Road transport and diet affect metabolic response to exercise in horses[1]

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ABSTRACT: This study investigated the effects of transport and diet on metabolic response during a subsequent race-like test in Standardbred horses in training fed a forage-only diet and a 50:50 forage:oats diet. Six trained and raced Standardbred trotter mares were used. Two diets, 1 forage-only diet (FONLY) and 1 diet with 50% of DM intake from forage and 50% from oats (FOATS), were fed for two 29-d periods in a crossover design. At Day 21, the horses were subjected to transport for 100 km before and after they performed an exercise test (transport test [TT]). At Day 26, the horses performed a control test (CT), in which they were kept in their stall before and after the exercise test. Blood samples were collected throughout the study, and heart rate and water intake were recorded. Heart rate and plasma cortisol, glucose, and NEFA concentrations were greater for the TT than for the CT ($P = 0.008$, $P = 0.020$, $P = 0.010$, and $P = 0.0002$, respectively) but were not affected by diet. Plasma acetate concentration was lower during the TT than during the CT ($P = 0.034$) and greater for the FONLY than for the FOATS ($P = 0.003$). There were no overall effects of the TT compared with the CT on total plasma protein concentration (TPP), but TPP was lower with the FONLY than with the FOATS ($P = 0.016$). There was no overall effect of the TT compared with the CT on water intake, but water intake was greater with the FONLY than the FOATS ($P = 0.011$). There were no overall effects of transport or diet on BW, plasma lactate, or plasma urea concentration. It was concluded that both transport and diet affect metabolic response during exercise in horses. Aerobic energy supply was most likely elevated by transportation and by the FONLY. The FONLY also decreased exercise-induced effects on extracellular fluid regulation. These results highlight the importance of experimental design in nutrition studies. If the aim is to examine how a diet affects exercise response in competition horses, transport should preferably be included in the experimental design, because horses are likely to be transported before a competition.

Key words: acetate, cortisol, forage only, glucose, nonesterified fatty acids, Standardbred trotters

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INTRODUCTION

Sport horses are generally subjected to road transport before competitions, but little is known about the effect of transportation on the subsequent metabolic response to exercise. However, several studies have shown that heart rate (Smith et al., 1996; Doherty et al., 1997; Schmidt et al., 2010) and plasma lactate (Stull, 1999; Werner and Gallo, 2008), plasma glucose (Stull and Rodiek, 2002; Oikawa et al., 2004), and cortisol concentrations (White et al., 1991; Smith et al., 1996; Stull and Rodiek, 2000; Fazio et al., 2008; Stull et al.,

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2008; Schmidt et al., 2010) increase during transportation, which indicates that substrate utilization could be altered during exercise. Studies on other animals (calf, heifers, dromedary camels, and sheep) have also shown that plasma NEFA concentrations increase as a result of road transport, an effect attributed in those studies to feed deprivation or glucocorticoid excretion due to stress (Locatelli et al., 1989; Earley and Murray, 2010; Saeb et al., 2010; Zhong et al., 2011).

Recent studies have also shown that forage-only diets alter the metabolic response in horses in training, both at rest (Connysson et al., 2010) and after exercise (Jansson and Lindberg, 2012), compared with the metabolic response to the high-concentrate diets commonly fed to competition horses (Jansson and Harris, 2013). Forage-only diets increase plasma acetate and NEFA concentration, lower plasma insulin concentrations at rest (Connysson et al., 2010; Jansson and Lindberg, 2012), and also lower lactate concentrations after exercise (Jansson and Lindberg, 2012).

The aim of this study was to investigate the effect of road transport on Standardbred horses in training fed a forage-only diet or a 50:50 forage:oats diet on metabolic response during a subsequent race-like test.

MATERIALS AND METHODS

The Umeå (Sweden) local ethics committee approved this study, and it was performed in agreement with European Union directives on animal experiments (2010/63/EU; European Union, 2010) and the laws (Swedish Constitution, 1988:534) and regulations (Swedish Board of Agriculture Constitution, 2010:28) governing experiments on live animals in Sweden.

Horses and Management

Six adult Standardbred mares in training were used. Their average age was 8 yr (range 6–10) and their mean BW at the beginning of the trial was 509 kg (range 410–562). The horses were trained by students at Wången National Center for Education in Trotting (Alsen, Sweden) under supervision of a professional trainer (license granted by the Swedish Trotting Association, Stockholm, Sweden) according to an ordinary training program for Standardbred trotters (Ringmark, 2014). This training program involved slow exercise 1 to 3 times/wk (approximately 1 h walk and slow trot [6–7 m/s]) and intensive exercise 1 to 2 times/wk (4,000-m slow trot warm-up, 2,000 m at 10–11 m/s on a race track or five 500-m uphill [25 m elevation/500 m] intervals at 9 m/s, and slow trot downhill). The horses were kept in individual stalls with sawdust bedding and spent 5 h/d outdoors in all-weather paddocks. All horses were experienced racing trotters (raced 7–40 times) and accustomed to road transport.

Experimental Design

Two diets were fed in 2 experimental periods of 29 d in a changeover design. The horses were divided into 2 groups and randomly allocated to diet, with 3 horses on each diet during each period. Each experimental period was preceded by a 3-d transition period in which the new diet was gradually introduced. Horses were fed 15, 20, 25, and 40% of the daily allowance at 0600, 1230, 1700, and 2100 h, respectively. At Day 21 and 26, the horses performed an exercise test on a 1,000-m oval, banked gravel race track. At Day 21, the horses were subjected to road transport for 100 km (1.5 h) before and after they performed the exercise test (transport test [**TT**]). At Day 26, the horses performed the same exercise test without being transported (control test [**CT**]). The horses performed their exercise tests at the same time of the day (1130 h) on all 4 occasions. Ambient temperature during exercise test days was 6.8, 5.2, 6.7, and 8.4°C.

Exercise Test

All horses did the test together in 1 group, and the same driver drove the same horse on all test occasions except one, when the driver was ill and had to be replaced. The exercise tests started with a warm-up consisting of 3,000 m of slow trot (6.3–6.7 m/s) and 1,000 m of trot (10 m/s). After the warm-up, the horses walked to the stable and had a 10-min recovery. Then, the horses trotted 2,140 m at 11.6 to 12.7 m/s followed by a cool-down of 1,000 m of slow trot (6.3–6.7 m/s). The track was estimated by experienced trainers to reduce velocity by 0.3 m/s compared with competition race tracks.

Transport and Control Treatment

The horses were transported using 2 commercial horse buses (Umesläp Renault master 2010 [Umesläp AB, Bygdsiljum, Sweden] and Equi-Trek Sonic, Peugeot Boxer 2010 [Equi-Trek, Sheffield, UK) and 1 commercial horse trailer (Umesläp BA45 2007; Umesläp AB), all with room for 2 horses. In the horse buses, the horses were transported facing backward (away from the driving direction), and in the trailer, the horses were facing forward. All horses were transported in the same place within the vehicle on both occasions, and all horses had cotton in their ears during road transportation (to reduce noise). During the TT, the horses were loaded at 0900 h before the exercise test and at 1430 h after the

exercise test. No feed or water was offered to the horses during the 1.5-h transport period. All horses were loaded and standing in the vehicle within 10 min from when the loading procedure started. During the CT, the horses remained in their stall.

Diets

One forage-only diet (**FONLY**) and 1 diet with 50% of DM intake from forage and 50% from oats (**FOATS**) were fed (Table 1). The same forage was used in both diets and comprised a haylage (mainly timothy and meadow fescue) with a DM content of approximately 76%. Feed samples were collected every week during the trial. Individual diets were calculated to fill ME, CP, and mineral requirements for athletic horses (NRC, 2007). The diets were supplemented (24 g/100 kg BW) with a commercial mineral and vitamin feedstuff (55 g Ca/kg, 65 g P/kg, 60 g Mg/kg, 125 g NaCl/kg, 900 mg Cu/kg, 15 mg Se/kg, 100,000 IU vitamin A/kg, 10,000 IU vitamin D_3/kg, and 5,000 mg vitamin E/kg; Krafft Miner Vit; Lantmännen KRAFFT AB, Malmō, Sweden), limestone (4.7 g/100 kg BW), and additional salt (total NaCl intake 10 g/100 kg BW). The horses were offered water ad libitum in graded buckets in the stall.

Table 1. Mean daily intake of DM, dietary components, and ME; mineral supplements not included

	Diet[1]		
Dietary components	FONLY	FOATS	SEM
DM, kg per 100 kg BW	2.0	1.9	0.1
Water in diets, kg per 100 kg BW	0.6[a]	0.4[b]	0.03
CP, g per 100 kg BW	217	212	12
ME, MJ per 100 kg BW	19	21	1
NDF, g per 100 kg BW	1,068[a]	778[b]	58
ADF, g per 100 kg BW	596[a]	427[b]	32
Lignin, g per 100 kg BW	99[a]	81[b]	5
Glucose, g per 100 kg BW	48[a]	24[b]	3
Fructose, g per 100 kg BW	121[a]	61[b]	7
Sucrose, g per 100 kg BW	2.1[a]	1.1[b]	0.2
Fructans, g per 100 kg BW	73[a]	36[b]	4
Starch, g per 100 kg BW	0[a]	408[b]	4
Water-soluble carbohydrates,[2] g per 100 kg BW	244[a]	122[b]	13
Ash, g per 100 kg BW	177[a]	116[b]	10
Calcium, g per 100 kg BW	11[a]	6.0[b]	0.6
Phosphorus, g per 100 kg BW	5.0[a]	6.3[b]	0.3
Magnesium, g per 100 kg BW	3.9[a]	3.2[b]	0.2
Potassium, g per 100 kg BW	50[a]	29[b]	3
Sulfur, g per 100 kg BW	4.6[a]	3.8[b]	0.3

[a,b]Means within rows with different superscripts significantly differ ($P < 0.05$).

[1]FONLY = forage-only diet; FOATS = Forage-oats diet.

[2]Free glucose, free fructose, sucrose, and fructans.

Measurements, Sampling, and Analysis

During Days 14 through 29, the horses were weighed daily (Tru-Test E2000S[2]; Tru-Test Ltd., Auckland, New Zealand) and daily water intake was measured using data from the graded buckets. Rectal temperature was measured every day at 0700 h. Before and after each experimental period, the BCS of all horses was evaluated according to Carroll and Huntington (1988) by 2 individuals with long experience of horses and blinded to the study treatments.

During the days of the exercise tests (Days 21 and 26), blood samples were collected in 6-mL lithium-heparinized tubes (102 IU) through a catheter inserted in the vena jugularis after local anesthetic (Tapin [25 mg/g lidocaine and 25 mg/g prilocaine]; Orifarm Generics, Stockholm, Sweden) approximately 1 h before the first blood sampling. During the TT, blood samples were collected at rest before the first transport (rest); in the stable directly after the first transport (**AT1**); after the finish line (**FL**); after 10, 30, and 90 min of recovery (**R10**, **R30**, and **R90**, respectively); and in the stable directly after the second transport (**AT2**). During the CT, blood samples were collected at the same times of day and the same postexercise time points as during the TT. At 24 h after the exercise tests, blood samples were collected by venipuncture (6-mL lithium-heparinized tubes [102 IU]) from the jugular vein.

Heart rate was recorded from rest until AT2, using a heart rate recorder (Polar CS600X; Polar Electro Oy, Kempele, Finland), and the data were analyzed using Polar ProTrainer 5 Equine Edition software (Polar Electro Oy). For values at rest, recordings from when the horses were in their stalls before transportation were used. Mean transportation heart rate was calculated using 30-min recordings from when the horses were transported in the TT, or in the stall at the same time of the day in the CT.

Blood samples were kept on ice until centrifuged (920 × *g* for 10 min at 18°C), and plasma was then frozen (−20°C). In all samples, analysis of total plasma protein concentration (**TPP**) was performed in the field using a handheld refractometer (Atago Co., Ltd., Tokyo, Japan). All plasma analyses except for TPP were performed at the laboratory at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences (Uppsala, Sweden). Plasma lactate concentration was analyzed in all samples except those from 24 h after exercise using an enzymatic (L-lactate dehydrogenase and glutamate-pyruvate transaminase) and spectrophoto-

metric method (Boehringer Mannheim/R-Biopharm AG, Darmstadt, Germany) with an intra-assay CV of 10.3%. Plasma acetate concentration was analyzed in all samples. Plasma cortisol, plasma NEFA, and plasma urea concentration were analyzed in samples taken at rest, AT1, R10, R90, AT2, and 24 h after exercise. Plasma glucose was analyzed in samples taken at rest, FL, R10, AT2, and 24 h after exercise. Plasma insulin was analyzed in samples taken at rest, AT2, and 24 h after exercise. Plasma acetate, plasma urea, and plasma glucose concentrations were analyzed with an enzymatic colorimetric/UV method (Boehringer Mannheim/R-Biopharm AG) with an intra-assay CV of 2.2% for glucose. Plasma cortisol concentration was analyzed using ELISA (IBL International GmbH, Hamburg, Germany) with an intra-assay CV of 4.7%. Plasma insulin concentration was analyzed using ELISA (Mercodia equine insulin kit; Mercodia AB, Uppsala, Sweden) with an intra-assay CV of 3.8% in this study. For quantitative determination of NEFA in plasma, an enzymatic colorimetric method was used (Wako Chemicals GmbH, Neuss, Germany) with an intra-assay CV of 3.1% in this study. A method evaluation was performed to the compare NEFA concentration (0–0.16 mmol/L) in horse plasma with EDTA anticoagulant (recommended by Wako Chemicals GmbH) with the concentration in plasma with heparin anticoagulant, and the correlation was found to be high ($R^2 = 0.9914$).

Preparation and conventional chemical analysis of feeds were performed as described by Palmgren Karlsson et al. (2000). Minerals were determined by boiling samples in nitric acid (7 *M*) and measurements were made by inductively coupled plasma optical emission spectrometry (Ametek Spectro Analytical Instruments, Kleve, Germany).

Statistical Analysis

Analysis of variance was performed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Variables from the exercise tests were analyzed by a statistical model including fixed effects (diet, test, sample within period, interactions between these, and period). The model for an observed variable of horse i in period j, given diet k at test l and sample m, was

$$y_{ijklm} = \mu + \eta_i + \pi_j + \gamma_k + t_l + s_m + (\gamma t)_{kl} + (\gamma s)_{km} + (ts)_{lm} + (\gamma ts)_{klm} + e_{ijklm}.$$

The model components are the overall mean μ; the effect of horse η_i; the effect of period π_j; the effect of diet γ_k; the effect of test t_l; the effect of sample within period s_m; the effect of interaction between diet and test $(\gamma t)_{kl}$, diet and sample $(\gamma s)_{km}$, test and sample $(ts)_{lm}$, and diet, test, and sample $(\gamma ts)_{klm}$; and the random error e_{ijklm}. The random part included horse, horse × diet, and horse × test. Observations within each horse × period × test combination were modeled as repeated measurements. Because the time between measurements varied, the repeated measurements were modeled using a spatial power covariance structure for the R side random effects. Post hoc comparisons were adjusted for multiplicity using the Bonferroni method.

Water intake, nutrient intake, rectal temperature, BCS, and BW were analyzed by a statistical model including fixed effects (diet, day, and period). The model for an observed variable of horse i in period j, given diet k and day m, was

$$y_{ijkm} = \mu + \eta_i + \pi_j + \gamma_k + s_m + e_{ijkm}.$$

The model components are the overall mean μ, the effect of horse η_i, the effect of period π_j, the effect of diet γ_k, the effect of day within period s_m, and the random error e_{ijklm}.

Pairwise *t*-tests were performed to separate the main effect means for test within each diet, for diet within each test, and for comparisons of sample within each test. Values are presented as least squares means with the pooled SEM. Differences were considered statistically significant at $P < 0.05$.

RESULTS

General

Due to a major rhabdomyolysis episode (after 14 d on the FOATS), 1 horse had to be excluded from the study. Daily DM and ME intake were similar on both diets (Table 1), but there were more leftovers with the FONLY than with the FOATS (1.4 vs. 0.8 kg haylage [SEM 0.2, $P = 0.017$]). Daily water intake was greater with the FONLY than with the FOATS (27 vs. 18 L [SEM 1, $P = 0.0002$]). There were no differences between the diets in mean horse BW (502 kg for the FONLY and 501 kg for the FOATS [SEM 26]) or rectal temperature at rest (37.1°C for the FONLY and 37.2°C for the FOATS [SEM 0.1]). There were no statistically significant differences in BCS between before the study started and after each diet (3.0 before the study, 2.8 after the FONLY, and 2.9 after the FOATS [SEM 0.3]).

General Effects of Transport

Overall heart rate and plasma cortisol, plasma glucose, and plasma NEFA concentrations were greater in

the TT than in the CT (84 vs. 79 beats/min [SEM 1.0, $P = 0.008$], 181 vs. 144 mmol/L [SEM 15, $P = 0.020$], 6.6 vs. 5.7 mmol/L [SEM 0.2, $P = 0.010$], and 0.42 vs. 0.16 mmol/L [SEM 0.02, $P = 0.0002$], respectively). The overall plasma acetate concentration was lower in the TT than in the CT (0.27 vs. 0.34 mmol/L [SEM 0.02, $P = 0.034$]). There were no overall effects of the TT compared with the CT on TPP (63 vs. 63 g/L [SEM 2]), plasma lactate (7.4 vs. 7.5 mmol/L [SEM 0.7]), plasma insulin (0.08 vs. 0.08 µmol/L [SEM 0.01]), or plasma urea (5.7 vs. 5.6 mmol/L [SEM 0.4]) concentration.

During test days and until 72 h after exercise, there was no overall effect of the TT compared with the CT on water intake or BW (16 vs. 17 L [SEM 1] and 497 vs. 496 kg [SEM 28], respectively).

General Effect of Diet

There was no overall effect of the FONLY compared with the FOATS on heart rate (80 vs. 82 beats/min [SEM 1]) or plasma lactate (7.4 vs. 7.4 mmol/L [SEM 0.7]), plasma cortisol (157 vs. 168 mmol/L [SEM 15]), plasma glucose (6.0 vs. diet 6.3 mmol/L [SEM 0.2]), plasma NEFA (0.33 vs. 0.26 mmol/L [SEM 0.02]), or plasma urea (5.8 vs. 5.5 mmol/L [SEM 0.4]) concentration. The overall plasma acetate concentration was greater for the FONLY than for the FOATS (0.40 vs. 0.20 mmol/L [SEM 0.02, $P = 0.003$]). The overall plasma insulin concentration and TPP were lower for the FONLY than for the FOATS (0.06 vs. 0.10 µmol/L [SEM 0.01, $P = 0.016$]) and 61 vs. 64 g/L [SEM 2, $P = 0.039$], respectively).

During test days and until 72 h after exercise, water intake was greater with the FONLY than with the FOATS (20 vs. 13 L [SEM 1, $P = 0.011$]), whereas there was no overall effect of the FONLY compared with the FOATS on BW (497 vs. 496 kg [SEM 28]).

Significant Sample Effects of Transport

During the FO, plasma acetate concentrations were lower during the TT than during the CT (Fig. 1). With both diets, plasma NEFA concentrations were greater during the TT than during the CT after transport 1 (AT1) and transport 2 (AT2; Fig. 2). With the FO, the plasma glucose concentration was greater FL and R10 during the TT than during the CT (Table 2). With the FONLY, heart rate was greater during the TT than during the CT during transport 1 and transport 2 (Table 3). With the FO, heart rate was greater during the TT than during the CT only during transport 2 (Table 3). With the FO, plasma cortisol concentration was greater during the TT than during the CT after both transport 1 and transport 2, whereas with the FONLY plasma cortisol concentration was greater only after transport 2 (Fig. 3).

Significant Sample Effects of Diet

During the TT, the plasma insulin concentration was lower with the FONLY than with the FOATS R10 (Table 2). During the CT, the plasma insulin concentration at rest was lower with the FONLY than with the FOATS (Table 2). During both the TT and the CT, the plasma acetate concentration was greater with the FONLY than with the FOATS at rest and AT1 (Fig. 1). During the CT, but not the TT, the plasma acetate concentration was greater in the FONLY than in the FOATS at the finish line and R10 and R30 (Fig. 1). During the TT, TPP was lower with the FONLY than with the FOATS at the finish line and R10 (Table 2). During the TT, plasma cortisol concentration was lower with the FONLY than the FOATS AT2 (Fig. 3).

DISCUSSION

The aim of this study was to assess the effect of road transport and 2 diets on the metabolic response in trotters to race-like, high-intensity exercise. Interestingly, however, in the overall treatment response, more parameters linked to metabolism were affected by transport (glucose, NEFA, acetate, and cortisol) than by diet (acetate and insulin).

The results indicated that transport could cause effects that promote performance. High plasma glucose concentrations during exercise are associated with increased performance (Farris et al., 1998; Lacombe et al., 2001), and in this study, transport increased FL and R10 the plasma glucose concentration compared with no transport, especially with the FOATS. The main reason for this might be the release of cortisol in horses subjected to transport. Cortisol increases the fatty acid concentration by stimulating degradation of adipose tissue, stimulates gluconeogenesis in muscle and the liver, inhibits glucose utilization, and increases the plasma glucose concentration. In the present study, there was a marked increase in NEFA after transport, both before and after exercise. Similar increases in NEFA have been reported after light exercise (Pösö et al., 1983) and moderate exercise (Jansson and Lindberg, 2012). Availability of energy substrate is one of the factors that determines the energy substrate used in muscle cells; for example, increased concentrations of FFA during exercise result in greater plasma glucose concentrations after exercise in rats due to decreased glucose muscle uptake and increased energy supply from fatty acid oxidation (Rennie et al., 1976). In humans in training, increased FFA concentration

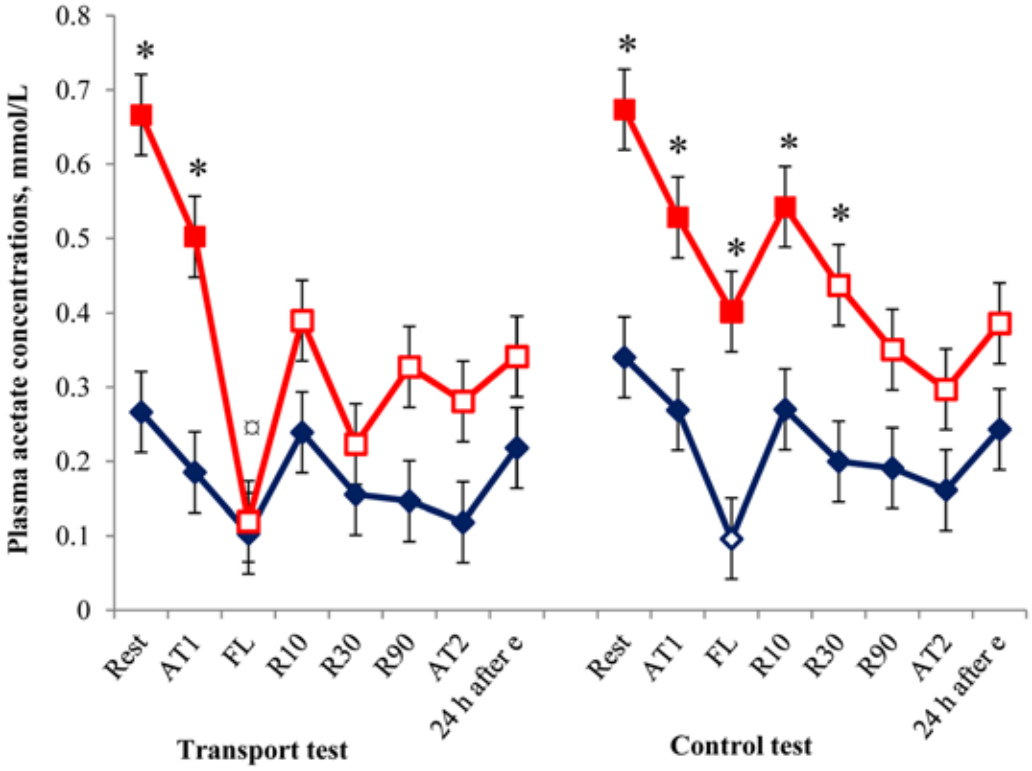

Figure 1. Plasma acetate concentration in horses fed a forage-only diet (F; red squares) and a forage–oats diet (diet with 50% of DM intake from forage and 50% from oats [FO]; blue diamonds) and transported (transport test) or not transported (control test) before and after an experimental race. AT1 = after the first transport; AT2 = after the second transport; FL = after the finish line; R10 = after 10 min of recovery; R30 = after 30 min of recovery; R90 = after 90 min of recovery; 24 h after e = 24 h after exercise. *Significant ($P < 0.05$) difference between diets. □Significant ($P < 0.05$) difference between exercise tests within the F. Unfilled markers: significant ($P < 0.05$) difference between exercise tests within the FO. Unfilled markers: significantly ($P < 0.05$) different from Rest values.

has been found to increase fat oxidation at 85% of maximal oxygen consumption (Romijn et al., 1993). Increased fat availability has also been shown to alter energy substrate oxidation during low-intensity exercise in horses (Pagan et al., 2002).

The contribution of fat oxidation to the total energy expenditure decreases as the intensity of exercise increases but seems to be greater in humans when exercise (warm-up) is performed before intense exercise (van Loon et al., 2001; Chenevière et al., 2012) and greater in horses when exercise is prolonged (Rose et al., 1991). It has been speculated that this increased fat oxidation after warm-up or prolonged exercise is due to decreased muscle glycogen. The decreased fat energy contribution during intense exercise has been suggested to be an effect of limited availability of fatty acids or limited availability of O_2. A short warm-up before exercise has been shown to increase the use of aerobic energy during intense exercise, indicating that even a short warm-up increases O_2 availability in muscle (Tyler et al., 1996). The greater plasma glucose concentrations after transport could be explained by glucose saving and greater fat oxidation as energy. The elevated NEFA concentration with the FONLY on the day after exercise and transport confirms findings by Jansson and Lindberg (2012) and could be a sign of prolonged recovery of energy balance. In that study, muscle glycogen content was still slightly (10%) lower on a FONLY than on a high-concentrate diet 3 d after intensive exercise. A reduced postexercise rate of glycogen repletion on a low-starch diet has also been observed by Lacombe et al. (2004).

Acetate is produced during fermentation of fibers in the hindgut, and the concentration in the colon and in feces fluid has been shown to increase in horses on forage diets compared with those on grain (starch) diets (Jansson and Lindberg, 2012). Acetate is taken up by most cells in the body and is converted by acetyl-CoA synthetases to acetyl-CoA and metabolized through the tricarboxylic acid cycle. In the present study, plasma acetate concentrations were greater in horses on the FONLY than in horses on the high-concentrate diet (the FOATS) during almost all the tests but markedly dropped after exercise when the horses had been transported. A similar drop in plasma acetate concentration after transportation and exercise has been shown in another study comparing the effect of a FONLY and a high-concentrate diet on exercise performance, in which the horses were transported before the exercise test (Jansson and Lindberg, 2012).

Table 2. Plasma concentrations of insulin, glucose, and urea after a forage-only diet (FONLY) and a forage–oats diet (diet with 50% of DM intake from forage and 50% from oats [FOATS]) and transported (transport test [TT]) or not transported (control test [CT]) before and after an experimental race

	TT		CT			*P*-value			
Sample[1]	FONLY	FOATS	FONLY	FOATS	SEM	Diet (within TT)	Diet (within CT)	Test (within FONLY)	Test (within FOATS)
Plasma urea, mmol/L									
Rest	5.6	6.2	5.6	5.6	0.6	1.000	1.000	1.000	1.000
AT1	4.7	5.0[a]	5.0	4.3[a]	0.6	1.000	1.000	1.000	1.000
R10	5.8	6.5	6.4	6.3	0.6	1.000	1.000	1.000	1.000
R90	5.4	5.2	5.9	5.1	0.6	1.000	1.000	1.000	1.000
AT2	6.9[a]	5.7	6.9[a]	5.9	0.6	1.000	1.000	1.000	1.000
24 h after e	5.9	5.5	5.3	5.2	0.6	1.000	1.000	1.000	1.000
Plasma glucose, mmol/L									
Rest	4.9	5.3	4.4	5.0	0.4	1.000	1.000	1.000	1.000
FL	7.9[a]	8.1[a]	6.8[a]	6.3	0.4	1.000	1.000	0.550	0.010
R10	9.4[a]	9.9[a]	7.9[a]	8.2[a]	0.4	1.000	1.000	0.056	0.016
AT2	4.8	5.6	4.6	5.0	0.4	1.000	1.000	1.000	1.000
24 h after e	5.0	4.8	4.4	4.8	0.4	1.000	1.000	1.000	1.000
Plasma insulin, µg/L									
Rest	0.04	0.11	0.06	0.15	0.02	0.059	0.002	1.000	0.570
R10	0.07	0.17	0.07	0.11	0.02	0.002	0.411	1.000	0.141
AT2	0.09[a]	0.11	0.06	0.05[a]	0.02	1.000	1.000	1.000	0.061
24 h after	0.03	0.04	0.04	0.07[a]	0.02	1.000	1.000	1.000	1.000
Plasma lactate, mmol/L									
Rest	1.2	0.7	1.3	0.8	1.5	1.000	1.000	1.000	1.000
AT1	0.8	0.9	1.3	0.6	1.5	1.000	1.000	1.000	1.000
FL	20.9[a]	21.6[a]	22.3[a]	21.9[a]	1.5	1.000	1.000	1.000	1.000
R10	14.5[a]	16.6[a]	16.0[a]	15.9[a]	1.5	1.000	1.000	1.000	1.000
R90	8.6[a]	10.3[a]	9.31[a]	8.8[a]	1.5	1.000	1.000	1.000	1.000
AT2	2.5	1.8	3.1	1.4	1.5	1.000	1.000	1.000	1.000
24 h after e	1.3	1.2	1.1	0.7	1.5	1.000	1.000	1.000	1.000
Total plasma protein, g/L									
Rest	57.2	57.8	58.2	60.6	2.6	1.000	1.000	1.000	1.000
AT1	57.8	60.8	57.8	60.6	2.6	1.000	1.000	1.000	1.000
FL	70.7[a]	76.6[a]	72.2[a]	73.4[a]	2.6	0.045	1.000	1.000	1.000
R10	63.0[a]	70.6[a]	65.4[a]	68.6[a]	2.6	0.003	1.000	1.000	1.000
R30	59.0	62.6[a]	60.6	64.0	2.6	1.000	1.000	1.000	1.000
R90	60.8	63.0[a]	59.4	61.6	2.6	1.000	1.000	1.000	0.208
AT2	58.4	61.8	61.0	64.2	2.6	1.000	1.000	1.000	1.000
24 h after e	60.5	63.4[a]	56.6	62.0	2.6	1.000	0.118	0.650	1.000

[a]Mean significantly differs from "Rest" values in the same column ($P < 0.05$).

[1]AT1 = after the first transport; AT2 = after the second transport; FL = after the finish line; R10 = after 10 min of recovery; R30 = after 30 min of recovery; R90 = after 90 min of recovery; 24 h after e = 24 h after exercise.

This drop could be due to reduced absorption/production or increased utilization of acetate during exercise in transported horses. Earlier studies have shown that acetate is the major metabolite in the hind limb at rest (Pethick et al., 1993) and also the major substrate used during submaximal exercise (Pratt et al., 2005). General plasma acetate concentrations were lower during the TT than during the CT, indicating that acetate was used as an energy substrate during transport, together with NEFA. There is no reason to believe that acetate absorption was reduced because of the transport, because the concentration immediately after transport was high and similar to that during the no-transport treatment. Therefore, we suggest that acetate utilization during exercise was stimulated after transport on the FONLY and that the high availability of acetate together with greater fatty acid utilization, initiated by the long period of elevated NEFA before exercise (1.5–2 h transport time), increased the use of acetate.

Increased utilization of acetate during exercise in horses on a high-energy FONLY has been previously suggested (Jansson and Lindberg, 2012). In that study,

Table 3. Heart rate in horses fed a forage-only diet (FONLY) and a forage–oats diet (diet with 50% of DM intake from forage and 50% from oats [FOATS]) and transported (transport test [TT]) or not transported (control test [CT]) before and after an experimental race

	Heart rate, beats/min								
	TT		CT						
Sample[1]	FONLY	FOATS	FONLY	FOATS	SEM	Diet (within TT)	Diet (within CT)	Test (within FONLY)	Test (within FOATS)
Rest	32	31	29	30	2	1.000	1.000	1.000	1.000
AT1	45[a]	45[a]	33	37	2	1.000	1.000	0.006	0.155
Exercise maximum	211[a]	212[a]	213[a]	213[a]	2	1.000	1.000	1.000	1.000
AT2	47[a]	50[a]	36	39[a]	2	1.000	1.000	0.007	0.030

[a]Mean significantly differs from "Rest" values in the same column ($P < 0.05$).

[1]AT1 = after the first transport; AT2 = after the second transport.

the lactate threshold also tended to be greater on a high-energy forage diet compared with a conventional oats–forage diet. In the present study, diet did not affect the plasma lactate concentration or heart rate. The lack of difference in plasma lactate might be due to the design of the exercise test, which was a race-like field test. It is likely that changes in the production and oxidation rate of lactate would have been easier to observe using a standardized incremental exercise test.

Interestingly, the lowest finish line plasma glucose concentration was observed with the FOATS in nontransported horses, indicating that these conditions might not be optimal for performance. Transported horses on the FOATS also showed greater TPP and no reduction in the plasma acetate concentration at the finish line, indicating that horses might have been exercising with a smaller plasma volume and with less contribution of acetate and aerobic metabolism during the race. There was no overall decrease in TPP in horses on the FONLY. However, TPP was numerically lower with the FONLY, and when the horses were transported, TPP at the finish line and R10 was significantly lower with the FONLY compared with the FOATS. With the FO, TPP remained elevated until the last sample, taken

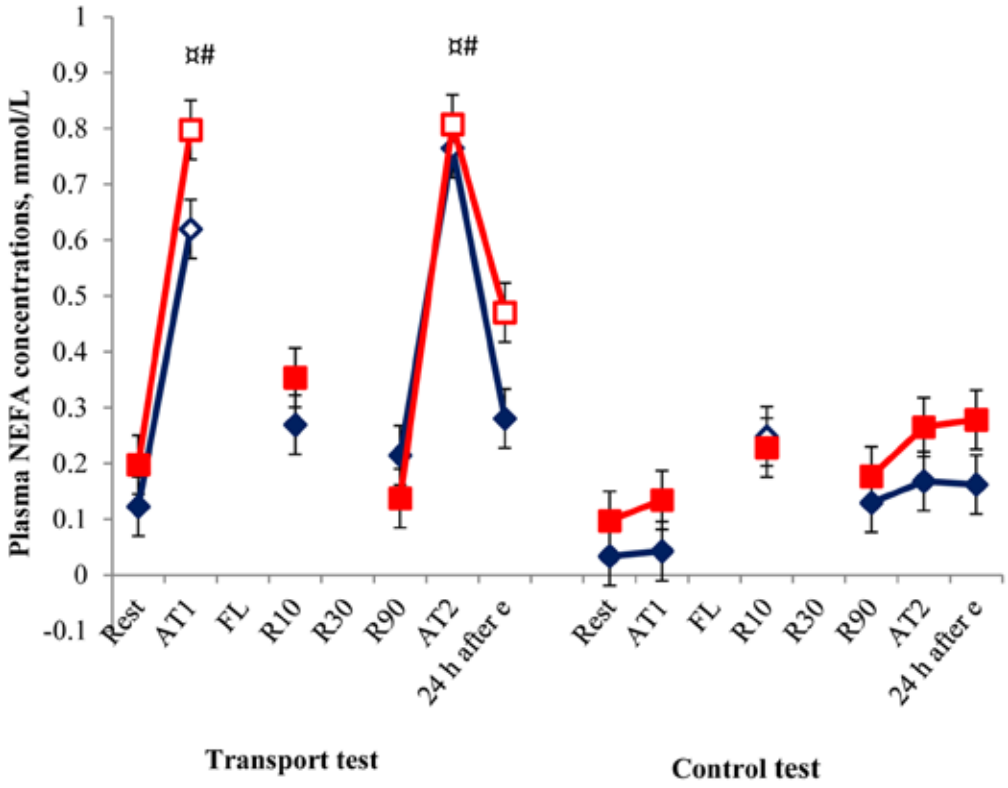

Figure 2. Plasma NEFA concentration in horses fed a forage-only diet (F; red squares) and a forage–oats diet (diet with 50% of DM intake from forage and 50% from oats [FO]; blue diamonds) and transported (transport test) or not transported (control test) before and after an experimental race. AT1 = after the first transport; AT2 = after the second transport; FL = after the finish line; R10 = after 10 min of recovery; R30 = after 30 min of recovery; R90 = after 90 min of recovery; 24 h after e = 24 h after exercise. □Significant ($P < 0.05$) difference between exercise tests within the F. #Significant ($P < 0.05$) difference between exercise tests within the FO. Unfilled markers: significantly ($P < 0.05$) different from Rest values.

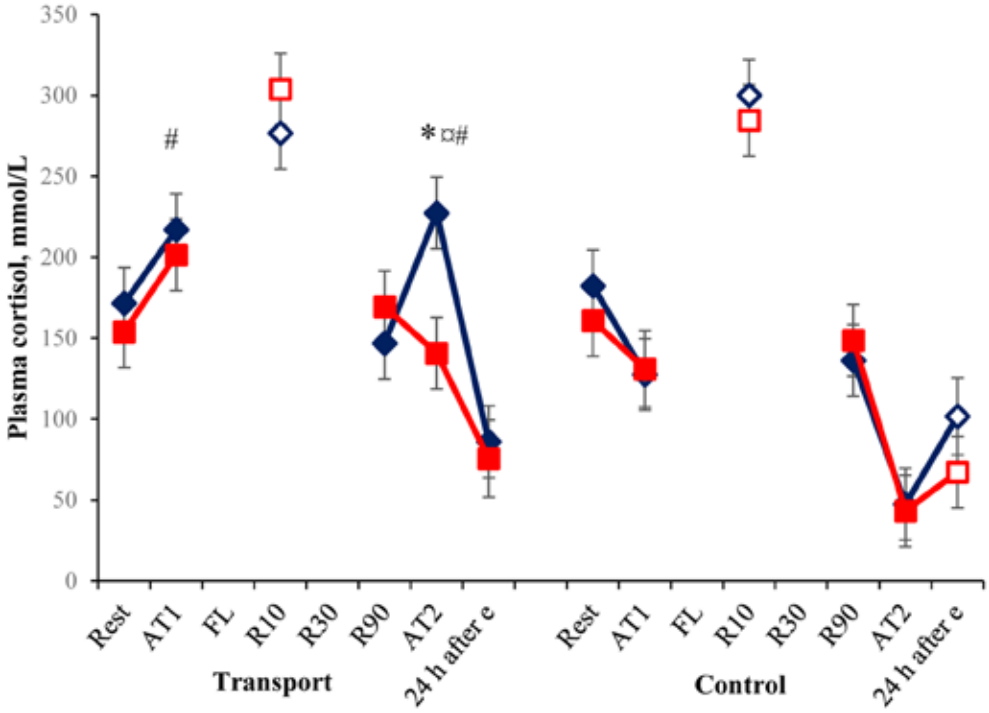

Figure 3. Plasma cortisol concentration in horses fed a forage-only diet (F; red squares) and a forage–oats diet (diet with 50% of DM intake from forage and 50% from oats [FO]; blue diamonds) and transported (transport test) or not transported (control test) before and after an experimental race. AT1 = after the first transport; AT2 = after the second transport; FL = after the finish line; R10 = after 10 min of recovery; R30 = after 30 min of recovery; R90 = after 90 min of recovery; 24 h after e = 24 h after exercise. *Significant ($P < 0.05$) difference between diets. □Significant ($P < 0.05$) difference between exercise tests within the F. #Significant ($P < 0.05$) difference between exercise tests within the FO. Unfilled markers: significantly ($P < 0.05$) different from Rest values.

at 24 h after exercise. This might be due the larger gastrointestinal fluid reservoir available (Meyer, 1995) on the FONLY being used to compensate for fluid losses. Maintenance of plasma volume is likely to be beneficial for performance. Water intake was greater with the FONLY than with the FO, both in general and during exercise and recovery days, as also reported in other studies comparing forage-only diets with forage–grain diets (Connysson et al., 2010; Jansson and Lindberg, 2012). In contrast to earlier studies (Connysson et al., 2010; Jansson and Lindberg, 2012), there was a small (3 kg) but significant increase in BW in horses at rest but there was no significant overall increase in BW with the FONLY. This indicates that the digestibility of the forage was high.

Cortisol concentration in plasma is used as an indicator of stress. The general plasma cortisol concentration was greater when the horses were transported, which is in agreement with previous findings that transport of animals elevates plasma, fecal, and saliva cortisol concentrations (Clark et al., 1993; Stull and Rodiek, 2002; Fazio et al., 2008; Schmidt et al., 2010). There was a clear effect of diet on the cortisol concentration immediately AT2, when the cortisol concentration was lower with the FONLY than with the FOATS. The reason for this is unclear, but there have been reports of effects of a high-starch diet, compared with a high-fiber diet, on reactivity, heart rate, and handling behavior (Bulmer et al., 2015), indicating that diet can affect neurophysiology.

As found in earlier studies, insulin concentration was greater at rest (before transportation) in horses on the high-concentrate diet (FOATS) compared with horses on the FONLY (Connysson et al., 2010; Jansson and Lindberg, 2012) and heart rate increased during transport (Smith et al., 1996; Doherty et al., 1997; Schmidt et al., 2010). However, exercise heart rate and plasma glucose concentration were not affected by diet, as previously observed (Pagan et al., 1987; Jansson et al., 2002; Jansson and Lindberg, 2012). The reason for this lack of dietary effect on plasma glucose is unclear but could be due to differences in the timing of feeding in relation to exercise (Duren et al., 1999; Pagan and Harris, 1999). It has been shown that heart rate during transport is strongly correlated (0.4–0.9) with muscular activity (Giovagnoli et al., 2002), and some of the elevation observed in the present study could, therefore, be exercise induced. Besides the effect of exercise, decreased parasympathetic and increased sympathetic activity probably contributed to increased heart rate. However, heart rate during transport was low (45–50 beats/min) compared with those reported in some other studies (75–77 beats/min reported by Clark et al. [1993], 59 beats/min reported by Doherty et al. [1997], and 50–

116 beats/min reported by Giovagnoli et al. [2002]) but similar to that observed by Waran et al. (1996). There might be several explanations for the low heart rate, for example, the horses were accustomed to being transported and were relaxed, the drivers were experienced (Giovagnoli et al., 2002), and the physical comfort in the transports was perhaps better than in earlier studies. The fact that noise disturbance was limited by cotton in the ears could also have contributed to the low heart rate. It is common practice among trainers of Standardbred trotters to use ear plugs because of the "calming" effect they are considered to have. In addition, there was no increase in plasma lactate concentration during transportation in the present study, in contrast to earlier observations on transported horses (5.5 mmol/L reported by Stull [1999], 1.2–1.3 mmol/L reported by Stull and Rodiek [2002], and 1.91 mmol/L reported by Werner and Gallo [2008]). This was in line with the low heart rates observed, confirming that the level of physical activity, eccentric or isometric, performed during the transport was low.

In horses on the FO, the plasma insulin concentration tended to be lower at the time of the second transport in horses that had not been transported (CT) than in horses that had been transported (TT). This might have been due to a delay in digestion and absorption of starch when horses were transported. Plasma urea concentrations were greater than values at rest on the FONLY AT2 for both the TT and the CT, indicating increased utilization of protein as an energy source. This observation is in agreement with findings in an earlier study in which feed deprivation increased urea concentration faster in horses fed a FONLY (6 h) compared with horses fed a forage–oats diet (12 h; Connysson et al., 2010). In the present study, the increase was observed after approximately 9 h without feed. The importance of these observations needs further investigation.

In conclusion, this study showed that both transport and diet affect the metabolic response during exercise in horses. Aerobic energy supply was most likely elevated by transportation and by the FONLY. The FONLY also decreased exercise-induced effects on extracellular fluid regulation. These results highlight the importance of experimental design for nutrition studies. If the aim is to increase knowledge of how diet affects exercise response in competition horses, transport should preferably be included in the experimental design, because horses are likely to be transported before a competition.

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