycogen Content (Experiment 1)
(mmol/kg dry wt)

<u>sampling time</u>	<u>High CHO</u>	<u>High Fat</u>
pre exercise	595±45 ^a	540±37
immed. post	475±51 ^b	425±36 ^b
1 day post	490±18 ^b	444±44 ^b
2 day post	495±31 ^b	493±22 ^b
3 day post	523±9	504±40

^amean±SE

^bdifferent from rest (p<.05)

Table 3. Muscle Glycogen Content (Experiment 2)
(mmol/kg dry wt)

<u>sampling times</u>	<u>High CHO</u>	<u>High Fat</u>
pre exercise	555±53 ^a	503±8
immed. post	394±44 ^b	400±44 ^b
7-10 days post	679±24 ^c	579±50 ^c

^amean±SE

^bdifferent from pre exercise (p<.05)

^cdifferent from pre exercise (p<.10)