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Influence of Diet on Substrate Metabolism during Exercise

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ABSTRACT. Six horses were fed a normal (N-diet), a fat rich (Fat-diet) or a carbohydrate rich (CHO-diet) diet, each for 5 weeks. The horses performed a standardised exercise tolerance test (SET) and a submaximal exercise test to fatigue (SEF) on a treadmill during the last week on each diet. Blood samples were taken in connection with SET and SEF and muscle biopsies in connection with SEF. The speeds producing a blood lactate value of 4 mmol l⁻¹ (V_{Lact}) and a heart rate of 200 bpm (V₂₀₀) were within the normal ranges on all diets during SET. Diet had no influence on the duration of SEF. The average durations of exercise of horses fed N-, CHO-, and Fat-diets were 56, 51 and 52 min, respectively. Muscle triglycerides varied 5-fold among horses irrespective of diet. Resting muscle glycogen was 12% higher in CHO-diet compared to N- and Fat-diets. In all diets, glycogen decreased during SEF with a marked depletion in type I and IIA fibres at fatigue. Muscle lactate and glucose-6-phosphate levels were higher at the end of exercise in CHO- and Fat-diet than in N-diet period. Plasma concentrations of free fatty acids (FFA), glucose, lactate, ammonia, alanine, branched-chain amino acids, tyrosine and tryptophan increased during SEF. Higher concentrations of glucose, lactate, ammonia, alanine and branched-chain amino acids were seen at end of exercise in CHO- and Fat-diet than in N-diet period. At the beginning of exercise FFA concentration was higher in CHO-diet compared with N-diet. The results indicated that dietary manipulations could affect glycogen storage and change the substrate utilisation during submaximal exercise. These changes did not appear to affect time to fatigue during this type of exercise.

Key words. Horses; diet; exercise test; muscle glycogen; plasma metabolites.

INTRODUCTION

Carbohydrates, especially glycogen, in skeletal muscle of man and horse have been shown to be an important energy source during various intensities of exercise.^{4,10,14,26} During submaximal exercise in man, fatigue is associated with glycogen depletion and a close relationship is observed between initial glycogen content and work time.¹⁰ A shorter time to fatigue during submaximal exercise has been observed in horses as in humans when the glycogen level in muscle has been markedly lowered.²⁵ Lipids also contribute to energy utilization during submaximal ex-

ercise.^{4,6} From a study on rats, it was suggested that an increased availability of free fatty acids (FFA) in blood may have a glycogen sparing effect, thus delaying the time to fatigue during prolonged running.⁸ Protein, as an energy source during exercise, is known to be of minor importance. It has been estimated that under normal conditions the metabolism of protein and amino acids contributes less than 10% to the total energy yield during exercise in human subjects.¹³ Protein and amino acid metabolism has been suggested to have other important functions, e.g. to serve as a metabolic link

between skeletal muscle and the brain, which could explain the development of central fatigue during sustained exercise.¹

It is possible to alter substrate availability in blood and muscle of man and rats by dietary means and to influence performance capacity. Carbohydrate (CHO) rich diets increase glycogen stores in muscle of man and have a beneficial effect on performance time.¹⁰ In contrast, rats exposed to a high fat and low carbohydrate diet for several weeks have low glycogen stores, but a prolonged exercise time to exhaustion, presumably related to their increased ability to oxidize fat.¹⁶ One dietary factor of importance for protein metabolism has been reported to be the supply of carbohydrates.¹³ An increase in net protein degradation, based on measurements of nitrogen excretion, was indicated when exercise was performed with low muscle glycogen levels or without a supply of carbohydrates during the exercise.¹³

An increase in the glycogen content is observed also in horses after CHO-rich diets. Horses have higher resting muscle glycogen content than humans.^{4,14,25,26} No beneficial effect on performance and work time has been shown in horses after CHO-rich diets.²⁵ Higher heart rates (HR) and greater lactate production were observed during intense exercise when horses were fed a CHO-rich diet as compared to those fed a fat rich diet.^{17,25} Fat rich diets fed to horses are never so extreme as can be obtained in human or rat studies. However, some studies on horses have indicated that fat rich diets may have a beneficial effect on performance times since elevated glucose concentrations have been observed following different types of exercise.^{3,7,9} Whether this is associated with a glycogen or glucose sparing effect during submaximal exercise is unknown.

The aim of this study was to investigate the influence of both a CHO-rich and a fat rich diet on the metabolic response to submaximal exercise and evaluate the effects on endurance. The concentrations of glycogen, triglyceride, lactate and glucose-6-phosphate in muscle and the concentrations of glucose,

FFA, ammonia, lactate, alanine, branched chain amino acids, tyrosine and tryptophan in plasma were analysed in 6 horses performing a submaximal exercise test to fatigue (SEF) on a treadmill after being on different diets. A standardised exercise tolerance test (SET) was also performed after the different dietary regimens.

MATERIALS AND METHODS

Animals. Six clinically healthy Standardbred horses (1 mare, 1 stallion and 4 geldings) with a mean age of 8 years (range 5–14) were used.

Diets. All horses were fed 3 different diets which were calculated to be isocaloric: a normal (N-diet), a fat rich (Fat-diet) and a carbohydrate rich (CHO-diet) diet. Each diet was given for a 5 week period. During the first 5 weeks, all horses were given N-diet and thereafter, 3 of the horses were fed CHO-diet while the other 3 horses were fed Fat-diet. The Fat- and CHO-diets were then switched and fed to the horses for another 5 weeks. During each dietary period the horses were fed 3 times a day and given a total of 6 kg of timothy hay and 3 kg of pelleted feed. The contents of the pelleted feed and the calculated amount of protein, fat and carbohydrate (starch and sugar) given to the horses each day are shown in Table 1. The estimated digestible energy intake was 88.5 MJ on N-diet, 89.4 MJ on Fat-diet and 86.4 MJ on CHO-diet.

Experimental protocol. The horses were weighed once a week. During the first 4 weeks on each diet, the horses exercised 3–4 times a week on a treadmill (5 min walk and 20 min trot at 5–6 m s⁻¹) in order to increase the energy requirement. During the last week of each dietary period the horses first performed a standardised incremental exercise tolerance test (SET) and later, with at least a 24 hour period in between, a submaximal (with respect to intensity) exercise test to fatigue (SEF).

SET. This test was performed on a high speed motor-driven treadmill (Sikob, Stock-

Table 1. Composition of the pelleted feed and the daily amount of protein, fat and carbohydrate (starch and sugar) for a normal (N-diet), a fat rich (Fat-diet) or a carbohydrate rich (CHO-diet) diet

	N-diet	Fat-diet	CHO-diet
<i>Pelleted feed (%)</i>			
Oats/barley (crushed)	86.8	43.4	45.6
Sucrose	—	—	32.5
Soya meal	—	8.7	8.7
Vegetable oil	—	13.0	—
Straw treated with lye	—	21.7	—
Molasses	10.9	10.9	10.9
Minerals and vitamins	2.3	2.3	2.3
<i>Hay + pelleted feed (g day⁻¹)</i>			
Protein	579	585	591
Fat	216	531	168
Carbohydrate	1 680	1 203	2 037

holm) with an incline of 6.25%. After 2 min warm up of walking, the horses trotted at incremental speeds. The duration of trotting at each speed was 2 min. Depending on the fitness of the horse, the test was started at either 5 or 6 m s⁻¹ and ended at either 8 or 9 m s⁻¹ in order to reach HR of at least 200 bpm. HR was continuously monitored and recorded during the last 15 seconds at each speed using a bipolar ECG lead (Mingograph 804 Siemens Elema, Stockholm). Venous blood for lactate analyses was collected from a jugular catheter at rest and during the final 15 seconds at each speed. Blood volume determination was performed immediately after the test using the Evans blue dye dilution technique and the red cell volume (CV) was calculated as the difference between the total blood and plasma volumes.¹⁹ The speed giving HR of 200 bpm (V₂₀₀) and a blood lactate (plasma lactate values were re-calculated to whole blood values by using the following regression: LA = 0.74 + 0.55 × plasma lac-

tate concentration) response of 4 mmol l⁻¹ (V_{La4}) as well as the CV kg⁻¹, were calculated from SET.

SEF. This test was performed on the horizontal treadmill at a speed of 7 m s⁻¹. The horses trotted as long as they were able to keep pace with the treadmill. The point of fatigue was associated with the horses beginning to move backwards on the treadmill and losing speed in spite of vigorous encouragement. HR was recorded at 5 or 10 min intervals. Venous blood from a jugular catheter was collected at rest and after 5, 10, 15 and 20 min, and thereafter with 10 or 15 min intervals until fatigue.

Muscle biopsies from m. gluteus were taken at rest and after 15 and 35 min (45 min in 2 horses) and at the end of exercise. The biopsies for the biochemical analyses were immediately frozen in liquid N₂ and stored at -80°C until analysed. Muscle biopsies obtained at rest and after exercise were prepared for histochemical analyses. These pieces were rolled in talcum powder before freezing in liquid N₂.

Biochemical analyses The muscle tissue was freeze dried and dissected free of fat, connective tissue and blood. For glycogen determination, 1–2 mg of tissue were heated in a water bath (100°C) for 2 hours in 1 M hydrochloric acid in order to form glucose residues. The concentration of glucose, as well as the concentration of glucose-6-phosphate (G-6-P) and lactate in neutralized perchloric acid extracts, were all analysed using fluorimetric enzymatic techniques.¹⁵ The concentration of triglyceride was analysed in duplicate samples.⁴

Histochemical analyses Serial cross sections were cut in a cryostat. Sections were stained for myofibrillar ATP-ase after pre-incubation at pH 4.6² in order to identify type I, IIA and IIB fibres. Glycogen within each fibre was evaluated by staining with periodic acid Schiffs (PAS) reaction.¹⁸ The fibres on the PAS stained cross sections were evaluated by the same person according to their staining intensity as high (H), medium (M) or low (L). The PAS stains were then

Table 2. Mean \pm SD for the red cell volume kg^{-1} (CV kg^{-1}) the speed giving a blood lactate response of 4 mmol l^{-1} ($V_{\text{La}4}$) and a heart rate of 200 bpm (V_{200}) obtained from a standardised exercise tolerance test performed by 6 horses after 5 weeks on either normal (N-diet), fat rich (Fat-diet) or carbohydrate rich (CHO-diet) diet

	N-diet	Fat-diet	CHO-diet
CV kg^{-1}	64.9 \pm 6.7	63.8 \pm 6.0	64.9 \pm 6.8
$V_{\text{La}4}$	7.87 \pm 0.58	7.23 \pm 0.58 ^a	7.11 \pm 0.64 ^a
V_{200}	7.98 \pm 0.46	7.80 \pm 0.37	7.56 \pm 0.58 ^a

^a Significantly different from N-diet.

compared with the ATP-ase stain to identify the fibre types.

Plasma analyses. Glucose was analysed using a seralyser reflectance photometer (Ames Division, Miles Laboratories, IN). Lactate was analysed using a lactate analyser (Analox Instruments Ltd, London). FFA and ammonia concentrations were determined with modified fluorimetric techniques.^{12,24} Amino acids were analysed on plasma samples which were deproteinized with 5% trichloroacetic acid (1:5) and centrifuged at 9000 g for 2 min. The supernatant was stored at -70°C until the concentration of amino acids was measured by reversed-phase HPLC,²¹ with orthophthaldehyde (OPA) as the derivatizing agent.

Statistics Conventional methods have been used to calculate means and standard deviations (SD). Statistical analyses of the data were carried out with the Statistical Analysis System (SAS Institute Inc 1985).²³ Differences between the dietary regimens regarding the rate of increase or decrease in plasma and muscle concentrations vs exercise time (linear regression coefficient) were evaluated by a two-way analysis of variance. To evaluate the resting levels between dietary regimens the intercepts were compared. These statistical calculations include all available data. Most of the horses were

able to perform 50 min of exercise and therefore a GLM procedure for analyses of variance, using horse, diet and time as main effects was used to evaluate dietary effects during this period. This statistical model was also performed with body weight of the horses included as a covariate. Statistical significance was declared at $p < 0.05$.

RESULTS

The different diets were accepted by all horses. Mean body weight of the horses was $456 \pm 29 \text{ kg}$ at the beginning of the experiment, $458 \pm 31 \text{ kg}$ after 5 weeks, $463 \pm 34 \text{ kg}$ after 10 weeks and $477 \pm 35 \text{ kg}$ after 15 weeks at the end of the experimental period. Body weight was thus increased 3–4% at the end of the third 5 week period compared to the first and second 5 week period.

SET. All horses reached HR of 200 bpm. Values for V_{200} , $V_{\text{La}4}$ and CV kg^{-1} are shown in Table 2. The CV kg^{-1} did not differ due to diet. No difference was seen in V_{200} or $V_{\text{La}4}$ when CHO- and Fat-diets were compared. V_{200} was lower in CHO-diet and $V_{\text{La}4}$ was lower in both Fat- and CHO-diets compared to N-diet.

SEF. HR response differed somewhat between the horses and the diets. From 5 to 50 min of exercise mean HR increased in all diets and mean HR at 5 and 50 min was 142 and 151 bpm in N-diet, 151 and 159 bpm in Fat-diet and 154 and 165 bpm in CHO-diet. No differences were found between CHO- and Fat-diets in HR response. HR response in these diets was slightly higher compared to that in N-diet.

The time to fatigue ranged between 35 and 75 min and did not vary with diet. It was $56 \pm 10 \text{ min}$ in N-diet, $51 \pm 8 \text{ min}$ in CHO-diet and $52 \pm 10 \text{ min}$ in Fat-diet.

Plasma samples Exercise caused an increase in the concentration of lactate, glucose, ammonia and FFA regardless of the dietary regimen. The responses of glucose, lactate and ammonia concentrations to exercise did not differ between CHO- and Fat-diet. These diets showed a different metabol-

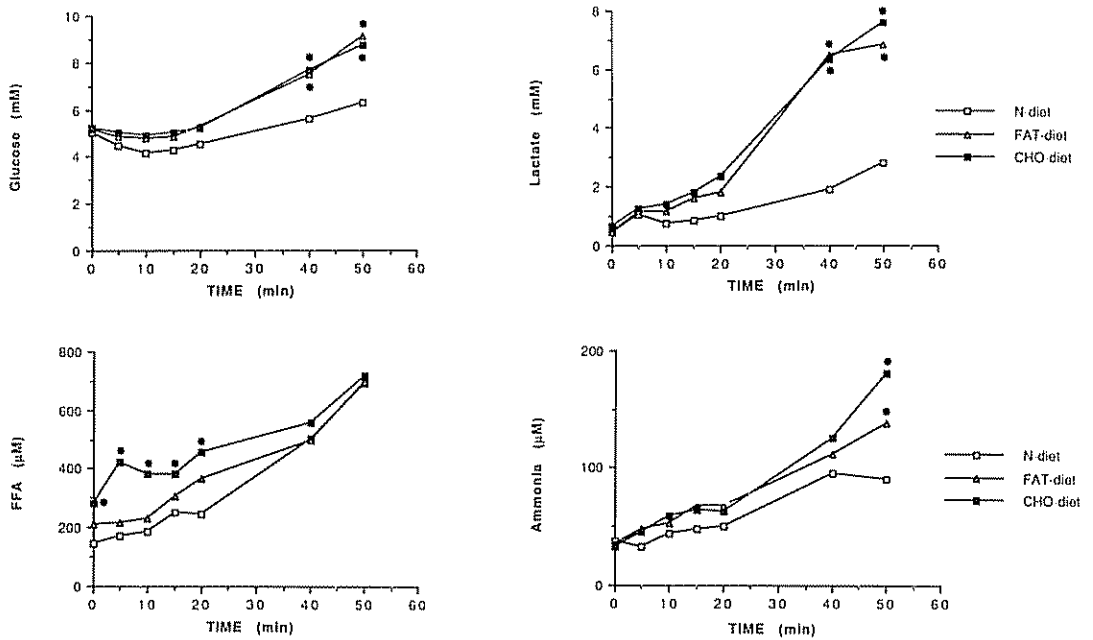


Fig 1 Mean values for plasma free fatty acid, glucose, lactate and ammonia concentrations dur-

ing 50 min of submaximal exercise at 7 m s^{-1} . An asterisk denotes significant difference to N-diet.

ic response to that in N-diet (Fig. 1). Lactate and glucose concentrations were higher after 40 and 50 min of exercise and ammonia concentration was higher after 50 min of exercise in both Fat- and CHO-diets compared to N-diet. The rate of increase in FFA concentration was lower in CHO-diet compared to both Fat- and N-diet. In CHO-diet, FFA concentrations were higher at rest and during the first 20 min of exercise compared to N-diet.

Regardless of the dietary regimen, exercise caused an increase in the concentration of all amino acids measured, except tryptophan, which was unchanged after N-diet (Fig. 2). The rate of increase in the concentration of alanine and the branched-chain amino acids during exercise did not differ between CHO- and Fat-diets. These diets showed a different metabolic response to that in N-diet. In CHO- and Fat-diets alanine concentration was 50% higher and branched chain amino acid concentration was 25% higher, compared with N-diet after 50 min of exercise.

In Fat-diet the resting level of the branched-chain amino acids was increased by 26% and in CHO-diet a tendency to higher resting level (8%) was found (5 of the 6 horses had higher levels) compared to N-diet. Resting levels and the metabolic response during exercise of the other amino acids were not affected by the different dietary regimens (Fig. 2).

Muscle samples Mean values of muscle glycogen, triglyceride, lactate and G-6-P concentrations are shown in Fig. 3. Muscle triglyceride concentration varied markedly among the horses. In one horse the values ranged between 9 and $48 \text{ mmol kg}^{-1} \text{ dw}$ in all diets, whereas in another horse the range was from 49 to 138 mmol kg^{-1} . No effect of diet on response to exercise was seen in the triglyceride concentration. The resting muscle glycogen concentration was 12% higher in CHO-diet compared to both Fat- and N-diets. Glycogen had decreased at the end of exercise on all diets. The rate of decrease in the concentration of glycogen and the rate of

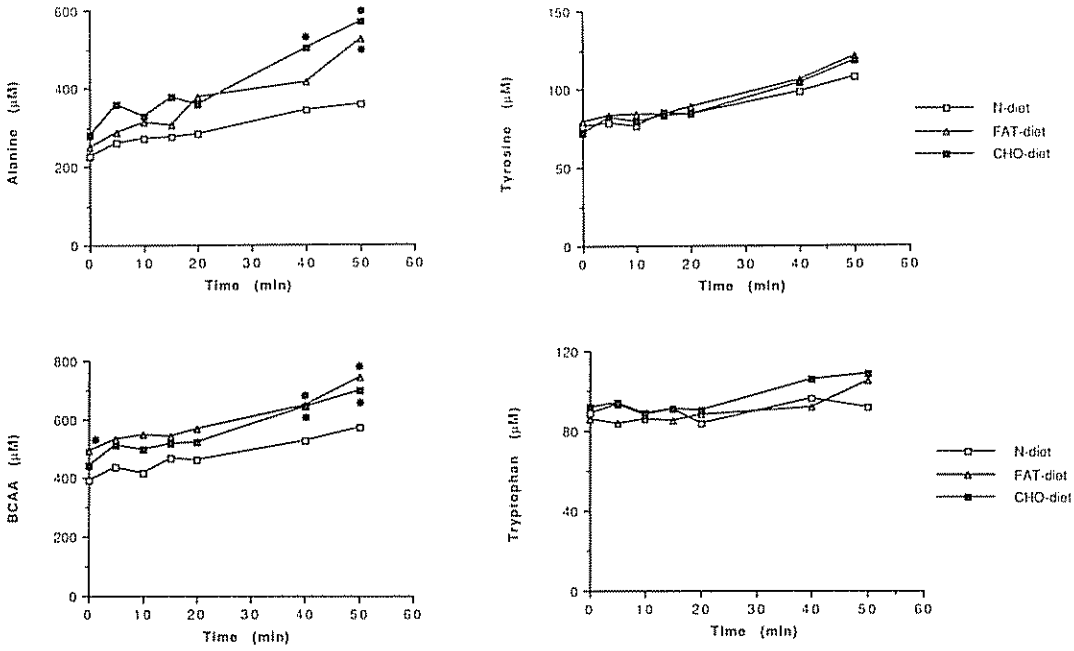


Fig. 2 Mean values for alanine, branched-chain amino acids, tyrosine and tryptophan concentrations during 50 min of submaximal exercise at 7 m s⁻¹. An asterisk denotes significant difference to N-diet.

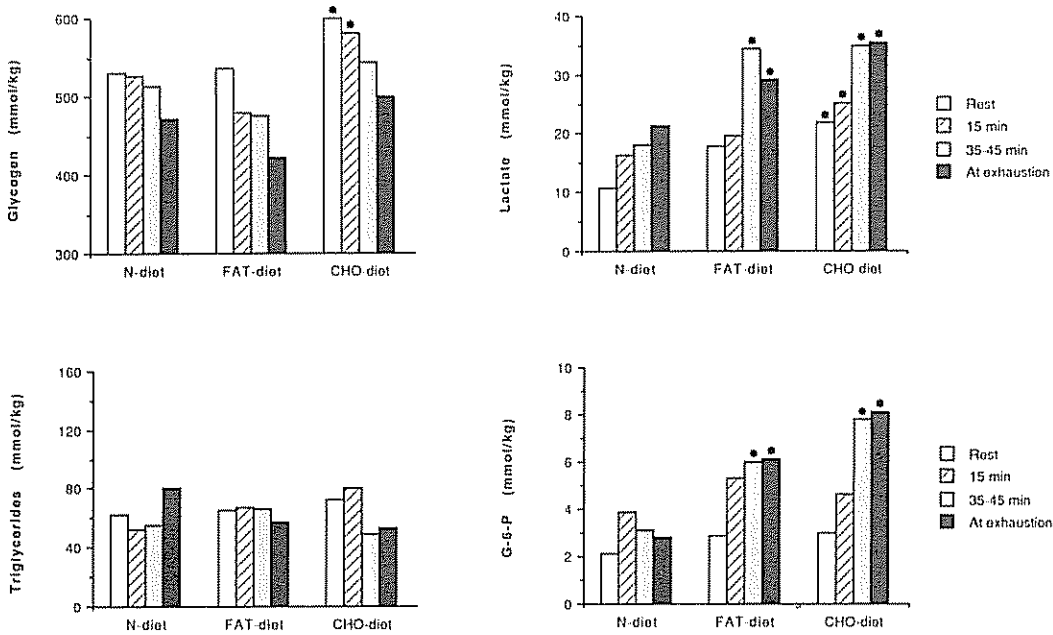


Fig. 3 Mean values for glycogen, triglyceride, lactate and G-6-P concentrations in m. gluteus during submaximal exercise at 7 m s⁻¹. An asterisk denotes significant difference to N-diet.

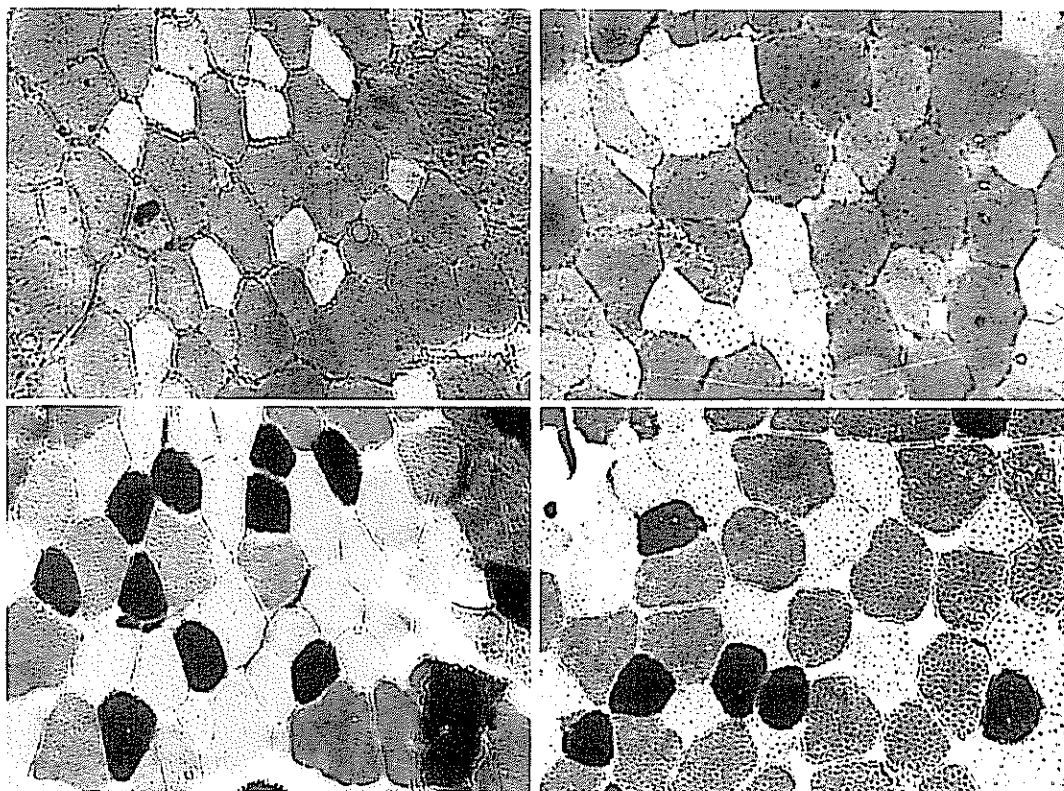


Fig 4 Serial cross-sections from m. gluteus of two horses. To the left is after 75 min and to the right is after 35 min of a submaximal exercise test to fatigue (7 m s^{-1}). The top sections illustrate the PAS stain and the bottom sections the ATP-ase

stain after preincubation at pH 4.6. Note that fibres which have low staining intensity on the PAS stain are identified as type I (black) or type IIA (white) on the ATP-ase stain

increase in lactate during exercise did not differ between diets. At rest, lactate concentrations were higher in CHO-diet compared to N-diet. The rate of increase in G-6-P concentration during exercise did not differ between CHO- and Fat-diet. A higher rate of increase was seen in CHO-diet compared with N-diet. G-6-P concentration was increased by 100% in both Fat- and CHO-diets after 35 to 45 min, whereas no change was seen in N-diet. Lactate concentration was increased 100% at the end of exercise in N- and Fat-diet and by 70% in CHO-diet. At end of exercise lactate and G-6-P concentrations were 50% and 100% higher, respectively, in CHO- and Fat-diets compared with N-diet.

Histochemical analyses The PAS stain showed that at rest all fibres contained a high amount of glycogen as they were all classified as having high staining intensity. At the end of exercise all type I fibres and about half of the type IIA fibres had a low glycogen content as they were classified as having either a low or medium staining intensity. In contrast almost all type IIB fibres had a high glycogen content at the end of exercise as they were classified as having a high staining intensity (Fig. 4, Table 3)

DISCUSSION

The results of this study show that horses tolerate marked alterations in their diets

Table 3. Percentage of low and medium PAS-stained fibres in *m. gluteus* at end of a sub-maximal exercise test to fatigue performed by 6 horses after 5 weeks on either a normal (N-diet), a fat rich (Fat-diet) or a carbohydrate rich (CHO-diet) diet

Fibre type	N-diet (n=4)	Fat-diet (n=5)	CHO-diet (n=6)
I	99	99	97
IIA	73	79	51
IIB	6	6	3

without any adverse effects. The horses were in positive energy balance during the whole experimental period as seen from the maintained and even slightly increased body weights by the end of the study. The N-diet was fed to all horses in the first 5 week period to standardise conditions, whereas CHO- and Fat-diets were both fed during the second and third 5 week period. It can be questioned if it is valid to compare these diets with N-diet as one cannot exclude a period effect. However, except for the slight increase in body weight of the horses no obvious period effect was observed. Since horses also performed exercise tests in N-diet it was of interest to compare these results with those in CHO- and Fat-diets. The change in body weight has therefore been taken into consideration when comparing N-diet with Fat- and CHO-diets.

HR and blood lactate responses to an exercise tolerance test as well as $CV\text{ kg}^{-1}$ may all indicate fitness and performance potential of horses.^{19,20} In all diets the values for $CV\text{ kg}^{-1}$, $V_{L_{3.4}}$ and V_{200} were within the normal range.²⁰ However, the lower values for $V_{L_{3.4}}$ and V_{200} in CHO-diet and for $V_{L_{3.4}}$ in Fat-diet compared to N-diet may in part be related to the fact that the horses had increased somewhat in body weight. When body weight of the horses was included in the statistical analyses as a covariate, no differences were observed in $V_{L_{3.4}}$ and V_{200} due to

diet. A previous study showed that CHO-rich diet in comparison to fat-rich diet gave rise to a higher blood lactate and HR response in 3 horses performing intense exercise.¹⁷ Therefore, one cannot exclude a possible influence of CHO-diet on $V_{L_{3.4}}$ and V_{200} during an exercise tolerance test.

A lower HR response was observed in N-diet during SEF compared with CHO- and Fat-diets. When the body weight change was considered, HR response was similar in all diets. Even though there was no marked effect of diet on the duration of submaximal exercise, the metabolic response differed somewhat. Differences between CHO- and Fat-diets were only seen in resting muscle glycogen and plasma FFA concentrations.

The lower rate of increase in plasma FFA concentration with exercise in CHO-diet may be related to the higher initial levels. When N-diet was compared with CHO- and Fat-diets the plasma concentrations of glucose, ammonia, lactate, alanine and the branched chain amino acids and the muscle concentrations of G-6-P and lactate were higher at end of exercise in CHO- and Fat-diets. Again it can be questioned if this is related to the body weight being greater on CHO- and Fat-diets. However, both diets and especially CHO-diet showed a different metabolic response to that seen in N-diet even when body weight was considered.

Glucose uptake from blood might have been slower in both CHO- and Fat-diets due to increased G-6-P levels in muscle, as it has been shown that G-6-P has an inhibitory effect on hexokinase, the enzyme which phosphorylates glucose.¹⁰ The high G-6-P and lactate concentrations in CHO-diet period may be related to the higher initial glycogen concentrations in this diet period compared to Fat- and N-diets. Studies on man and rat have shown that the rate of glycogen breakdown during exercise is accelerated by high pre-exercise glycogen levels giving rise to an inhibition of glucose uptake as well as an increase in the release of lactate from the muscle.^{6,22} An accelerated glycogenolysis would lead to an increase in the production

of pyruvate and consequently of both lactate and alanine in the muscle, which could explain the high levels of plasma lactate and alanine concentrations found after CHO-diet. The high G-6-P and lactate concentrations in muscle and the high plasma lactate and alanine concentrations in Fat-diet may not only be related to a high rate of glycogenolysis, but also to a reduced rate of pyruvate oxidation due to an inhibitory effect of lipid oxidation on pyruvate dehydrogenase.¹⁰ In both man, rat and horse there is a greater dependence on lipid metabolism during submaximal exercise after a fat rich diet.^{11,16,17} It has been shown in humans that several weeks on a fat rich diet increase muscle lipoprotein lipase activity, the enzyme that hydrolyses plasma triglycerides, and in horses, plasma triglyceride levels decrease with increasing levels of dietary fat.^{3,11} Thus, it is likely that adaptation to a fat rich diet in horses may also be related to a higher capacity for uptake of FFA from circulating plasma triglycerides.

The marked variation seen in muscle triglyceride content between horses in the present study agrees with earlier observations.^{4,14} The different diets did not seem to alter muscle triglyceride content and no changes were seen during SEF. It has been shown that muscle triglycerides can be utilized in connection with submaximal exercise but of lower intensity and longer duration than in the present study.^{4,14}

In all diets, muscle glycogen was used as a substrate for energy production during SEF, as seen from the lowered glycogen content at the end of exercise. In agreement with earlier studies, an increase was observed in glycogen content at rest after CHO-rich diet.^{17,25} Studies on humans show that CHO-rich diet enhances submaximal exercise performance and the larger the initial glycogen content the longer the work time.¹⁰ It was therefore interesting to note that in spite of increased glycogen levels in CHO-diet, none of the horses performed longer in the submaximal exercise test as compared to Fat- and N-diets. It is, however, notable that even if the

carbohydrate content differed between the diets, these were all sufficient for maintaining high glycogen stores at rest. It is only when glycogen stores are markedly reduced that a negative effect on performance time is observed.²⁵ Limited glycogen stores as a cause of fatigue were not indicated by the biochemical analyses which showed that as much as 400–500 mmol kg⁻¹ of glycogen (glucose units) was still available at the end of exercise. However, it could be seen from the histochemical stains that almost all type I fibres were depleted of glycogen at the end of exercise and that most type IIA fibres showed a reduction in glycogen content. This pattern was seen in all horses irrespective of diet and is in agreement with earlier studies in which horses have performed similar types of work.^{14,26} Glycogenolysis in type I and IIA fibres thus seems to be of great importance for energy production during submaximal exercise of the intensity used in this study. The intensity was moderate as indicated by the low HR and plasma lactate responses and this suggests that depleted fibres may play a role in development of fatigue.

There seemed to be an abundance of other substrates available for the glycogen depleted fibres in all diets as both blood glucose, lactate, FFA and branched chain amino acid concentrations increased with exercise. It can, therefore, be questioned whether continuous glycogenolysis is needed in type I and IIA fibres in order to support the energy demand during treadmill exercise at submaximal intensities. The repetitive nature of treadmill exercise may limit the ability of the horses to work with the muscles and fibres in the most efficient pattern.

Of interest was the increase in plasma ammonia concentration during exercise in all diets. During intense exercise when ATP levels are low and lactate levels are high, ammonia levels are shown to increase both in plasma and in muscle.⁵ It is thus likely that ammonia can be produced in glycogen depleted type I and IIA fibres during submaximal exercise, if resynthesis of ATP is impaired in

these fibres. This will give rise to increased AMP and ADP levels activating AMP-deaminase. This reaction, which is part of the purine nucleotide cycle, produces IMP and ammonia. Another possible metabolic pathway in which ammonia may play a role is amino acid metabolism.

The results of this study indicate that protein metabolism was influenced by different diets. It is important, however, to point out that only changes in plasma concentrations of amino acids were measured. This means that the observed changes in concentrations of amino acids can only indicate an altered uptake or release from various tissues. The results in this study indicate that different diets may alter the liver and/or muscle metabolism of alanine and branched chain amino acids whereas tyrosine does not seem to be affected by dietary regimen. Since tyrosine is an essential amino acid and is not metabolized by muscle, an increase in its plasma level suggests a net protein degradation in muscle or a decrease in the uptake of tyrosine by the liver during exercise. The plasma concentration of branched-chain amino acids increased during exercise after all 3 diets, but the increase was larger in CHO- and Fat-diets. This indicates that 5 weeks on either CHO- or Fat-diet may alter the liver and/or muscle metabolism of branched-chain amino acids which leads to elevated levels both at rest and during exercise.

A hypothesis to explain central fatigue has been based on the exercise-induced increase in the plasma ratio of tryptophan/branched chain amino acids found in human subjects after prolonged severe exercise.¹ An increase in this ratio could lead to an increase in the transport of tryptophan into the brain and also an increased production of the neurotransmitter 5-hydroxytryptamine, which has been suggested to contribute to the development of central fatigue. In the present study the increase in the plasma concentration of tryptophan was less than or similar to the increase in that of the branched chain amino acids after all 3 diets. It has been suggested,

however, that the free concentration of tryptophan, rather than the total concentration, regulates the transport into the brain. It is shown both *in vivo* and *in vitro* that a marked increase in the FFA concentration releases part of the tryptophan from albumin, thereby increasing the concentration of free tryptophan.¹ When the horses fatigued fatty acid concentrations in plasma were elevated to a great extent which may indicate that also the free tryptophan is increased. One could speculate that an increase in the concentration ratio of free tryptophan to branched-chain amino acids may have occurred and played a role in development of fatigue.

In conclusion, the results of the present study indicate that exercise time to fatigue during submaximal trotting on a treadmill for approximately one hour is not influenced by the different diets employed, while amino acid, lipid and carbohydrate metabolism during exercise may be altered by changing the dietary regimen. The question that still needs to be answered in the future is whether CHO- and Fat-rich diets have a positive influence on performance either with short maximal exercise as in races or with prolonged submaximal exercise intensities as during endurance rides.

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