Metabolic adaptation to a fat-supplemented diet by the thoroughbred horse

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Following 10 weeks of fat supplementation a group of aerobically trained thoroughbred horses exhibited a significant decrease in postprandial plasma triacylglycerol concentration. This decrease was associated with a mean 50 % increase in plasma total lipase activity following pentosan polysulfate administration and an increase in postprandial plasma cholesterol concentration. A significant increase in the activity of muscle citrate synthase (*EC* 4.1.3.7), expressed as a ratio to the total fractional area occupied by type I and type IIa muscle fibres, was also observed. No significant change in the concentration of resting muscle glycogen or triacylglycerol occurred as a result of fat supplementation. These results suggest that there was improved management of the fat load and that the triacylglycerol-clearing capacity of the horses was increased as a result of fat supplementation. It is suggested that the increase in plasma total lipase activity following pentosan polysulfate administration may have reflected an increase in muscle lipoprotein lipase (*EC* 3.1.1.34) activity, which would increase the capacity of muscle for free fatty acid uptake from circulating triacylglycerol-rich plasma lipoproteins. Fat supplementation may also enhance the oxidative capacity of muscle, as suggested by the significant increase in muscle citrate synthase and the trend towards an increase in β-hydroxyacyl CoA dehydrogenase (*EC* 1.1.1.35) following 10 weeks of fat supplementation.

Dietary fat: Lipase activity: Horse

Previous work has suggested that the inclusion of fat in the equine diet may have beneficial effects on exercise performance, either through direct substrate effects or through some other indirect mechanism as yet unidentified. Shifts in substrate utilization from carbohydrate to fat, during prolonged low intensity (Hambleton *et al.* 1980; Hintz *et al.* 1987), moderate (Oldham *et al.* 1990) or high intensity exercise (Duren *et al.* 1987; Harkins *et al.* 1992) have been suggested. However, the mechanisms involved in this increase in fat utilization and/or enhanced performance during exercise, as a result of fat supplementation in the horse, remain unresolved. An increase in resting muscle glycogen concentration has been reported by a number of workers following fat supplementation in the horse (Meyers *et al.* 1989; Oldham *et al.* 1990; Harkins *et al.* 1992; Scott *et al.* 1992) enabling increased glycogen utilization with subsequent high intensity exercise (Oldham *et al.* 1990; Scott *et al.* 1992). However, other authors have failed to show a similar increase in resting muscle glycogen concentration (Pagan *et al.* 1987; Greiwe *et al.* 1989; Hodgson *et al.* 1995).

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Endurance exercise performance was preserved, despite dietary carbohydrate restriction and subsequent reduction in resting muscle glycogen concentration, in human subjects and rats fed on a high-fat diet (Phinney *et al.* 1983; Conlee *et al.* 1990). Increased endurance capacity has also been described in trained cyclists (Lambert *et al.* 1994), sledge dogs (Hammell *et al.* 1977) and rats (Miller *et al.* 1984) fed on diets containing 38, 70 and 78 % of the dietary energy as fat respectively, when compared with control groups fed on an isoenergetic high-carbohydrate diet. It is suggested that under conditions of carbohydrate restriction, a shift occurs in the C source for the tricarboxylic acid cycle from carbohydrate to free fatty acids (FFA) resulting in a rise in the oxidative capacity and enhancement of the β-oxidizing capacity (Simi *et al.* 1991). An increase in the activity in muscle of both citrate synthase (*EC* 4.1.3.7; CS) and β-hydroxylacyl CoA dehydrogenase (*EC* 1.1.1.35; β-HAD) has been reported in rats in response to diets containing 78 % of the energy content in the form of fat (Miller *et al.* 1984; Simi *et al.* 1991). The supply of FFA to exercising muscle may also be increased as a result of feeding a high-fat diet. Kiens *et al.* (1987) and Conlee *et al.* (1990) have reported an increase in the concentration of muscle triacylglycerol in human subjects and rats in response to a high-fat diet. Furthermore, an increase in the activity of muscle lipoprotein lipase (*EC* 3.1.1.34; LPL) in response to fat supplementation in human subjects and rats suggests an increase in the capacity of muscle to utilize FFA derived from lipoprotein-associated triacylglycerols (Delorme & Harris, 1975; Jacobs *et al.* 1982; Kiens *et al.* 1987).

The relative amount of fat contained in fat-supplemented equine diets, when expressed as a percentage of the total energy intake, is much lower in comparison with those used with other species (Delorme & Harris, 1975; Jacobs *et al.* 1982; Kiens *et al.* 1987). Fat-supplemented equine diets contain typically between 5 and 35 % of the total energy content of the diet in the form of fat (Hambleton *et al.* 1980; Hintz *et al.* 1987; Pagan *et al.* 1987, 1995; Harkins *et al.* 1992; Hollands & Cuddeford, 1992), although a lack of standardization of the expression of the fat content makes comparisons difficult.

The aim of the present study was to investigate the effect of fat supplementation on the resting concentration of certain plasma and muscle metabolites, which may form part of an adaptive response to fat supplementation. Specifically, the study aimed to assess the capacity for increased fat utilization by: (a) determining the capacity for the uptake of FFA derived from plasma lipoprotein-associated triacylglycerol, by muscle and other tissues, using measurements of post pentosan polysulfate total lipase activity (post PP T. Lip); (b) investigation of the effect of fat supplementation on muscle triacylglycerol concentration and on the activities of key oxidative enzymes in muscle.

MATERIALS AND METHODS

Seven thoroughbred horses weighing between 405 and 543 kg (three fillies and four geldings) aged between 3 and 8 years were all trained for a period of 10 weeks. A regimen of walking and trotting only was employed for the first 6 weeks of training, for approximately 1 h/d, except on Sundays. On the 7th week of training, and throughout the remainder of the experimental period, all horses additionally undertook a 10 furlong (1200 metre) canter (10–12 m/s), three times weekly. All exercise was undertaken in the morning before the first feed of the day. During the last 7 weeks of the training period, designated the baseline period, all horses were fed on a low-fat pelleted control diet (diet C, Table 1). After the baseline period, the horses were randomly divided into a control group (diet C: two fillies and one gelding) and a fat-supplemented group (group F: one filly and three geldings). Horses in group C were fed on diet C throughout the study. Horses in

group F were fed on a pelleted fat-supplemented diet (group F, Table 1) for a period of 10 weeks, designated the treatment period. Diets C and F were calculated to be isoenergetic and isonitrogenous with a metabolizable energy (ME) content of 9·8 MJ/kg which relates to an approximate digestible energy (DE) content of 12·4 kJ/kg (as calculated using Format International, Dalgety Agriculture, Bristol, Avon). Both diets were fed at 13·7 g/kg body weight together with hay at 10 g/kg body weight. The pelleted diet and hay fed throughout this study were from a single batch. Diets C and F supplied 0·22 and 1·0 g fat/kg body weight respectively. The total amounts of DE supplied by the fat contents of diets C and F, including hay, were calculated to be 3 and 20 % respectively. Subsamples of the pelleted diet and hay were commercially analysed by the same laboratory for oil (petroleum ether extract), protein, crude fibre, Ca and P in the case of the pelleted diet and for oil (petroleum ether extract), protein, DM, Ca and P in the case of the hay (Dalgety Agriculture Ltd, Table 2). Following the 10-week period of fat supplementation the horses in group F reverted to the control diet for a further 5 weeks. This phase was designated the washout period.

Feed intake

The pelleted feed and hay rations were divided into two meals that were weighed separately, each being fed at the same time each day throughout the experimental period. The pelleted portion of the diet was fed at 09.30 hours and 16.30 hours respectively, whilst each ration of hay was given 2 h before feeding the pelleted portion of the diet. Any residual feed that remained after the morning meal was carried over and added to the evening meal. Any feed that had then not been eaten by the following morning was removed and weighed. The actual feed intake and energy intake were calculated on a daily basis. No palatability problems or significant feed refusals were encountered with either diet.

Table 1. *Ingredients used in the manufacture of the control (low fat) and the fat-supplemented diets (g/kg DM)*

Ingredient	g/kg DM	
	Control	Fat-supplemented
Wheatfeed	293·0	334·0
Untreated straw	10·0	141·9
Grass	150·0	102·7
Barley	300·0	—
Wheat	29·5	—
Sunflowerseed extract	—	50·0
High-protein soyabean	—	38·0
Oat meal	—	200·0
Sugarbeet pulp	75·0	—
Soyabean oil	—	80·3
Lysine/lysine-HCl	0·7	—
Molasses	88·0	—
Limestone	13·0	23·9
Dicalcium phosphate	25·0	10·5
Salt	7·0	8·9
Minerals and vitamins	8·0	9·0
Mould inhibitor	0·8	0·8

Table 2. *Oil, crude protein, crude fibre, calcium, phosphorus, sugar and starch contents of the control and fat-supplemented diets (g/kg DM)*

Constituent	g/kg DM	
	Control	Fat-supplemented
Oil	28	95
Crude protein	114	129
Crude fibre	92	154
Calcium	16	17
Phosphorus	8	7
Starch*	223	80
Sugar*	116	50

* Calculated values.

Blood samples and body weight

At the end of the 7th week of the baseline period and at the end of every week thereafter, blood samples were obtained from all horses by jugular venepuncture. Blood samples were analysed for plasma glucose, FFA, triacylglycerol and cholesterol. Body weight was measured at the end of the 1st week of the baseline period and each week thereafter. All blood samples and body weight measurements were taken in the morning before feeding.

Muscle samples

Muscle biopsy samples were taken from the middle gluteal muscle, according to the method of Snow & Guy (1976) using a 6 mm Bergstrom–Stille needle (Bergstrom, 1962), at the end of the baseline period, after 3, 6 and 10 weeks of the treatment period and at the end of the washout period. The biopsy samples were taken alternately from the left and right middle gluteal muscle. Standardization of the biopsy site and sample depth was carried out in order to minimize the variation in the muscle fibre composition of the biopsy samples. A sub-sample of the biopsy was retained for histochemical fibre analysis and the remainder was used for the analysis of glycogen concentration. The baseline and 10-week treatment period samples were analysed for triacylglycerol concentration and CS and β-HAD activities. All muscle samples were obtained at least 24 h after exercise. In order to account for differences in the fibre composition of serial muscle biopsy samples, triacylglycerol concentration and CS and β-HAD activities were expressed as a ratio to the combined fractional area occupied by types I and IIa fibres.

Post pentosan polysulfate total lipase activity

Plasma samples were obtained for the analysis of total lipase (T. Lip) activity, both before and 10 min after the intravenous injection of pentosan polysulfate (PP, 1·3 mg/kg body weight), a synthetic heparinoid-type substance, which has previously been reported to cause the release of both LPL and hepatic lipase (*EC* 3.1.1.3; HL) into the circulation (Fischer *et al.* 1982*a*, *b*; Barrocliffe *et al.* 1986). T. Lip activity following PP injection was measured at the end of the baseline period, after 3, 6 and 10 weeks of the treatment period and at the end of the washout period. Plasma T. Lip activity was analysed according to the method of Watson *et al.* (1992). Activity was measured in the presence of a low

concentration of NaCl (0·1 M) without pre-incubation with SDS. Activities are reported as μmol FFA produced/ml plasma per h.

Plasma analysis

Plasma FFA (Wako NEFA C, Alpha Laboratories, Eastleigh, Hampshire), glucose (Randox Laboratories, Co. Antrim, N. Ireland) and cholesterol (Randox Laboratories) were analysed using commercially available kits, adapted for use on a Kone specific autoanalyser (Labmedics, Stockport, Ches.). Plasma triacylglycerols were saponified using alcoholic KOH, Plasma (200 μl) was added to ethanol (500 μl, 950 ml/l) and KOH (20 μl, 8 M), vortex-mixed and heated at 70° for 20 min. After cooling, $MgSO_4$ (1000 μl, 0·15 M) was added, and the samples vortex-mixed and centrifuged. The aspirate was analysed for glycerol, according to the method of Bergmeyer (1986), to give the plasma total glyceride-glycerol concentration. Plasma triacylglycerol concentrations include the contribution made by plasma glycerol concentration. The resting concentration of plasma glycerol in the thoroughbred horse has previously been reported to be relatively low, typically about 25 μmol/l (Orme *et al.* 1995).

Muscle analysis

All muscle analysis was carried out on freeze-dried powdered muscle which had been dissected free of any visible blood, fat or connective tissue. Glycogen was analysed according to the method of Essen & Henriksson (1974). CS activity was analysed at 25° according to the method of Alp *et al.* (1976). Determination of muscle β-HAD activity was made on freeze-dried muscle homogenized in phosphate buffer (K_2HPO_4 and KH_2PO_4, 50 mmol/l, pH 7·5). The homogenate was added to a reagent solution containing triethanolamine (80 mmol/l, pH 7·5); EDTA (0·6 mmol/l); Na-NADH (0·18 mmol/l) and acetoacetyl CoA (0·05 mmol/l). The conversion of NADH to NAD with its associated absorbance change at 340 nm was monitored over a 20 min period at 25° and enzyme activity per min calculated. Activities are reported as IU per kg dry muscle, where 1 IU is equivalent to the consumption of 1 mmol substrate per min.

Triacylglycerol. Lipid extracts of freeze-dried muscle (5 mg) were prepared by sonication in 3 ml chloroform–methanol (2 : 1, v/v) on ice, using an ultrasonic disintegrator (MSE Soniprep 150, Fisons, Crawley, Sussex, 18 μm, 4 × 30 s). NaCl (0·15 M, 400 μl) was then added and the samples centrifuged at 4000 rev./min for 5 min. The lipid-containing layer was aspirated and dried under a stream of N_2 before being reconstituted into 550 μl chloroform. Tri-, di- and monoacylglycerols were separated together by solid-phase extraction using glass columns packed with aminopropyl bonded phase (Isolute, Jones Chromatography Ltd, Hengoed, Mid Glamorgan), according to the method of Renuka-Prasad *et al.* (1988). The tri-, di- and monoacylglycerol-containing eluate was dried down under N_2 and reconstituted in ethanol (950 ml/l, 250 μl) and KOH (8 M, 10 μl), vortex-mixed and heated at 70° for 20 min. After cooling, $MgSO_4$ (0·15 M, 500 μl) was added and the extracts vortex-mixed and centrifuged at 4000 rev./min for 5 min. The supernatant fraction was removed and analysed for glycerol according to the method of Bergmeyer (1986).

Table 3. *Daily energy intake (MJ/kg) and body weight (kg) during the baseline, treatment and washout periods in horses in the control and fat-supplemented dietary groups*

(Mean values and standard deviations for three (control) or four (fat supplemented) horses)

Dietary group ...	Daily energy intake (MJ/kg body wt)				Body wt (kg)			
	Control		Fat supplemented		Control		Fat supplemented	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	0·226	0·025	0·238	0·009	435·9	43·7	461·4	62·7
Treatment	0·243	0·012	0·248	0·005	434·6	43·4	456·3	63·0
Washout	0·243	0·006	0·245	0·006	442·3	43·9	460·8	59·1

Histological staining of muscle biopsy sections. Muscle samples for histochemical analysis were mounted in OCT embedding compound (Tissue Tek II, Miles Labs. Inc., Elkhart, IN, USA) and serial 15 μm sections were cut using a Bright OFT motor-driven cryostat (Bright Instruments, Huntingdon, Cambs.). Sections were stained for myofibrillar actinomyosin ATPase (*EC* 3.6.1.32) after pre-incubation at pH 4·5 (Brooke & Kaiser, 1970). Stained sections were mounted and photographed using a Leitz Dialux 20 stereo microscope (Leitz, Wetzlar, Germany) with a Wild MPS 51S camera (Wild, Heerbrugg, Switzerland) and MPS 45 automatic controller (×10 magnification). From the photographs, all fibres within a given field were classified as either type I, IIa or IIb fibres. The individual fibres on the photographs were then individually cut out and the total number of type I, IIa and IIb fibres weighed. Fractional fibre area occupied was calculated by expressing the weight of a given fibre type within a given field as a fraction of the total fibre weight (I + IIa, IIb) within that same field.

Statistical analysis

A two-factor ANOVA for repeated measures was used to identify significant effects of diet × time interactions during the sampling period. Dietary treatment was designated as factor 1 with two levels: control and fat-supplemented. The second factor, the repeat measure, was time in weeks. Significance was declared at $P < 0{\cdot}05$. Where significance was established a multiple comparison test (Fisher's protected least significant difference test) was applied, and again significance reported at $P < 0{\cdot}05$. An unpaired two-tailed Student's *t* test was used to identify differences between diets. A paired two-tailed Student's *t* test was used to identify differences in muscle triacylglycerol, β-HAD and CS within dietary group.

RESULTS

Feed intake and body weight

With the exception of one horse feed intake was sufficient for maintenance of body weight at the level of work undertaken and was in these maintained at initially designated levels. In the remaining horse, a 3-year-old gelding, a slow decline in body weight was observed, during the first 5 weeks of the treatment period. The pelleted portion of the feed of this

horse was therefore increased by 1·2 kg and the hay by 0·8 kg. In all other horses, body weight fluctuated randomly within 5 % of initial body weight with no apparent effect of diet (Table 3). There was no significant effect of diet on body weight during the treatment period ($P = 0·3$). Horses in group C, however, showed a small but significant increase in body weight by the 4th week of the washout period with respect to week 1 ($P = 0·029$).

Plasma glucose and free fatty acids

There was no significant effect of diet during the treatment period between dietary groups with respect to plasma glucose ($P = 0·106$, Fig. 1(a)) or plasma FFA ($P = 0·362$, Fig. 1(b)).

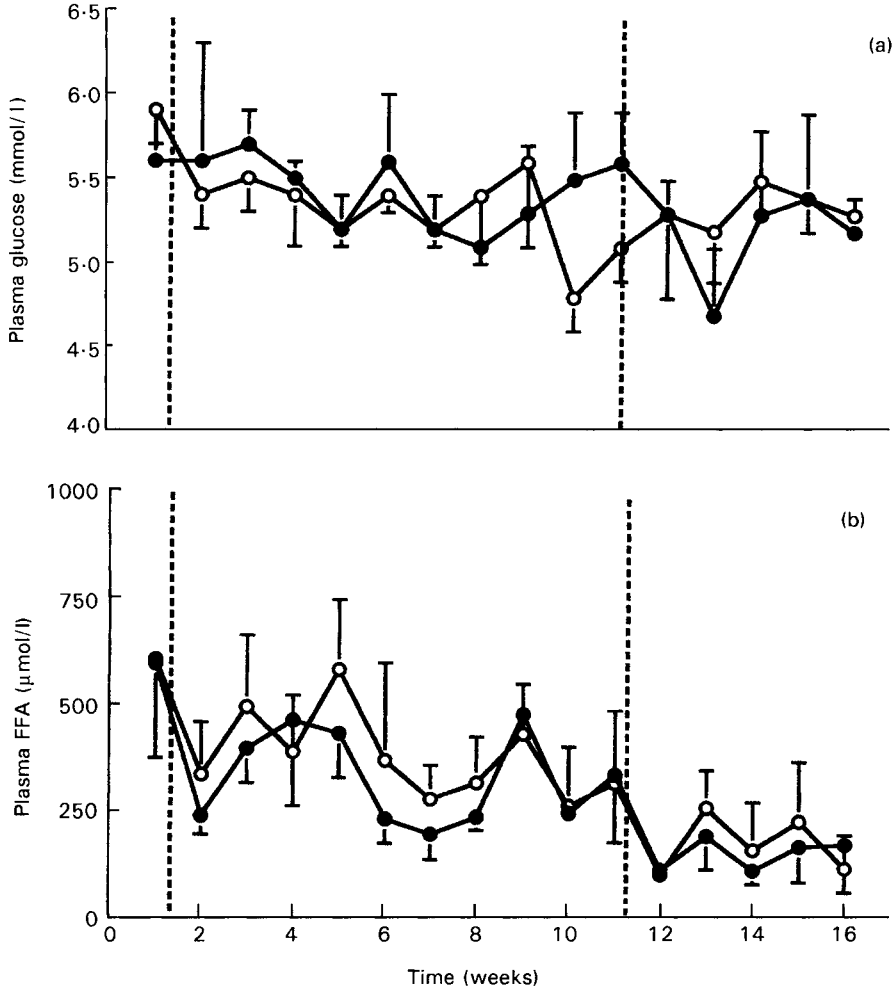

Fig. 1. Resting (a) plasma glucose and (b) plasma free fatty acids (FFA) concentrations of horses receiving a control diet (●) or a fat-supplemented diet (○). Week 1, end of baseline period; weeks 2–11, treatment period; weeks 12–16, washout period. Values are means for three (control) or four (fat-supplemented) horses, with standard deviations represented by vertical bars.

Plasma post pentosan polysulfate total lipase activity

Plasma T. Lip activity measured before the injection of PP was negligible ($< 0{\cdot}1$ μmol/ml per h) and was unchanged in either dietary group during the sampling period. There was a highly significant effect of diet during the treatment period between the two dietary groups ($P = 0{\cdot}0004$). Horses in group F exhibited a significant increase in post PP T. Lip activity during the period of fat supplementation (Fig. 2(a)). The post PP T. Lip activity was significantly elevated above that measured during the baseline period following 3 weeks of fat supplementation. Peak enzyme activity was reached after either 6 or 10 weeks of fat supplementation in individual horses. Mean post PP T. Lip activity was no longer significantly different from the baseline activity 5 weeks after the withdrawal of the fat-supplemented diet.

Plasma triacylglycerol

A significant effect of diet was identified during the treatment period between dietary groups ($P = 0{\cdot}038$). The plasma triacylglycerol concentration of the horses in group F decreased significantly during the period of fat supplementation and was significantly lower than the baseline concentration after 9 weeks of fat supplementation. The concentration was also significantly lower than that of the horses in group C during this period (Fig. 2(b)).

Plasma cholesterol

Plasma cholesterol concentration was unchanged throughout the sampling period in the horses in group C ($P = 0{\cdot}13$) but a highly significant effect of diet was identified within group F ($P = 0{\cdot}0001$) (Fig. 2(c)). Within group F, the cholesterol concentration was significantly greater than the baseline concentration after 1 week of fat supplementation, but this was no longer significantly different 3 weeks after withdrawal of diet F. Although the plasma cholesterol concentration was increased during fat supplementation, the concentration remained within the normal concentration range at all times (1·9–3·9 mmol/l; diagnostic laboratory, Animal Health Trust) and there was no significant difference between the two dietary groups ($P = 0{\cdot}64$).

Muscle metabolites and enzyme activities

The mean fractional area occupied by type I, IIa (high oxidative) and IIb (glycolytic) fibres in muscle biopsy samples is reported in Table 5. There was no significant effect of either diet on muscle glycogen concentration ($P = 0{\cdot}1726$, Table 4). Similarly, there was no change in muscle triacylglycerol in either dietary group C or F ($P = 0{\cdot}24$, $P = 0{\cdot}50$ respectively, Table 6). There was, however, a significant increase in muscle CS activity (expressed as a ratio to the combined fractional area occupied by type I and IIa fibres) after 10 weeks of fat supplementation in dietary group F ($P = 0{\cdot}0139$, Table 6). There was no corresponding change in CS activity in dietary group C ($P = 0{\cdot}95$). There was a trend towards an increase in muscle β-HAD activity after 10 weeks of fat supplementation in dietary group F (three out of four horses); however, this increase was not significant ($P = 0{\cdot}56$, Table 6).

Table 4. *Muscle glycogen concentration during the baseline (pre), treatment (3, 6 or 10 weeks fat supplementation in the treatment group) and washout (following 5 weeks withdrawal of the fat-supplemented diet) periods in horses in the control and fat-supplemented dietary groups**

(Mean values and standard deviations for three (control) or four (fat-supplemented) horses)

	Muscle glycogen (mmol/kg DM)			
	Control		Fat supplemented	
Dietary group	Mean	SD	Mean	SD
Pre	727	80	596	26
3 weeks	600	66	575	46
6 weeks	679	29	610	53
10 weeks	672	49	620	25
Washout	653	26	647	29

* For details of diets and procedures, see Tables 1 and 2 and pp. 444–447.

Table 5. *Fractional area occupied by type I, IIa and IIb muscle fibre types in biopsy samples taken from horses in the control (C) and fat-supplemented (F) dietary groups both before (pre) and after the period of fat supplementation*

(Mean values and standard deviations for three (control) or four (fat-supplemented) horses)

Muscle fibre ...	Type I		Type IIa		Type IIb		Type I + IIa	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre								
Group C	0·16	0·10	0·36	0·06	0·48	0·15	0·52	0·15
Group F	0·14	0·04	0·51	0·09	0·36	0·13	0·65	0·13
10 weeks supplementation								
Group C	0·11	0·03	0·40	0·13	0·52	0·12	0·48	0·12
Group F	0·08	0·04	0·40	0·13	0·52	0·12	0·48	0·12

DISCUSSION

Alterations in muscle metabolism during exercise, following a period of exposure to a fat-supplemented diet, have been widely reported in the horse although the mechanisms involved have not as yet been elucidated. It is possible that a direct substrate effect due to a change in the diet, or an adaptational response that affects the ability to utilize fat during exercise may explain the observed response. Alternatively, a shift in exercise metabolism may occur as a consequence of hormonal changes, arising as a result of the change in the fat : soluble-carbohydrate ratio in the diet.

An elevation in resting plasma FFA concentration, before exercise, has been previously reported to increase fat utilization during subsequent low–moderate intensity exercise, in the horse (Orme *et al.* 1995) and other species (Costill *et al.* 1977; Hickson *et al.* 1977: Ravussin *et al.* 1986). A diet-induced increase in resting plasma FFA concentration was not, however, demonstrated in response to the period of fat supplementation in the current study. Postprandial plasma glucose concentration was also not significantly influenced by dietary treatment.

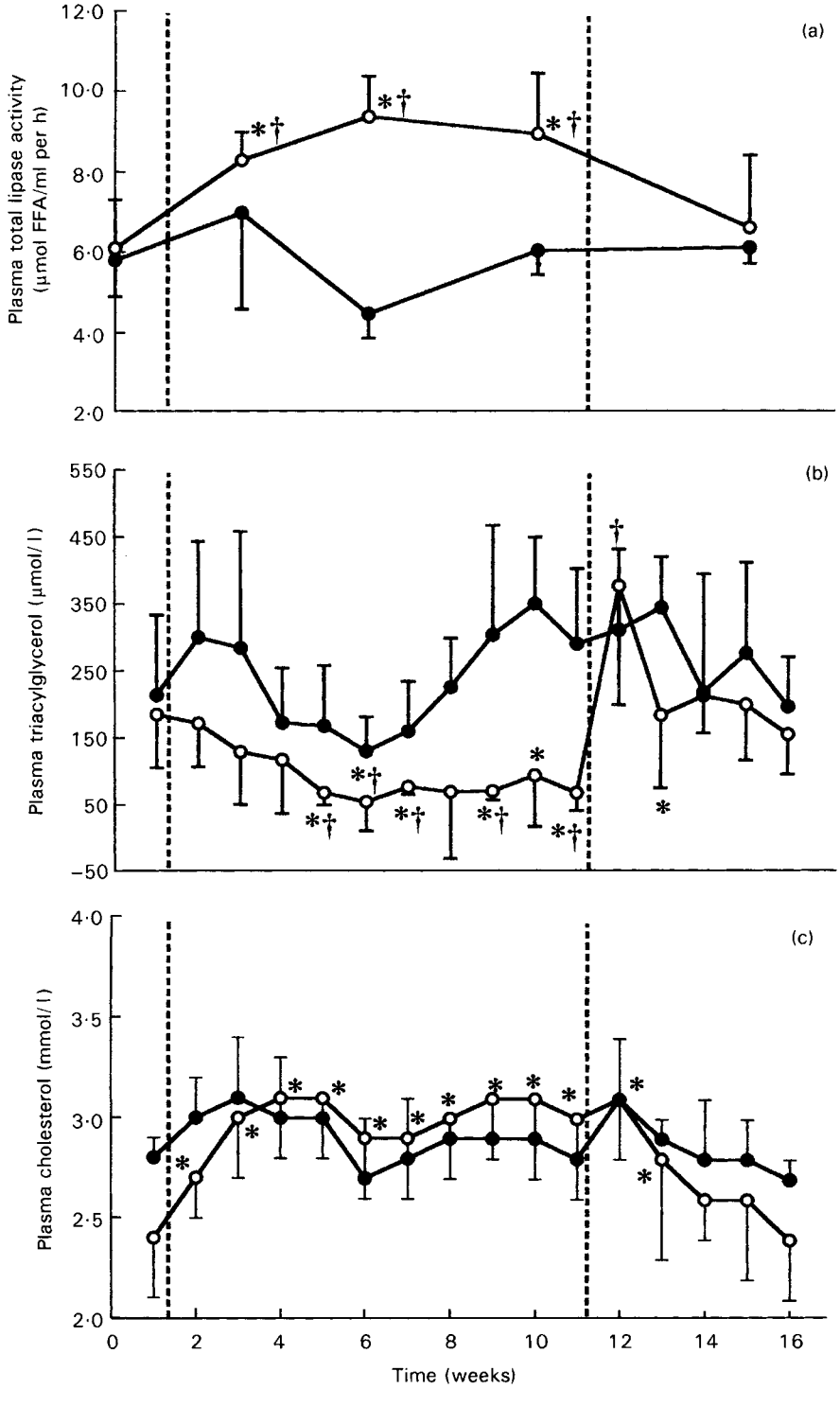

Table 6. *Muscle citrate synthase (CS) and β-hydroxyacyl CoA dehydrogenase (β-HAD) activities (IU/kg DM), muscle triacylglycerol concentration (mmol/kg DM) and muscle CS and β-HAD activities and triacylglycerol expressed as a ratio to the combined fractional area occupied by type I and IIa fibres in biopsy samples taken from horses in the control (C) and fat-supplemented (F) dietary groups, before (pre) and after 10 weeks of fat supplementation*†

(Mean values and standard deviations for three (control) or four (fat-supplemented) horses)

	CS (IU/kg DM)		β-HAD (IU/kg DM)		Triacyl-glycerol (mmol/kg DM)		Ratio					
							CS (IU/kg DM)		β-HAD (IU/kg DM)		Triacyl-glycerol (mmol/kg DM)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre												
Group C	122·8	41·1	18·9	7·8	18·0	18·7	238·0	46·5	39·8	23·8	35·8	31·8
Group F	118·5	27·2	22·7	5·3	7·0	4·9	186·8	48·6	36·2	10·0	11·1	8·7
10 weeks supplementation												
Group C	114·9	29·4	21·0	1·2	7·5	5·7	201·4	28·9	38·4	11·4	12·8	7·9
Group F	108·6	9·3	18·7	3·7	5·4	3·6	236·3*	52·4	40·4	8·7	12·9	10·3

* Mean value was significantly different from the corresponding Pre concentration or activity ($P = 0{\cdot}0139$).
† For details of diets and procedures, see Tables 1 and 2 and pp. 444–448.

A significant increase in the plasma post PP T. Lip activity occurred in response to fat supplementation and was associated with a significant reduction in plasma triacylglycerol concentration. The post PP T. Lip enzyme activity was increased in the horses in group F by an average of 50 % as a result of fat supplementation. Plasma post PP T. Lip activity represents the sum of heparin-releasable LPL and HL. Triacylglycerols are not taken up directly into tissues but are first hydrolysed to their constituent FFA and glycerol. LPL is involved in the removal of FFA and glycerol from the triacylglycerol moiety of triacylglycerol-rich lipoprotein particles (Cryer, 1987). Its functional location is the endothelial cell surface of extra-hepatic tissue capillaries, mainly adipose tissue and skeletal muscle (Robinson, 1987). HL is responsible for remodelling circulating lipoproteins and is involved in the removal of cholesterol and phospholipids from HDL and LDL (Jansen *et al.* 1980; Watson *et al.* 1993*b*). The observed increase in plasma post PP T. Lip activity therefore suggests an increase in either or both adipose or muscle tissue LPL, or an increase in the activity of HL. The increase in T. Lip activity is likely to be due to an increase in LPL since it was associated with a reduction in postprandial plasma triacylglycerol concentration. An increase in muscle heparin releasable LPL activity has been reported to occur as the result of feeding diets containing between 65 and 78 % of the energy as fat, in human subjects and in rats (Delorme & Harris, 1975; Jacobs *et al.* 1982).

Fig. 2. Resting (a) post pentosan polysulfate plasma total lipase activity (as determined by the release of free fatty acids, FFA), (b) plasma triacylglycerol concentration and (c) plasma cholesterol concentration of horses receiving a control diet (●) or a fat-supplemented diet (○). Week 1, end of baseline period; weeks 2–11, treatment period; weeks 12–16, washout period. Values are means for three (control) or four (fat-supplemented) horses, with standard deviations represented by vertical bars. *Mean values for fat-supplemented horses were significantly different from week 1, $P < 0{\cdot}05$. † Mean values for the two dietary groups were significantly different, $P < 0{\cdot}05$.

Furthermore, an increase in the activity of heparin-releasable muscle LPL, as a result of dietary manipulation (Delorme & Harris, 1975), fasting (Delorme & Harris, 1975; Lithell *et al.* 1978) or exercise (Borensztajn *et al.* 1975; Taskinen *et al.* 1980) is also associated with a reciprocal decrease in the activity in adipose tissue. In other words, as the capacity for uptake of FFA by skeletal muscle from lipoprotein-associated triacylglycerols is increased, there is a reciprocal decrease in their uptake by adipose tissue.

The fasting activity of post-heparin plasma LPL in suckling foals is also higher compared with that reported in adult ponies (Watson *et al.* 1993*a*) which may reflect the increased fat content of the diet in the form of mares' milk. A triacylglycerol-rich chylomicron-like lipoprotein fraction has also been identified in the non-fasting plasma of suckling foals (Watson *et al.* 1991) in contrast to adult horses. The absence of a chylomicron lipoprotein fraction in adult horses may be a reflection of their diet, which is normally low in fat and lacking in long-chain fatty acids (Watson *et al.* 1991).

Summerfield *et al.* (1984) reported that HL activity was lower in rats fed on a high-fat diet in comparison with those fed on a high-carbohydrate diet. The reduced HL activity, however, was associated with a lower plasma cholesterol concentration (Summerfield *et al.* 1984). This is the opposite to that observed in the present study. HL acts on lipoprotein particles with a much lower triacylglycerol content than are acted on by LPL and hence the reduction in plasma triacylglycerol observed is probably the result of an increase in LPL activity. The increase in the activity of post PP T. Lip was probably the result of an increase in muscle LPL that was larger than a reciprocal decrease in adipose tissue LPL activity. The decrease in plasma triacylglycerol concentration, associated with the fat-supplemented diet, indicates a better management of the fat load and has been previously reported in the horse (Duren *et al.* 1987). Similarly, Harris & Felts (1973) reported an increase in the rate of removal of infused labelled triacylglycerol from the plasma of rats fed on a high-fat diet compared with those fed on a control diet.

The stimulus for the increase in post PP T. Lip activity is unknown. The suggested changes in LPL activity may have been induced by hormonal changes occurring as a result of the change in diet. Insulin has previously been implicated in the regulation of adipose tissue and muscle LPL, although its effect on LPL activity appears to be tissue dependent (Jacobs *et al.* 1982; Kiens & Lithell, 1989). Recent findings suggest that increased plasma insulin concentrations may down-regulate muscle LPL activity, possibly as a result of an insulin-mediated increase in tissue glucose metabolism (Kiens & Lithell, 1989). In contrast insulin appears to stimulate adipose tissue LPL activity, again possibly as a result of a change in tissue glucose metabolism. Fat supplementation appears to be associated with a lower plasma insulin concentration in the horse following feeding (Stull *et al.* 1987; Pagan *et al.* 1995) and this may also influence tissue LPL activities.

Plasma cholesterol concentration was significantly elevated as a result of fat supplementation, although it remained within the normal concentration range at all times. An increase in total plasma cholesterol evoked by a high-fat or fat-supplemented diet has been observed in the horse (Apter *et al.* 1995) in response to supplementation with animal fat. This has also been widely reported in other species in response to polyunsaturated fat (see review by Rudney & Sexton, 1986). An increase in cholesterol concentration may arise as a consequence of increased dietary intake or as a result of increased cholesterol biosynthesis. The cholesterol content of both the control and fat-supplemented diets in this study is likely to be minimal since their main ingredients, cereal grains, forages, grain by-products and soyabean oil have an extremely low cholesterol content (Lentner, 1981). It is therefore likely that the increase in plasma cholesterol concentration was due to endogenous synthesis, possibly as a result of increased production of acetyl CoA due to

an increased flux through β-oxidation. It has previously been suggested that cholesterol biosynthesis may represent an alternative pathway for acetyl CoA when lipogenesis is suppressed (Hill *et al.* 1960). Alternatively, an increase in the triacylglycerol : cholesterol ratio of VLDL, intermediate density lipoproteins and LDL may indirectly stimulate cholesterol biosynthesis via a change in the activity of 3-hydroxy-3-methylglutaryl CoA reductase (*EC* 1.1.1.88), a key enzyme in the cholesterol biosynthetic pathway (Van Zuiden *et al.* 1983). As triacylglycerols are removed from the latter lipoproteins, they are replaced with a cholesterol ester in order to maintain lipoprotein stability; an increase in triacylglycerol load may therefore necessitate an increase in cholesterol biosynthesis.

No significant increase in muscle glycogen concentration was observed in response to fat supplementation in this study. This is in contrast to the work in horses carried out by Meyers *et al.* (1989), Oldham *et al.* (1990), Harkins *et al.* (1992) and Scott *et al.* (1992). Muscle glycogen concentration was comparable to those equine studies similarly reporting no significant change in muscle glycogen concentration in response to a comparable level of fat supplementation (Pagan *et al.* 1987; Greiwe *et al.* 1989; Hodgson *et al.* 1995). The elevation in muscle glycogen concentration reported in the former studies may have been influenced by the relatively low concentrations reported both before and after the period of fat supplementation. Random variation in muscle glycogen concentration probably reflects differences in biopsy site, which may, at least in part, be attributed to variation in the fibre composition of biopsy samples. Type I fibres may contain less muscle glycogen than type II fibres (White & Snow, 1987). Snow & Harris (1991) estimated that the variance between sampling sites, within horses undertaking race-type training, was 57 mmol glycosyl units/kg dry muscle. Nutritional and training status are other factors which may influence muscle glycogen content.

An increase in the oxidative capacity of muscle has been previously suggested to occur as the result of a high level of fat supplementation in rats (Miller *et al.* 1984; Simi *et al.* 1991). In agreement with these latter studies, fat supplementation in the present study was associated with an increase in both muscle CS and β-HAD activity. Statistical significance was, however, only achieved with respect to muscle CS activity. Although an attempt was made to correct for differences in the fibre composition of biopsy samples, definitive confirmation of the increase in these oxidative enzymes could potentially only be achieved using measurements in single muscle fibres.

The absolute fat content of diet F used in this study, although comparable to that used in other equine studies, is much lower than that in diets which have been used in human and rodent studies. In the present study, the addition of fat to the diet represented a 5-fold increase in the relative fat content compared with that in the control diet. In comparison, the addition of fat to a level of about 78 % of the total energy intake of the diet of a human athlete, may represent only a 2-fold increase in the relative fat content. This is because the average consumption of fat, even in athletes, may be as high as 38 % of the total energy intake (Wilmore & Costill, 1994). The relative increase in fat content may be more important that the absolute content *per se* in terms of adaptation.

In summary, fat supplementation has been shown to be associated with improved metabolic management of the fat load as illustrated by a decrease in the resting plasma triacylglycerol concentration. An increase in total cholesterol concentration, which remained within the normal range at all times, also occurred as a result of fat supplementation. The authors suggest that the reduction in plasma triacylglycerol concentration may have occurred as the result of an increase in the ability of muscle to utilize lipoprotein-associated plasma triacylglycerol, as a result of an increase in the activity of muscle LPL. Additionally fat supplementation may have resulted in an increase

in muscle oxidative capacity as indicated by an increase in muscle CS and possibly β-HAD activity in the horses in dietary group F.

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