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Effect of training duration and exercise on blood-borne substrates, plasma lactate and enzyme concentrations in Andalusian, Anglo-Arabian and Arabian breeds

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Summary

Metabolic responses to exercise differ between Andalusian horses and other breeds, although changes in plasma muscle enzymes have not been reported and most useful information is obtained from animals subjected to different training programmes. The objectives of this study were to 1) describe the changes in plasma enzymes during exercise in different horse breeds in relation to other biochemical parameters (*Experiment A*) and 2) assess the effect of training duration on these measures (*Experiment B*).

Twenty stallions, 9 Andalusian (AN), 7 Arabian (A) and 4 Anglo-Arabian (AA), age 5–10 years, were studied. They performed 3 exercise tests (ET), consisting of a warm-up of 800 m at 0.7 km/h and 4 workloads at 15, 20, 25 and 30 km/h, at respective distances of 1250, 1670, 2080 and 2500 m, with 5 min active recovery between each workload (*Experiment A*). Three ETs were performed at the beginning and after 2 and 6 months of training (*Experiment B*). Venous blood samples were collected during the ETs and plasma glucose (GLU), free fatty acids (FFA), lactate (LA), creatine kinase (CK), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBHD), aspartate aminotransferase (AST), Na^+, K^+ and Cl^- were measured.

AN horses responded to exercise with greater increases in GLU, HBHD, LDH, CK and AST compared to the other breeds. An unexpected result in *Experiment A* was the lack of interbreed differences in plasma peak LA concentrations, since it is commonly accepted that AA and A horses have greater athletic potential. Although the glycolytic response to exercise was reduced after 2 months of training in the AA and A horses, and after 6 months of training in the AN horses, at the end of *Experiment B*, AN horses produced more lactate than the other 2 breeds. Most of the adaptations linked to training were found in the AN breed. The more striking changes in plasma enzyme activities corresponded to CK in AN horses after 2 months of training. The attenuation of CK response to exercise was related to lower extrafibrilar GLU utilisation with LA formation and greater fat metabolism. The results show that plasma muscle enzyme concentrations for the diagnosis of equine myopathies must be interpreted in relation to breed and training.

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Introduction

Myopathies are an important clinical entity in performance horses, and different classifications have been reported recently (Rivero 1999), after investigating their aetiology and pathophysiology (De la Corte *et al.* 1999; Valberg *et al.* 1999; Divers *et al.* 2001). Among the more recently described muscle pathologies are equine polysaccharide storage myopathy and equine motor neuron disease.

Although the importance of evaluation of muscle biopsy samples is now recognised, clinical and rapid field diagnosis relies basically on clinical signs and plasma enzyme activities. Increased plasma activities of muscle enzymes are believed to reflect skeletal muscle damage in both man (Clarkson and Ebbeling 1988) and horses (Lindholm 1987; Siciliano *et al.* 1995). In this context, the enzymes most commonly used are cytosolic creatine kinase (CK; EC 2.7.3.2), lactate dehydrogenase (LDH; EC 1.1.1.27) and cytosolic and mitochondrial aspartate aminotransferase (AST; EC 2.6.1.1). CK is an enzyme found in skeletal muscle, myocardium and brain, although very little exchange occurs between cerebrospinal fluid and plasma. CK is rapidly released from muscle after injury, with a peak activity at 4–12 h after a muscle insult and removed from blood in 2 h ($T_{1/2}$). By contrast, AST and LDH are found in most soft tissues, with peak concentrations after 24 h and a much longer half-life (7 days) (Cardinet *et al.* 1963; Clarkson and Ebbeling 1988; Harris 1998).

Plasma muscle enzymes (PME) of healthy, unexercised horses have been reported to range from 90–275 u/l for CK, 230–311 u/l for AST and 150–240 u/l for LDH, while in horses with exertional rhabdomyolysis, enzyme activities might increase 10–900 and 5–100-fold for plasma CK and AST, respectively (McEwen and Hulland 1986; Valberg *et al.* 1993). However, many different factors could influence the plasma activity of these enzymes, such as age, sex and the characteristics of exercise and training programmes. Harris *et al.* (1990) showed the highest incidence of elevated PME in 2-year-old Thoroughbred horses. This apparent effect of age on the incidence of increased PME activities was associated with loss of training days, due either to overt muscle problems or subclinical problems in the horses' 2-year-old season. By contrast, another study of over 100 Thoroughbred mares age 2–4 years failed to show any age effect on resting AST and CK activities (Gigli *et al.* 1996). In relation to sex, it has been demonstrated that elevated PME is more common in mares than in male horses, suggesting a hormonal predisposition. However,

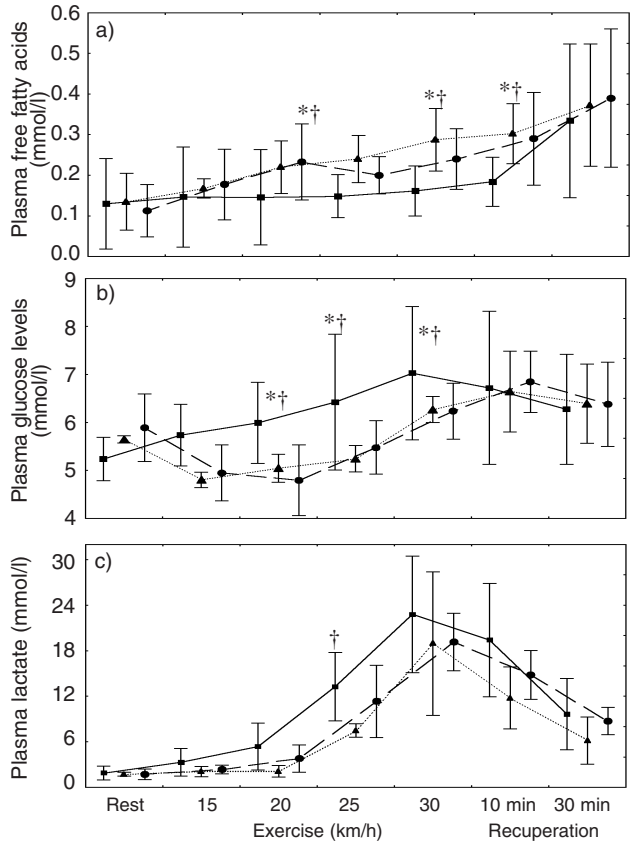

*Fig 1: Mean ± s.d. plasma (a) free fatty acid, (b) glucose and (c) lactate concentrations during an increasing intensity exercise test in untrained Andalusian (AN; ■), Anglo-Arabian (AA; ▲) and Arabian (A; ●) horses. *Significant differences between AN and AA horses; †differences between AN and A horses.*

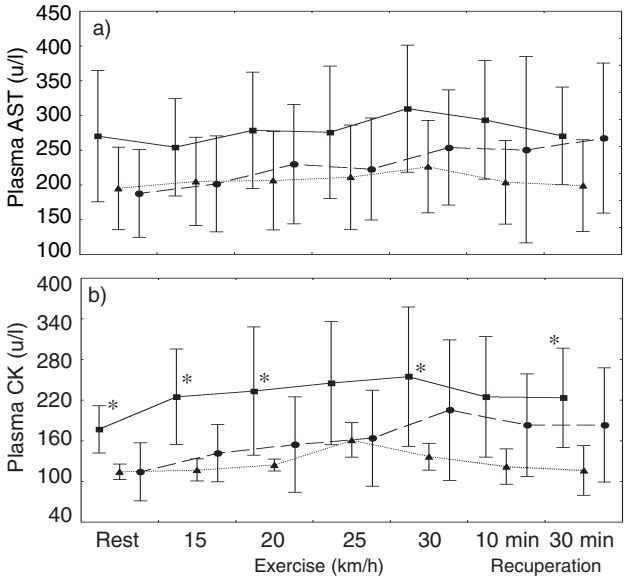

*Fig 2: Mean ± s.d. plasma (a) AST and (b) CK activities during an increasing intensity exercise test in untrained Andalusian (AN; ■), Anglo-Arabian (AA; ▲) and Arabian (A; ●) horses. *Significant differences between AN and AA horses; †significant differences between AN and A horses; §significant differences between AA and A horses.*

Frauenfelder *et al*. (1986) found no relationship between increased PME, plasma progesterone concentrations and the incidence of rhabdomyolysis episodes.

The type of exercise and fitness level are also known to affect postexercise PME elevations. In this way, exercise models involving eccentrically biased muscle concentrations produce the greatest elevation in postexercise PME (Armstrong *et al.* 1983). However, in human subjects, exercise duration has a greater influence on increased postexercise PME than does exercise intensity, perhaps due to the production of reactive oxygen species by aerobic metabolism or alterations in electrolyte concentrations by a greater sweating rate (Noakes, 1987; Sjödin *et al.* 1990). Valberg *et al.* (1993) reported a greater change in serum AST and CK in horses following submaximal exercise compared to short-term high-intensity exercise.

The main problem in the evaluation of PME values is defining the normal increase in enzyme activity in response to a specific exercise regimen. Given that strenuous exercise can result in increased CK, AST and LDH plasma activities without any sign of muscle damage and stiffness, it is rather difficult to differentiate between borderline functional or physiological and pathological changes.

Significant cardiovascular, haematological, metabolic and muscular differences exist between Andalusian horses and other breeds, as demonstrated previously (Castejón *et al.* 1994; Rivero *et al.* 1995; Muñoz *et al.* 1998, 1999). The Andalusian breed responded to exercise with higher haematocrit, haemoglobin values, heart rate and plasma lactate concentrations (Muñoz *et al.* 1999). These differences could influence PME values but, to date, no data exist concerning changes in PME values after a controlled bout of exercise in Andalusian horses. Furthermore, because of the widespread use of this breed in Spain, mainly for breeding purposes or use as dressage and saddle horses, the training programmes are quite different to those reported by researchers in other countries, and comparisons are not, therefore, possible. In the present study, we had the opportunity to evaluate the effect of similar training in this breed compared to Anglo-Arabians and Arabians.

The main purposes of this research were to evaluate whether PME differences during exercise exist in relation to 1) breed (*Experiment A*) and 2) duration of training (*Experiment B*). The intensity of exercise and training was quantified by the metabolic response to exercise (glucose, free fatty acids and lactate). As myopathies have been associated with electrolyte disturbances, plasma sodium, potassium and chloride concentrations were also determined.

Materials and methods

Animals

Twenty stallions, 9 Andalusian (AN), 7 Arabian (A) and 4 Anglo-Arabian (AA), age 5–10 years (mean ± s.d. 6.4 ± 3.2 years) were studied. All horses belonged to the same Military Stud Selection and Horse Training Centre and, therefore, were subjected to the same breaking-in period, training programme, diet, vaccination and anthelmintic procedures. No special criteria were followed to select these animals, although only males were included in the study to avoid sex effect on PME response to exercise. In rats, it has been suggested that oestradiol attenuates CK efflux by changing intracellular calcium homeostasis (Amelink *et al.* 1990).

During the study, horses were carefully monitored for signs of injury, such as muscular pain, stiffness or lameness, that could influence the training effect. The horses that lost training days because of other diseases (digestive or respiratory problems) were not included in the study (n = 20 after eliminating 4 horses). Routine veterinary examinations were performed before each of the exercise tests.

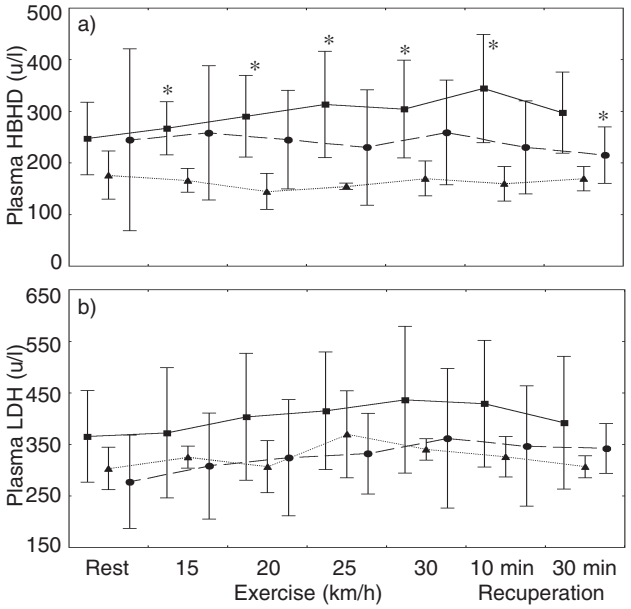

*Fig 3: Mean ± s.d. plasma (a) HBDH and (b) LDH activities during an increasing intensity exercise test in untrained Andalusian (AN; ■), Anglo-Arabian (AA; ▲) and Arabian (A; ●) horses. *Significant differences between AN and AA horses; †significant differences between AN and A horses; §significant differences between AA and A horses.*

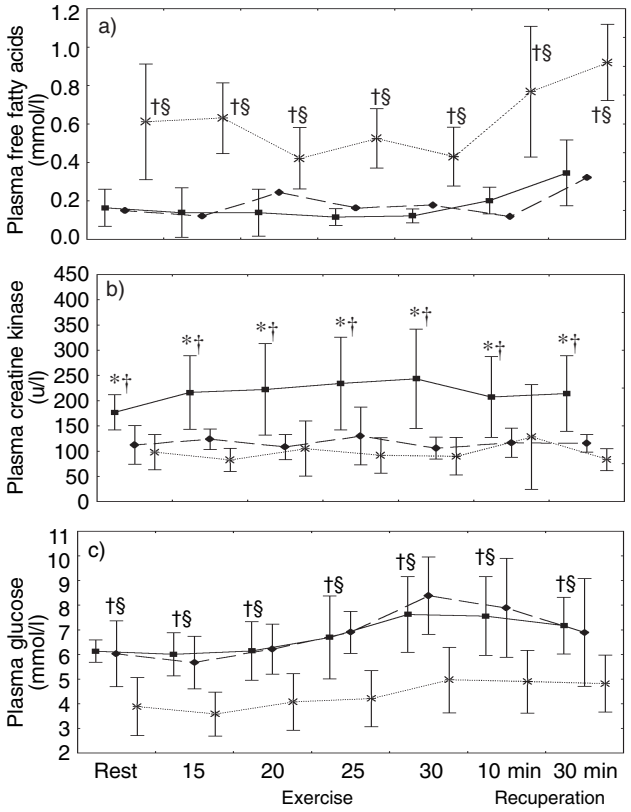

*Fig 4: Mean ± s.d. plasma (a) free fatty acids, (b) creatine kinase and (c) glucose concentrations in untrained AN horses (ET1) and after 2 and 6 months of training (ET2 and ET3). *Significant differences between ET1 and ET2; †significant differences between ET1 and ET3; §significant differences between ET2 and ET3.*

Dietary information

During the experiment, the horses were fed with a commercial fodder (3–4 kg according to the amount of work and reproductive activity), oats, bran (3 kg) and hay *ad libitum* (at least 1% bwt). This diet was supplemented with different mineral and vitamins complexes, especially during the first months of training and the reproductive season (April–July). They were fed twice a day, in the morning after exercise and in the early evening. The caloric distribution of nutrients was 12–18% nitrogenated substances, 2–4% fat and 15–25% grass fibre.

Exercise test (ET)

The ET was preceded by a warm-up period at a walk, at an approximate velocity of 0.7 km/h, covering about 800 m. Immediately after warming-up, the horses covered distances of 1250, 1670, 2080 and 2500 m at velocities of 15, 20, 25 and 30 km/h (i.e. 4.15, 5.50, 6.95 and 8.30 m/s). The workloads were separated by a 5 min rest period at a walk. A 30 min active recovery (approximate walk velocity 0.556 km/h) was completed after each exercise test.

In order to avoid the influence of track characteristics on exercise responses, ETs were conducted on the same oval-shaped track, built on a flat sandy surface and used to train the horses. The riders in charge of the training performed the tests (mean ± s.d. weight 71.9 ± 7.6 kg). Environmental conditions were noted and ranged between 15–29°C for temperature and 45–76% for relative humidity. All tests were carried out early in the morning.

The procedure to standardise velocity has been detailed previously (Muñoz *et al.* 1997). The method has been used successfully for years and, in all the tests, there were no differences between real or chronometered and expected times.

This exercise test was carried out 3 times during the experimental period, first in March, when the animals were relatively untrained (*ET1*; *Experiment A*: effect of exercise), then in May (*ET2*) and September (*ET3*), after 2 and 4 months of training (*Experiment B*: effect of training), respectively.

Training programme

As all animals included in this research were stallions, the training programme was designed to improve fitness to ensure that they could properly perform their reproductive function. This training programme, therefore, did not aim to condition the horses for determined exercises or competitions.

The 20 horses followed a breaking-in period and a training programme for 2 and 6 months, respectively, when they were 3-year-olds. After that, they were kept almost physically inactive in boxes or, occasionally, paddocks until the beginning of this study.

The training programme was divided in 2 main phases (A and B). Phase A was composed of 4 subphases (1A, 2A, 3A and 4A). During subphase 1A, or acclimation period (duration: one week), the horses were walked for 40 min without a rider. Subphase 2A, or basic training (duration: 3 weeks), consisted of 45 min trotting without a rider in the mornings at a frequency of 5 days/week. In the evenings, 3 days/week, animals performed walking exercises with three 5 min periods of trotting. Two days/week, the horses were subjected to a 60 min dressage period, with nonstandardised walking, trotting and cantering exercises. During subphase 3A, or special training (duration: 2 weeks), morning work consisted of 45 min of trotting without a rider, 5 days/week. In the evenings, the work carried out was similar to subphase 2A, except one day/week, when the horses were trotted in the country at an approximate velocity of 12 km/h for 60 min in order to improve stamina. ET2 was performed during subphase 4A. On the days on which the horses did not carry out the test, they were subjected to the same exercises as for subphase 3A (2 weeks).

Phase B was divided in 2 subphases (1B and 2B). During subphase 1B (duration: 8 weeks), the animals performed trotting exercises without a rider for 60 min in the mornings. In the evenings, 3 days/week, they were walked and trotted for 90 min and, on the remaining 2 days, they were subjected to dressage exercises for 60 min on a sandy track. Subphase 2B lasted for a further 8 weeks. In the mornings, the exercises were of the same intensity as in subphase 1B. In the evenings, 3 days/week, a 60 min exercise session was completed (15 min walking, 30 min trotting and 15 min galloping). On the other 2 days, the animals performed track exercises for 60 min. In subphase 2B, the horses performed ET3.

Sample collection

Blood samples were collected from the external jugular vein at rest, during the first 30 s immediately after each exercise velocity and at 10 and 30 min recovery. Special care was taken during this procedure, as inaccurate venepuncture technique can result in elevated CK values. The samples, poured into tubes containing heparin-lithium, were immediately centrifuged and plasma removed and kept refrigerated at 4–6°C during transportation to the laboratory.

Plasma analysis

Plasma was analysed within 6 h of collection. Glucose (GLU) and free fatty acid (FFA) concentrations and CK, LDH, HBDH and AST activities were determined by spectrophotometric techniques, using commercially available kits (Cromatest)[1]. The CK, LDH, HBDH and AST analyses were carried out at 37ºC and the values were corrected to 25ºC. Plasma lactate concentrations (LA) were measured on an analyser according to a method based on the reduction of pyruvate to lactate by the enzyme L-lactate oxidoreductase (Champion PLM5)[2]. Plasma sodium (Na^+), potassium (K^+) and chloride (Cl^-) concentrations were measured with an analyser with selective electrodes (Ciba Corning 644)[3]. GLU, FFA, LA, Na^+, K^+ and Cl^- concentrations were expressed in mmol/l and enzyme activities in u/l.

Statistical procedure

All results are expressed as mean ± s.d. The data have been evaluated using analysis of variance for a repeated measures design. The main effects for *Experiment A* were exercise level (after each velocity of the ET and during the recovery period) and breed (AN, AA and A). The main effects for *Experiment B* were training level (ET1, ET2 and ET3) and breed (AN, AA and A). When the main effect interactions were significant, the means were assessed by *t* tests. Finally, a linear correlation analysis was performed in order to evaluate the relationships between metabolic response to exercise and training and PME. Statistical significance was set at $P<0.05$.

TABLE 1: Mean ± s.d. plasma sodium, potassium and chloride concentrations (mmol/l) at rest and after an exercise test to 30 km/h in Andalusian (AN), Anglo-Arabian (AA) and Arabian (A) horses

	AN	AA	A
Pre-exercise Na	135.6 ± 6.5	138.80 ± 2.99	134.60 ± 4.12
Postexercise Na	141.4 ± 7.5	141.80 ± 4.79	141.60 ± 3.74
Pre-exercise K	3.388 ± 0.600	3.400 ± 0.340	3.481 ± 0.280
Postexercise K	4.193 ± 0.500	4.395 ± 0.710	4.390 ± 0.470
Pre-exercise Cl	102.70 ± 4.30	105.50 ± 3.11	101.90 ± 5.76
Postexercise Cl	99.67 ± 4.36	103.00 ± 3.16	103.00 ± 3.70

Results

Experiment A

The metabolic response to the ET1 in the 3 breeds is shown in Figure 1. The FFA concentrations increased with exercise in AA and A horses, but remained unchanged in AN horses. Moreover, plasma FFAs were significantly lower in AN, in the 2 last exercise bouts and after 10 min of recovery. In contrast, plasma GLU concentrations were significantly higher in AN horses during the last 3 workloads, but no statistically significantly differences were detected during the recovery period. No differences between the AA and the A horses were detected. Plasma LA concentrations increased with exercise intensity, as expected, and the only interbreed difference found was the higher values of the AN horses during the third exercise bout.

Changes of PMEs are presented in Figures 2 and 3. Resting AST activities were significantly higher in AN horses. The most evident interbreed differences were observed in CK and HBDH between AN and AA horses. The AN breed showed the highest PME values and s.d.

No interbreed differences were found in plasma chloride, potassium and sodium concentrations and all values were within the normal physiological range (Table 1). Correlations between metabolic and electrolyte responses to exercise and PME are

TABLE 2: Correlation coefficients of the metabolic and electrolyte responses to an increasing intensity exercise test and plasma muscle enzymes in Andalusian, Anglo-Arabian and Arabian horses

	GLUC	FFA	LA	CK	AST	LDH	HDBDH	Na^+	K^+
FFA	0.190*								
LA	0.580*	0.290*							
CK	0.350*	0.320*	0.290*						
AST	0.300*	0.200*	0.210*	0.610*					
LDH	-0.120	0.090	0.250*	0.240*	-0.070				
HBDH	0.260	-0.230*	0.200*	0.320*	0.450*	0.060			
Na	-0.000	-0.020	0.300 *	0.070	-0.070	0.150	-0.140		
K	-0.440*	-0.230*	-0.003	-0.070	-0.090	0.200*	-0.070	0.330*	
Cl	-0.180	-0.020	-0.240*	-0.040	-0.013	-0.130	-0.080	0.330*	0.230*

*Significant correlations. GLUC = Glucose; FFA = Free fatty acids; LA = Lactose; CK = Creatine kinase; AST = Aspartate aminotransferase; LDH = Lactate dehydrogenase; HBDH = α-hydroxybutyrate dehydrogenase.

TABLE 3: Mean ± s.d. plasma lactate concentrations at rest (LA0), after exercise (LA0') and after 10 min of recuperation (LA10'R) (mmol/l) in Andalusian (AN), Arabian (A) and Anglo-Arabian (AA) horses

	AN			A			AA		
	ET1	ET2	ET3	ET1	ET2	ET3	ET1	ET2	ET3
LA0	0.890 ± 0.51	0.730 ± 0.44	0.742 ± 0.44	0.726 ± 0.231	0.732 ± 0.28	0.715 ± 0.44	0.525 ± 0.25	0.605 ± 0.23	0.515 ± 0.27
LA0'	22.79 ± 6.75	22.77 ± 4.74	13.24 ± 4.67†§	19.16 ± 3.76	12.21 ± 4.20*	13.36 ± 2.36†	19.74 ± 5.44	12.88 ± 3.89*	12.59 ± 5.36†
LA10'R	15.65 ± 4.69	14.37 ± 5.71	6.752 ± 2.96†§	8.739 ± 1.81	9.372 ± 3.21	7.34 ± 2.09	6.968 ± 2.12	5.67 ± 1.45	5.113 ± 2.22

ET1 = before training; ET2 = after 2 months of training; ET3 = after 3 months of training. *Significant differences between ET1 and ET2; †significant differences between ET1 and ET3; §significant differences between ET2 and ET3.

presented in Table 2 (ET1). The data from all 3 groups were combined for calculation of correlations, as values computed using individual breeds produced similar results.

Experiment B

The most evident changes linked to training were observed in the AN breed for plasma GLU, FFA and CK (Fig 4). The only modification found after 2 months of training in AN horses was an attenuated CK response to exercise. The other biochemical adaptations appeared after 6 months of daily exercise. The rest of the studied parameters, i.e. AST, LDH, HBDH, Na^+, K^+ and Cl^- did not undergo any significant modification during the study period. Although some variations linked to training were found in FFA concentrations and CK activities in the A breed, it was not possible to determine a clear correlation. In contrast, glycolytic response to exercise was reduced after 2 months of training in both AA and A horses and after 6 months in AN horses (Table 3).

Discussion

None of the stallions included in the present study suffered from muscular pain, soreness or stiffness during ET or training periods. Those animals (n = 2) which underwent locomotor injury (not muscle origin) were excluded from the study. The main reason for low incidence of injuries was probably the low intensity of training, although this intensity was sufficient to improve fitness level, according to the riders' opinion and the results of the ETs, with a progressive decrease in the glycolytic response to exercise.

The PME ranged between limits considered as normal physiological limits for horses. Therefore, maximum and minimum plasma CK activities obtained immediately after exercise and at rest, respectively, were 417 and 63 u/l for AN, 213 and 66 u/l for AA and 364 and 53 u/l for A horses. No blood samples were withdrawn at the time corresponding to the peak postexercise values for CK activity (4–12 h after exercise), as no horses showed clinical signs of muscle disease. Those animals with the highest plasma CK activities were compared with the horses of the same breed, and no differences were found in other biochemical parameters.

Experiment A

Metabolic response to exercise was mainly glycolytic with LA formation, due to the muscle glycogen and/or blood GLU metabolism, although peripheral fat reserves were also mobilised, at least in AA and A horses.

The greater GLU concentrations in the AN horses may represent a more intense hepatic mobilisation or a reduced peripheral GLU uptake. Hepatic glucose mobilisation is affected by the relative exercise intensity and the extent of catecholamine release. There are currently no data related to endocrine response to physical activity in AN horses or if this is different to other breeds, although it has been suggested that A foals undergo greater catecholamine release during exercise (Rubio *et al.* 1995). If this finding is also true in mature subjects, the hormonal characteristics probably did not influence the greater GLU response of AN horses in our study. Instead, the higher GLU concentrations could reflect adaptation to a greater relative exercise intensity or limitation to muscle uptake. In fact, Blanco (1995), in a group of AN horses belonging to the same training centre as those introduced in the present research, reported low hexokinase activity which could be linked to lower oxidative muscle potential, as demonstrated by muscle composition (Rivero *et al.* 1995) and enzyme profile studies (Blanco 1995).

Plasma FFA concentrations did not change during exercise in AN horses, whereas they increased in the other 2 breeds, with the highest values during recovery. This delay in the start of lipolysis could have been due to the inability to increase oxygen uptake in this breed. To date, there are no data concerning oxygen uptake during exercise in this Spanish breed, although it is accepted that these horses have a reduced oxidative capacity. However, their *gluteus medius* muscle has similar percentages of the 3 main fibre populations (Rivero *et al.* 1995). For that reason, other factors, such as respiratory, haematological and cardiovascular systems, locomotor pattern and morphometry must be considered in order to explain the delay in beginning mobilisation of lipid from adipose tissues during exercise.

A surprising result was the lack of interbreed differences in LA concentrations after exercise, since AN horses have higher glycolytic capacity and lower oxidative potential in comparison to other breeds (Castejón *et al.* 1994; Muñoz *et al.* 1999). In the opinion of the authors, this result was related to the great interindividual differences, as confirmed by the higher s.d. The reason for this variation is unknown, despite the selection of a homogenous group. The analysis of the s.d. of different parameters, such as haemoglobin concentration, packed cell volume, heart rate, plasma lactate (Muñoz *et al.* 1998, 1999), stride frequency, length and duration (Muñoz *et al.* 1997), muscle composition (Rivero *et al.* 1995) and muscle enzyme profile (Blanco 1995), could provide further insight. In previous studies (Muñoz *et al.* 1997, 1999), a greater s.d. was found in the haematological and plasma biochemical variables and in the locomotor pattern. Therefore, the reported high vertical component of the stride and reduced stride length (horizontal component of the stride) may account for the large interindividual variance observed in the present study.

The lack of exercise-induced changes in PME could have been associated with the low exercise duration (20 min without considering warming-up period) and long recuperation time between workloads. Furthermore, according to Sjödin *et al.* (1990), the main reasons for elevated PME during exercise are

oxidative metabolism and the production of reactive oxygen species and alterations in the electrolyte concentrations by sweating, whereas our ET induced a predominant glycolytic adaptation and did not significantly modify the plasma electrolyte concentrations. However, correlation analysis showed significant correlations between LA and PME.

Plasma HBDH and CK activities were higher in the AN horses than in the AA horses, without differences between the AA and A horses. According to the results of the correlation analysis, these findings could have been linked to the greater relative exercise intensity of the AN breed (positive correlation with LA, GLU and FFA). This higher metabolic response, with hyperglycaemia and hyperlactacidaemia might have induced temporal changes in muscle cell permeability, permitting muscle enzyme leakage (Harris *et al.* 1998). The causes of the higher plasma resting CK and AST activities in AN horses are unknown.

Experiment B

A striking result of *Experiment B* was that the metabolic adaptations to training become evident after 2 months in A and AA and after 6 months in AN horses. The reason for the delay in these adaptations is unknown, and could have been linked to individual characteristics. However, previous reports have shown that heart rate and lactate responses to exercise after very similar training programmes are reduced after only 2 months of training (Agüera *et al.* 1995; Muñoz *et al.* 1998).

Plasma FFA, LA and CK in the 3 breeds, and GLU concentrations in AN, underwent the most notable changes as a consequence of training. The lower GLU increase during ET3 in AN horses, in comparison to ET1 and ET2, could indicate a reduction in catecholamine release or in relative exercise intensity, a greater FFA utilisation and/or an increased muscle oxidative potential. The 2 last factors were implicated in this result, as appreciated by the increase in FFA and decrease in LA during exercise after 6 months of training. The increase in FFA was probably related to the greater oxygen uptake after training, although the importance of reduced lactacidaemia should also be considered, as a significant negative correlation was found between FFA and LA. No important feeding changes were introduced during this research in order to explain the high FFA during ET3 at rest and during exercise and recuperation.

One of the most evident changes linked to training was the attenuated response of plasma CK to exercise in AN horses. One possible determinant of temporal alteration in the membrane permeability is reduction of ATP, needed to maintain membrane integrity (Cerny and Haralambie 1983). During maximal exercise, there is an ATP reduction in fast-twitch fibres (Valberg 1987). Although the exercise carried out by the AN horses had a great reliance on glycolytic metabolism, it cannot be considered as maximal (maximum mean LA concentration 13.28 mmol/l). Räsänen *et al.* (1996) showed that a certain degree of ischaemia exists during submaximal exercise, as shown by the oxidative form of the oxidase xynthase enzyme. The lack of oxygen might delay the contractile activity of *type I* fibres and, therefore, *IIB* fibres must be recruited. These changes may have occurred in our horses, as LA concentrations were moderately elevated.

Training effects on muscle composition include a conversion of *IIB* into *IIA* fibres, which are considered to be less susceptible to exercise-induced muscle damage (Friden and Lieber 1992). These effects have also been reported in animals very similar to those introduced in our study and subjected to the same training programme (Rivero *et al.* 1995). Therefore, an increase in muscle oxidative power is another factor to consider in order to explain the lower CK activities after training in AN horses. In fact, CK and LA values were positively related. Future studies should determine whether muscular acidosis after LA production is mechanistically related to an increase in muscle cell membrane permeability.

In summary, compared to AA and A, untrained AN horses responded to exercise with more marked increases in GLU, HBDH, LDH, CK and AST, without significant differences in LA concentrations. The effects of training were evident after 2 months in A and AA breeds and after 6 months in AN breed. The AN horses showed a reduction in CK response to exercise after 6 months of training, related to decreased extrafibrillar GLU utilisation with LA formation and greater fat metabolism. In spite of the improved exercise capacity after training, the apparent fitness of the AN horses remained lower than in the other 2 breeds.

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Manufacturers' addresses

[1]Cromatest, Bayer Diagnostics, Barcelona, Spain.
[2]Medizin & MedTech - Verlag, Munich, Germany.
[3]Ciba Corning, Inter Bio-Lab Inc., Orlando, Florida, USA.

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