DIETARY FAT SUPPLEMENTATION AND EQUINE PLASMA LIPID METABOLISM

Suzanne N.J. Geelen¹, Marianne M. Sloet van Oldruitenborgh-Oosterbaan¹ and A.C. Beynen².

¹Department of Equine Sciences, ²Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
Summary

Feeding of a fat-rich diet to horses may enhance the flux of fatty acids, in the form of triacylglycerols (TAG), through the circulation into skeletal muscle. This hypothesis was tested indirectly by measuring the concentration of plasma TAG and the activity of lipoprotein lipase (LPL) in post-heparin plasma. Six adult horses were fed a high-fat or a control diet according to a cross-over design with feeding periods of 6 weeks. The control diet contained 1.5% fat in the dry matter and the high-fat diet 11.8%. The high-fat diet was formulated by adding soybean oil to the control diet at the expense of an isoenergetic amount of corn starch plus glucose. Both diets consisted of hay and concentrate and were given on a restricted basis. Nine hours after feeding, whole-plasma TAG concentration decreased significantly by 84% following fat supplementation, whereas the whole-plasma concentrations of cholesterol and phospholipids were significantly increased by 53 and 26%, respectively. The level of HDL-cholesterol was raised by 54%. The changes in plasma lipids were accompanied by a 79% increase in LPL activity in post-heparin plasma. These results indicate that in the fasting state a high-fat diet raises the flux of fatty acids, in the form of TAG, into skeletal muscles as illustrated by the observed decrease in plasma TAG concentrations and increase in LPL activity. It is speculated that the increased flux of fatty acids is associated with an increased oxidative capacity of skeletal muscle which might be advantageous to exercising horses.
Introduction

Exercise performance in horses has been suggested to improve following a period of feeding a fat-supplemented diet (Eaton et al. 1995, Harkins et al. 1992, Oldham et al. 1990), but the mechanisms involved are still obscure. Equine muscle has a high aerobic capacity, resulting in a significant ability to use fatty acids as an energy source during high intensity exercise (Snow et al. 1983). The feeding of a high-fat diet could produce a substantial flux of fatty acids which will increase their use as fuel for aerobic metabolism. This may facilitate oxidation of fatty acids during exercise. In humans and rats, a lowering of plasma concentrations of triacylglycerols (TAG) and an increase in the activity of lipoprotein lipase (LPL) from the luminal surface of capillary endothelial cells of skeletal muscle are seen after fat feeding (Jacobs et al. 1982, Kiens and Lithell 1989, Delorme and Harris 1975). Since LPL hydrolyses TAG in chylomicrons and very-low-density lipoproteins (VLDL) so that fatty acids can be taken up by muscle tissue, an increase in LPL activity and a decrease in TAG concentrations may indicate an accelerated turnover of fatty acids. Interestingly, similar metabolic changes are induced by exercise (Jacobs 1981, Meyers et al. 1987). It appears that exercise leads to a specific adaptation of metabolism in order to use fatty acids efficiently when energy demands increase. In this light the feeding of a high-fat diet might be a suitable adjunct to training.

Orme et al. (1997) have demonstrated an increase in the total lipase activity of post-heparin plasma and a decrease in plasma TAG concentrations in trained horses given a fat-supplemented diet. LPL is released from the endothelial membranes into the circulation by intravenous administration of heparin (Watson et al. 1993). Thus, in horses, fat feeding may also increase the uptake of fatty acids by skeletal muscle which in turn activates fatty acid oxidation. High activities of LPL are generally associated with high levels of high-density lipoprotein (HDL) cholesterol (Kantor et al. 1987, Stanley et al. 1986). When TAG in VLDL are hydrolysed by LPL, surface material of VLDL particles, including apoproteins, cholesterol and phospholipids, is transferred to HDL (Stanley et al. 1986), explaining why LPL activity and HDL concentrations are directly correlated. Thus, it could be suggested that fat feeding raises HDL cholesterol in horses. Total lipase activity in post-heparin plasma includes hepatic triacylglycerol lipase (HTGL) which is located on hepatic endothelial cells, and thought to be involved in the hepatic uptake of cholesterol and phospholipids from HDL (Janssen et al. 1980, Bamberg et al. 1983, Watson et al. 1993). If fat feeding raises HDL cholesterol in...
horses, then the activity of HTGL may also be increased. Orme et al. (1997) did not measure HDL cholesterol and HTGL activity. The aim of the present study was to investigate in a cross-over design the effect of fat supplementation to the diet on a number of lipid variables in plasma which may form part of an adaptive response.

Materials and Methods

Animals and diets
Six horses (2 mares, 4 geldings) weighing 397-473 kg and aged 4-12 years were fed a high-fat or a control diet according to a cross-over design (2x2 Latin square) with feeding periods of 6 weeks. The horses were randomly allocated to the order of the diets. Thus, 3 horses received the low-fat diet followed by the high-fat diet and 3 horses had the opposite order. The diets consisted of hay and either a control or high-fat concentrate. At 08.00h and 20.00h the concentrates were offered, and at 10.00h and 22.00h the hay was provided. The high-fat concentrate was formulated by adding soybean oil to the control concentrate at the expense of an isoenergetic amount of starch plus glucose (Table 1). The diets were given on a restricted basis (i.e. at a level equivalent to 90% of the calculated amount of energy needed for maintenance of their initial body weight) to ensure that all feed was consumed. On average, the horses received 0.8 kg hay and 1.4 and 1.2 kg of the control and high-fat concentrates per meal, respectively. The control diet contained 1.5% fat in the dry matter and the high-fat diet 11.8%. The horses were individually housed in stands which were located in a ventilated stable. All animals walked each day for 60 min in a mechanical horse walker at a speed of 100 m/min.

Sampling and assay procedures
Blood samples were collected in heparinized tubes by jugular venepuncture at 07.00h each week. The samples were analysed for whole-plasma TAG, phospholipids and cholesterol concentrations and for their lipoprotein profile. Serum lipoproteins were isolated by density gradient ultracentrifugation (Terpstra et al. 1981) at the following densities (d, g/mL): VLDL, d<1.006; low-density lipoproteins (LDL), 1.019<d<1.063 and HDL, 1.063<d<1.210. Isolated lipoprotein samples were frozen and stored at -20°C until analysis. Whole plasma and lipoprotein lipid concentrations were measured enzymatically with an autoanalysers (COBAS-MI, Hoffmann-La Roche, Mijdrecht, The Netherlands) and test combinations purchased from Boehringer, Mannheim, Germany. Prior to feeding, but after regular sampling, blood samples were also obtained every 2 weeks for the analysis of LPL and HTGL at 5, 10, 20 and 60 min after intravenous injection of heparin (70 IU/kg body weight). Following plasma
preparation, samples were stored at -80°C until analysis. Total and hepatic lipase activities were determined according to Nilsson-Ehle and Schotz (1976) in the presence of a low and high concentration of NaCl, respectively. LPL activity was calculated as the difference.

Statistical analyses
Dietary effects on the plasma variables were sought using repeated measurements, two-way analysis of variance (ANOVA) with period, horse and experimental treatment as factors. The outcome is given in the legends to the figures. As the data were not significantly affected by period they were also evaluated with Student's paired t-test, using the values pooled per dietary treatment at the end of each experimental period. The results of this test are described in the text. The level of statistical significance was pre-set at $P<0.05$.

Table 1 Composition of the experimental concentrates (g)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low-fat concentrate</th>
<th>High-fat concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>193</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>140</td>
<td>-</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>Constant components *</td>
<td>850</td>
<td>850</td>
</tr>
<tr>
<td>Total</td>
<td>1183</td>
<td>1000</td>
</tr>
</tbody>
</table>

* The constant components consisted of the following (g): Alfalfa meal, dehydrated, 342.4; Corn starch, 150; Glucose, 150; Soya beans, extracted, 100; Molasses, beet, 50; Linseed expeller, 20, Ca$_3$PO$_4$, 15; NaCl, 15; MgO, 3.4, CaCO$_3$, 1.7; Premix **, 2.5

** The premix consisted of the following (g/kg): CoSO$_4$.7H$_2$O, 0.66; Na$_2$SeO$_3$.5H$_2$O, 0.76; KIO$_3$, 0.32; MnSO$_4$.H$_2$O, 172.4; CuSO$_4$.5H$_2$O, 27.2; ZnSO$_4$.H$_2$O, 192.4; Vitamin A, 12.0 (500.000 IU/gram); Vitamin D3, 5.2 (100.000 IU/gram); Vitamin E, 240.0 (500 IU/gram); Vitamin B1, 1.8 (purity 100%); Vitamin B2 (purity 100%), 1.8; Vitamin B12 (purity 0.1%), 1.8; Biotin (purity 100%), 0.4; Corn starch (Carrier), 343.26.
Results

Feed intake and body weight. The horses consumed all feed supplied. Due to the restricted feeding regimen all horses lost some weight during the course of the experiment. During the first period, weight loss averaged 7 and 6% and during the second period it was 1 and 4% for the control and high-fat diet, respectively. There was no significant diet effect on weight change.

Plasma triacylglycerols. Concentrations of whole plasma TAG (Fig. 1) did not show a significant period effect. Compared with the control diet, the high-fat diet produced significantly lower whole plasma TAG ($P=0.047$, Student's paired $t$-test).
Following the diet switch-over, the values decreased when the control diet was replaced by the high-fat diet and increased after substitution of the control diet for the high-fat diet. On average, 54% of whole plasma TAG was associated with VLDL particles.

**Whole-plasma and lipoprotein cholesterol.** Diet had a significant effect on whole-plasma cholesterol concentrations (Fig. 2). The level increased gradually during the first 6 weeks of fat feeding and then decreased gradually towards the baseline value after the diet switch-over. In the horses that were first given the control diet, whole plasma cholesterol concentration was stable until the diet switch-over and then rose when the high-fat diet was supplied. Student's paired t-test revealed a significant diet effect ($P=0.002$). Changes in whole plasma cholesterol paralleled those in HDL cholesterol (Fig. 2 and 3A). LDL cholesterol concentrations did not systematically respond to the diet changes (Fig. 3B).
Student's paired $t$-test showed a significant diet effect on HDL cholesterol ($P<0.0001$), but not on LDL cholesterol ($P=0.508$). On average, 91% of the increase in whole plasma cholesterol for the high-fat diet, was located in HDL cholesterol.

Fig. 3. Time course of cholesterol concentrations in HDL (panel A) and in LDL (panel B) isolated from plasma of the horses when they were fed the high-fat (●) or control (○) diet according to a cross-over design. ANOVA showed a significant effect of diet on HDL cholesterol ($P<0.0001$), but not on LDL cholesterol ($P=0.198$). Each point and bar represents the mean ± SEM for three horses. The triangular symbol depicts the starting values.
Plasma phospholipids. After fat loading, whole plasma phospholipids increased ($P<0.0001$, Student's paired $t$-test). The time course of whole plasma phospholipids (Fig. 4) resembled that of whole plasma cholesterol.

Post-heparin lipase activity. Total lipase activity showed no significant differences between sampling times at 5, 10 and 20 min after heparin injection. HTGL and LPL activities at 60 min post-heparin administration were on average 30 and 10% lower than those collected at 5 min after heparin injection.

There was a significant diet effect on LPL ($P<0.0001$, Student's paired $t$-test) and HTGL ($P=0.042$, Student's paired $t$-test) activities. Fat loading produced a rapid increase in LPL activity which also fell rapidly after switching over to the control diet (Fig. 5A). A similar pattern was found for HTGL activity, although the diet effect was less pronounced (Fig. 5B).
Fig. 5. Time course of LPL (panel A) and HTGL (panel B) activities in post-heparin plasma prepared from blood collected 5 min following heparin injection in the horses when they were fed the high-fat (●) or control (○) diet according to a cross-over design. ANOVA showed a significant effect of diet on LPL ($P=0.002$) and on HTGL ($P=0.009$). Each point and bar represents the mean ± SEM for three horses. The triangular symbol depicts the starting values.
Discussion

This study shows that feeding a high-fat diet to horses caused pronounced changes in lipid metabolism. Fat loading lowered the concentration of plasma TAG and raised LPL activity in plasma collected 9 hours after feeding. These observations support those of Orme et al. (1997) and indicate that lipid metabolism in horses responds to fat feeding in a fashion similar to that in humans (Jacobs et al. 1982, Kiens and Lithell 1989) and rats (Delorme and Harris 1975). The observed lowering of plasma TAG may be secondary to a fat-feeding-induced increase in LPL activity. It would follow that fat feeding raises the flux of fatty acids, at least in the form of TAG, which could facilitate the oxidation of fatty acids during exercise. Various new findings emerged from this study. Fat feeding produced an increase in both plasma concentrations of cholesterol and phospholipids. These effects probably are secondary to an increase in HDL which is the major carrier of cholesterol and phospholipids in horse plasma (Watson et al. 1993). The observed increase in HDL cholesterol is explained by the increased generation of VLDL surface material as a result of the increase in LPL activity. Fat feeding also raised the activity of HTGL so that the increased transfer of cholesterol and phospholipids to HDL may reach a new equilibrium with their removal.

In conclusion, fat feeding in horses increased post-heparin plasma LPL activity and decreased the concentration of circulating TAG, indicating an increased flux of fatty acids. The increased activity of LPL in post-heparin plasma could reflect an increase in muscle LPL activity because the samples were taken in the fasting state when only little LPL is derived from adipose tissue (Mackie et al. 1980, Terjung et al. 1982), but this needs to be demonstrated in horses fed a high-fat diet. An increase in muscle LPL could be associated with an increase in the oxidative capacity of the muscle which may be advantageous to exercising horses.

Acknowledgement

We are most grateful to Inez Lemmens for excellent technical assistance.
Fat feeding and lipid metabolism

References


