Pferdeheilkunde 23 (2007) 3 (Mai/Juni) 241-246

Fat adaptation affects insulin sensitivity and elimination of horses during an 80 km endurance ride

Tanja M. Hess[1], David S. Kronfeld[1], Kibby H. Treiber[1], Kathleen E. Crandell[2], Jeannie N. Waldron[3], Carrey A. Williams[1,4], W. Burton Staniar[1], Marco A. Lopes[1,5] and Patricia A. Harris[6]

Department of Animal and Poultry Sciences,Virginia Polytechnic Institute and State University, College of Agriculture and Life Sciences, Blacksburg, VA[1], KC Equine Consulting, The Plains, VA[2], Rectortown Equine Clinic, Rectortown, VA[3], Rutgers University, Cook Campus, New Brunswick, NJ, USA[4], Universidade Federal de Viçosa, Minas Gerais, BR[5] and WALTHAM Centre for Pet Nutrition, Melton Mowbray, UK[6]

Summary

The present study tested the hypothesis that insulin sensitivity would be lower in horses adapted to sugar and starch than those adapted to fat and fiber during an endurance race. Forty horses were divided into 3 dietary groups: one group of experimental feed (SS) was rich in starch (33%), low in fat (8%); another (FF) low in starch (6%) and rich in fat (15%); and a third group of commercial concentrates (CC) was intermediate in starch (16%) and fat (11%). Blood samples were taken the day before the race (PRE), within 3 minutes of arrival at each vet check (after 27, 48, 80 km), and three hours post completion or elimination. Plasma samples were analyzed for glucose, insulin, cortisol, glycerol, triglycerides, CK and AST. A proxy for insulin sensitivity as measured by the minimal model was calculated: RISQI = (1/sqrt [insulin]). Also, a proxy for pancreatic β-cell response to plasma glucose was calculated: MIRG =[800-0.30([insulin] -50)^2]/[glucose - 30]. Higher insulin in FF and CC horses, and in eliminated horses, combined with a lower RISQI in eliminated horses indicated that insulin resistance (low RISQI) was attenuated by fat and fiber feeding and decreased the likelihood of elimination. Higher insulin sensitivity in finishers and fat and fiber fed horses may have allowed a more efficient glucose uptake by muscles, allowing energy to be obtained through NEFA and TG. Fat and fiber feeding could avoid insulin resistance improving the efficiency of energy utilization and performance of horses during endurance races it also could reduce excitement and increases in muscle enzymes.

Keywords: Insulin, insulin sensitivity, endurance exercise, fat feeding

Die Adaptation von Pferden an eine fettreiche Ration beeinflusst die Insulin- Sensibilität und -Elimination während eines 80 km langen Distanzrittes

Im nachfolgend vorgestellten Versuch sollte geprüft werden, ob bei Pferden während eines Distanzrittes von 80 km Länge die Insulinsensitivität durch die Art der Ration, zucker- und stärkereich oder alternativ fett- und faserreich, beeinflusst wird. Hierzu standen 40 Pferde zur Verfügung, die zu Heu eines der folgenden Konzentrate erhielten: 1. stärkereich, fettarm (SS; 33 % Stärke, 8 % Fett), 2. stärkearm, fett- und faserreich (FF; 6 % Stärke, 16 % Fett), 3. intermediärer Futtertyp (CC) mit 16 % Stärke und 11 % Fett. Die Rationen waren isokalorisch und isonitrogen eingestellt (~3,3 Mcal bzw. 13,81 MJ und 120 g Rohprotein/kg Trockensubstanz). Die Pferde erhielten 3 Monate vor dem Distanzritt täglich je nach Leistung 1,7-5,1 kg Konzentrat, Heu und Wasser standen ad libitum zur Verfügung. Am Tag vor dem Wettkampf (PRE), bis zu 3 Minuten nach Ankunft an den Veterinärkontrollen bei Kilometer 27, 48 und 80 sowie 3 Stunden nach Zieleinlauf oder Ausschluss wurden Blutproben entnommen. Im Blutplasma wurden Glukose, Insulin, Kortisol, CK, AST, Glycerol und Triglyzeride bestimmt. Die Insulinsensitivität wurde entsprechend dem so genannten „Minimalen Modell“ (*Treiber* et al. 2005b) kalkuliert: RSIQI = (1/Quadratwurzel [Insulin]. Desgleichen wurde ein entsprechender Wert für die Antwort der ß-Zellen des Pankreas auf Glukose berechnet (*Treiber* et al. 2005b), MIRG = [800-0.30([Insulin]-50)^2]/[glucose-30]. Effekte der Entnahmezeit, Beendung des Rittes, Fütterungsgruppe sowie Interaktionen wurden durch Varianzanalyse (ANOVA) in einem gemischten Modell mit wiederholten Messungen analysiert; eine nicht parametrische ANOVA bezüglich der Parameter Glukose, Insulin, RISQI und MIRG wurde zum Vergleich der Pferde genutzt, die das Ziel ereichten, und der vom Wettbewerb ausgeschlossenen Pferde benutzt. Ergebnisse: Die ausgeschlossenen Pferde zeigten niedrigere (P = 0,037) RISQI-Werte (0,26 ± 0,01 mU/L$^{-0.5}$) und höhere Insulinkonzentrationen (P = 0,015; 21,14 ± 1,7 mU/L) als Pferde, die das Ziel erreichten (RISQI, 0.29 ± 0.01 mU/L$^{-0.5}$; Insulin 15.19 ± 1.0 mU). Im Blut die Tiere waren ferner (ohne Berücksichtigung von Pferden mit klinischer Myopathie, „Rhabdomyolyse“) höhere CK- und AST-Werte bei 48 km (P < 0,005), und bei REC (P = 0,002) sowie höhere Kortisolkonzentrationen (P = 0 036; 131,1 ± 10,2 ng/dl) zu beobachten als bei den im Ziel einlaufenden Pferden (Kortisol, 119.9 ± 4.7 ng/dl) Gegenüber dem Ruhewert stiegen die Plasmawerte für CK, AST, Kortisol, Glycerol, Triglyzeride und RISQI an, während für Insulin und MIRG ein Abfall zu verzeichnen war. Pferde, die mit SS und CC gefüttert wurden, hatten niedrigere RISQI-Werte (p=0,018) als die mit FF versorgtenTiere. Die MIRG-Werte waren für die FF-Gruppe lediglich tendenziell niedriger (P = 0,088) als bei den Gruppen SS und CC. Die Pferde dieser beiden Gruppen wiesen höhere Insulinwerte auf (SS 17,7 ± 1,6, CC 18,6 ± 1,8 mU/L) als die nach FF-Regime versorgten Pferde (13,6 ± 0,9 mU/L). Die Ergebnisse dieser Arbeit zeigen, dass eine Insulin Resistenz ein mit dem notwendigen Ausschluss von Pferden aus dem Wettbewerb in Zusammenhang stehender Faktor sein kann. Durch eine fett- und strukturreiche Fütterung kann, wie der Werte für die RISQI zeigen, eine Insulin Resistenz vermieden werden. Zwei Pferde wurden wegen klinischer Symptome einer Myopathie (Rhabdomyolyse) eliminiert; insgesamt zeigten vom Wettbewerb ausgeschlossene Pferde höhere Werte für muskelsensitive Enzymen bei Kilometer 27, 48 und bei REC als die unauffälligen Tiere. Auch die SS-Fütterung war mit höheren Enzymwerten assoziiert. Fettreiche und stärkearme Diäten sind danach geeignet, kritische Veränderungen in der Muskulatur während und nach Belastung bei Pferden mit wiederholter Myopathie zu unterdrücken (*McKenzie* et al. 2003). Solche Diäten sind erfolgreich verwendet worden, um eine abnorm rasche Glykogensynthese bei Pferden mit Glykogenspeicherkrankheit zu vermeiden (*Annandale* et al. 2004). Auffällig erregbare Pferde zeigen offenbar eine besondere Disposition für Myopathien und profitieren daher von einer fettreichen Diät (*Holland* et al. 1996). Die höheren Kortisolwerte bei ausgeschlossenen Pferden deuten auf vermehrte Stressbelastung dieser Tiere hin im Vergleich zu den erfolgreich ins Ziel kommenden Tieren. Ein forcierter Anstieg des Kortisols im Blut ist ebenfalls bei stärkereicher Fütterung arbeitender Pferde beobachtet worden (*Slade* et al. 1975, *Crandell* et al. 1999). Kortisol verstärkt die Insulin Resistenz (*Guyton* and *Hall* 2001) und intensiviert die Insulinsekretion wie anhand vorliegender Ergebnisse bestätigt werden konnte. Normalerweise führt Bewegung zu einer Erhöhung der Insulin Sensitivität (*Brun* et al. 1995, *Powell* et al. 2002). Der Glucosentransport in der arbeitenden Muskelzelle erfolgt über den entsprechenden Transporter „GLUT-4", abhängig vom ionisierten Calcium (Ca^{++}) und vom Insulin selbst (*Richter* et al. 2004). Diese Konstellation bedingt gegenüber der Ruhebedingung eine erhöhte Insulinsensitivität. Die hier ermittelten Werte belegen eine Zunahme der Insulinsensitivität im Verlauf der Belastung bei allen Pferden. Allerdings fiel die Insulin Sensitivität bei den ausgeschlossenen Pferden kleiner aus als bei den ankommenden Pferden. Die Plasmaglukose gab diese Unterschiede nicht wieder, offenbar infolge einer Kompensation mittels intensivierter Insulinsekretion (MIRG). Verstärkte Insulinsekretion kann zur Reduktion der Fettverbrennung führen (*Saltiel* und *Kahn*. 2001), während der Belastung wurde hierdurch bedingt eine verminderte Leistungsfähigkeit und erhöhte Ausschlussrate beobachtet, vergleichbar zu den Ergebnissen der eigenen Studie. Die verminderte Insulinsensitivität (RSIQI) bei Pferden der SS und CC Gruppe sowie bei den vom Rennen ausgeschlossenen Tieren zeigt an, dass Insulin eine Resistenz (niedriges RISQI) durch fett- und faserreiche Fütterung gemildert wurde. Eine hohe Insulinsensitivität kann andererseits die Glukoseaufnahme der Muskulatur verbessern und die Energiegewinnung durch Oxidation von Fettsäuren begünstigen. Dies dürfte dem Leistungsvermögen von Distanzpferden zugute kommen. Unter diesen Bedingungen (FF-Gruppe) ist offenbar auch die Schädigung der Muskelzelle vermindert. Diese Arbeit zeigt erstmalig die mögliche Verbindung zwischen einer Insulin Resistenz und der Leistung von Pferden während eines Distanzrittes sowie dem Ausschluss von eingeschränkt leistungsfähigen Tieren vom Wettkampf.

Stichwörter: Insulin-Sensitivität, Distanzritte, Fette, Fütterung

Introduction

Feeding feeds rich in fat and fiber (FF) may avoid the adverse effects on insulin sensitivity and insulin signaling associated with chronic adaptation to meals of grain and molasses (starch and sugar, SS) (*Hoffman* et al. 2003, *Treiber* et al. 2005a). This adverse effect of SS has been observed in resting horses but not when they are physically conditioned (*Treiber* et al. 2006). It was revealed, however, when the fit horses were subjected to endurance exercise on a treadmill, insulin sensitivity and related variables were lower in horses adapted to SS compared to those adapted to a fat and fibre enriched diet (*Treiber* et al. 2006). The present study tested the hypothesis that insulin sensitivity would be lower in horses adapted to SS than those adapted to FF during an endurance race.

Material and Methods

This study was undertaken during the Middleburg Research Ride 2002, which was held on April 14 and followed American Endurance Ride Conference (AERC) rules (1999). The effects of feeds rich in starch and fat on insulin sensitivity were compared in 40 endurance horses during an 80 km race in 3 dietary groups: one experimental feed (SS, n = 15) was rich in starch (33%), low in fat (8%); another (FF, n = 14) was low in starch (6%) and rich in fat (15%); and commercial concentrates (CC, n = 11) were intermediate in starch (16%) and fat (11%). Feeds were isoenergetic (~3.3 Mcal/kg DM) and isonitrogenous (~12% CP). Horses were fed 1.7 to 5.1 kg/d with hay ad libitum for 3 months prior to the race. The protocol was approved by the institutional animal care and use committee.

The race started at 07.00 and covered 80 km of rolling hills with altitudes varying from 135 to 450 m. Rest stops were provided at 27, 48 and 72 km with veterinary checks (AERC 1999). The trail between two rest stops is called a loop, and this trail consisted of four loops. Ambient temperature ranged from 25 to 32°C, and humidity from 60 to 100%.

The 40 horses with an average age of 11 ± 1 years included 37 Arabians, purebred or crossbred, 1 Quarter Horse, 1 Mustang, and 1 Thoroughbred.

Blood samples were taken the day before the race (PRE), within 3 minutes of arrival at each vet check (after 27, 48, 80 km), and three hours post completion or elimination (REC) into heparinized sample tubes (Vacutainer, Fisher Health Care, Chicago, IL). Blood samples were immediately centrifuged at 3000 g for 10 min. Plasma was removed within 30 min of collection and frozen at -4° C until analysis. Plasma samples were analyzed for glucose, insulin, cortisol, glycerol, triglycerides, CK and AST. Plasma glucose, glycerol, triglycerides, CK and AST were analyzed by enzymatic assay (Beckman Instruments, St. Louis, MO). Insulin and cortisol were determined using an RIA (Coat-A-Count Insulin, validated for equine insulin, *Freestone* et al. 1991; Cortisol, Diagnostic Products, Los Angeles, CA). The intraassay CV of duplicate samples was <1% for glucose, glycerol, triglycerides, CK, AST, and 5% for insulin and cortisol.

Horses were not fed grain at least five hours prior to the PRE blood sample. Information about feed intake during and until three hours after the race was recorded. A proxy for insulin sensitivity as measured by the minimal model was calculated: RISQI = (1/sqrt [insulin]) (*Treiber* et al. 2005b). Also, a proxy for pancreatic β-cell response to plasma glucose was calculated: modified insulin response to glucose (MIRG) =[800-0.30([insulin] -50)^2]/[glucose - 30] (*Treiber* et al. 2005b). Insulin sensitivity was graded according to reference quintiles previously determined in horses (*Treiber* et al. 2005b). Frequency of insulin sensitivity quintiles was assessed and compared among the different feed groups and between finishers and eliminated horses through odds ratio analysis (StataCorp 2003).

Effects of sampling time, completion of the ride, treatments and interactions were evaluated by ANOVA in a mixed model with repeated measures; non-parametric ANOVA compared finishers and eliminated horses for glucose, insulin, RISQI and MIRG (SAS Institute Inc., Cary, NC).

Results

Reasons for elimination of 15 of the 40 horses (37.5%) were lameness (3: 1FF, 2 CC), exertional rhabdomyolysis (2: 1 CC,

Table 1 Overall incidence of insulin response to glucose (MIRG) and insulin sensitivity as the reciprocal of the square root index (RISQI) in the five pre determined quintiles (*Treiber* et al. 2005b)
Verteilung der Insulin-Antwort auf Glucose (MIRG) und Insulinsensibilität gemessen an der inversen Quadratwurzel von Insulin (RISQI) in den fünf determinierten Quintilen.

Quintiles	1st	2nd	3rd	4th	5th
MIRG FINISHER	0	1	15	2	1
MIRG ELIMINATED	0	2	4	2	5
RISQI FINISHER	15	2	3	0	0
RISQI ELIMINATED	10	1	1	0	0

1 SS), failure to recover heart rate in 30 min (3: 2 SS, 1 CC), labile heart rate (2: 1 FF, 1 SS), arrhythmias (2: 1 FF, 1 CC), slow gut sounds (1 CC), sore back (1 FF), and rider option (1 CC). Average speed was 10.0 ± 0.4 km/h over 80 km, and 10.5, 10.8, 9.3, and 7.7 km/h for the 4 loops for finishing horses.

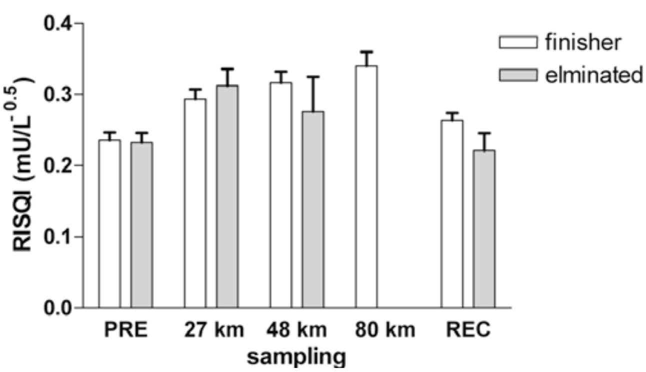

Fig 1 Completion and insulin sensitivity as measured by the reciprocal of the square root index (RISQI) during an 80 km endurance race. Overall lower RISQI (P = 0.037) in eliminated horses compared to finishers.
Insulinsensitivität, gemessen an der inversen Quadratwurzel der Insulinkonzentrationen (RISQI) vor, während und nach dem Distanzritt bei Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden (insgesamt niedrigere RISQI-Werte bei den ausgeschlossenen Pferden, P =0,037).

Average feed intake was 1 kg for SS (0-5 kg), 2 kg for FF (0.5-4 kg), and 2.5 kg (0-5.4 kg) for CC fed horses in the morning of the ride. During the ride all horses consumed in average 0.4 kg of grain at each vet check (range 0 -0.7kg), and 0.3 kg of grain (range 0 – 1.5 kg) at REC for SS, FF, and CC horses. Eliminated horses had overall lower (P = 0.037) RISQI (0.26 ± 0.01 mU/L$^{-0.5}$) than finishing horses (0.29 ± 0.01 mU/L$^{-0.5}$; Figure 1). Especially at REC, RISQI had a trend to be lower (P = 0.061) in eliminated horses. Eliminated horses had overall higher (P = 0.015) insulin (21.14 ± 1.7 mU/L) than finishing horses (15.19 ± 1.0 mU/L) (Figure 2). Eliminated horses had higher insulin specifically at 48 km (P = 0.012) and at REC (P = 0.002) compared to finishing horses (Figure 2). Analysis of the insulin sensitivity (RISQI and MIRG) quintiles frequency revealed an overall higher incidence of eliminated horses in the 5th quintile of MIRG (X^2 = 5.58, P = 0.018; Table 1), indicating a higher secretion of insulin for the present amount of glucose. Eliminated horses

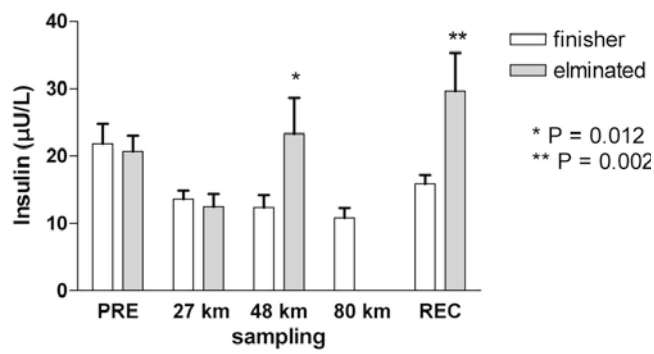

Fig 2 Completion and insulin concentration in blood of horses during an 80 km endurance race (*/** eliminated horses significantly higher insulin than finishers)
Insulinkonzentrationen im Blutplasma vor, während und nach dem Distanzritt bei Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden (/** signifikant höhere Insulinkonzentrationen bei den ausgeschlossenen Pferden).*

Table 2 Sampling effect on selected plasma variables and proxies for RISQI and MIRG. Different letter subscripts differ (P < 0.05).
Plasmaparameter und Kennwerte für die Insulinsensitivität (RISQI) und insulinbedingte Glukosereaktion (MIRG) zu den verschiedenen Zeitpunkten; unterschiedliche Buchstaben kennzeichnen signifikante Differenzen.

	PRE	27 km	48 km	80 km	REC	MSE
Glucose (mg/dl)	125.7 ^{ab}	135.61^{ac}	122.45^{b}	124.10^{b}	142.83^{c}	± 4.5
Insulin (µu/ml)	21.48^{a}	13.53^{b}	12.28^{b}	11.39^{b}	15.90^{b}	± 2.1
Cortisol (µg/dl)	76.43^{a}	145.88^{b}	154.70^{b}	156.95^{b}	78.53^{a}	± 7.7
Glycerol (mg/dl)	1.12^{a}	12.96^{b}	21.97^{c}	33.36^{d}	4.37^{a}	± 2.2
Triglycerides (mg/dl)	15.76^{a}	21.58^{a}	32.69^{b}	42.89^{c}	18.54^{a}	± 2.9
RISQI	0.23^{a}	0.30^{b}	0.31^{bc}	0.34^{c}	0.25^{a}	± 0.01 0.010.002
MIRG	5.41^{a}	3.57^{b}	3.79^{bc}	3.48^{b}	4.27^{c}	± 0.33

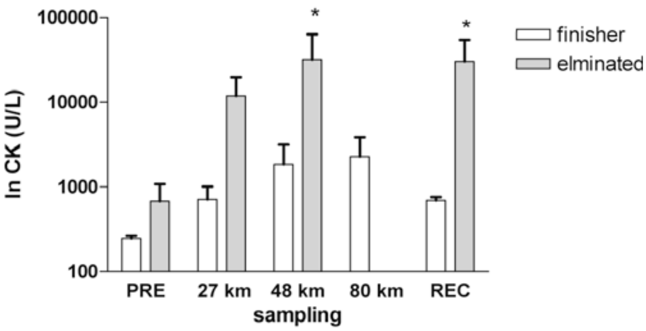

Fig 3 Completion and ln CK during an 80 km endurance race. * CK significantly higher in eliminated than in finishing horses ($P < 0.001$).
Logarithmus der CK-Aktivität im Blutplasma vor, während und nach dem Distanzritt bei Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden (Signifikant höhere CK-Aktivitäten bei den ausgeschlossenen Pferden, P <0,001)*

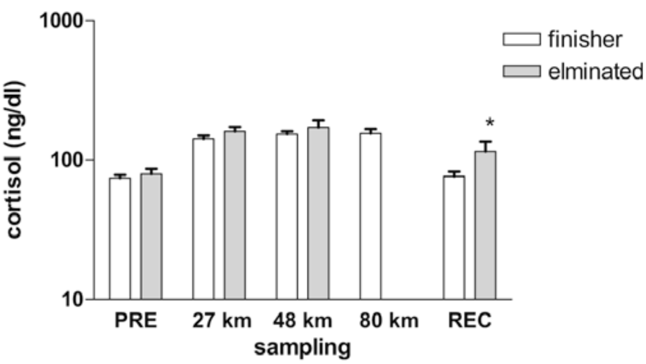

Fig 4 Completion and plasma cortisol during an 80 km endurance race. Overall higher cortisol in eliminated versus finishers ($P = 0.036$). * Higher cortisol in eliminated horses compared to finishers ($P = 0.005$) at recovery.
Kortisolkonzentrationen im Plasma vor, während und nach dem Distanzritt bei Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden (höhere Kortisolwerte bei den ausgeschlossenen Pferden, P = 0,036)*

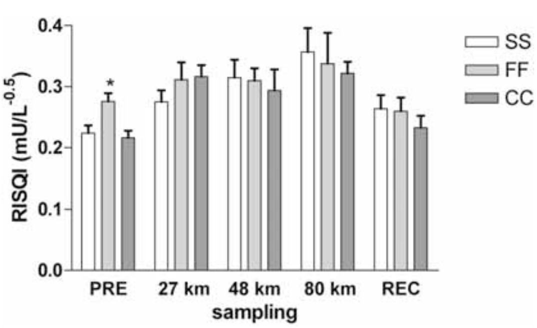

Fig 5 The reciprocal of the square root index (RISQI) in sugar starch (SS), fat and fiber (FF), and commercial concentrate (CC) fed horses during an 80 km endurance race. *Higher RISQI ($P = 0.018$) in FF fed compared to SS and CC fed horses at PRE.
Insulinsensitivität gemessen an der inversen Quadratwurzel der Insulinkonzentrationen (RISQI) vor, während und nach dem Distanzritt bei Pferden, differenziert nach dem Fütterungsregime SS: stärkereich/fettarm, FF: fett-, faserreich, CC intermediär, kommerzielles Mischfutter (höhere RISQI-Werte bei Pferden der FF-Gruppe im Vergleich zu den übrigen Pferden, P = 0,018)*

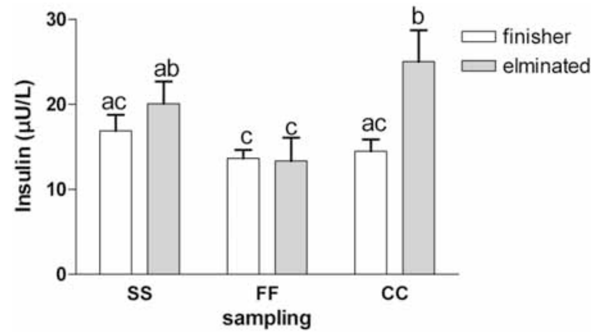

Fig 6 Plasma insulin in sugar starch (SS), fat and fiber (FF), and commercial concentrate (CC) fed horses during an 80 km endurance race. Overall lower plasma insulin ($P = 0.042$) in FF fed compared to SS and CC fed horses. Different letters differ ($P < 0.05$).
Insulin im Plasma von Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden, differenziert nach dem Fütterungsregime SS: stärkereich/fettarm, FF: fett-, faserreich, CC intermediär, kommerzielles Mischfutter. (unterschiedliche Buchstaben kennzeichnen signifikante Mittelwertsdifferenzen, P<0,05; insgesamt geringere Insulinwerte, P<0,042, bei den Pferden der FF-Gruppe im Vergleich zu den übrigen Tieren)

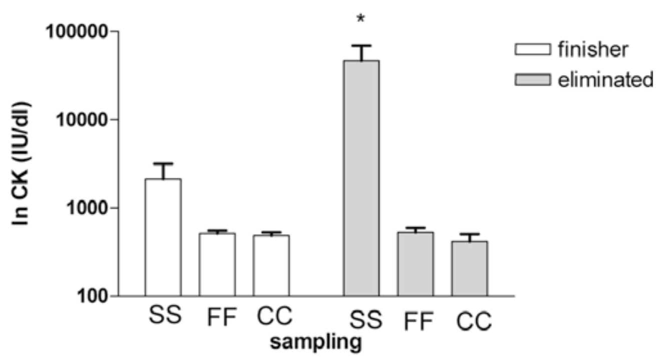

Fig 7 Logarithm of plasma CK in finishing and eliminated horses fed sugar starch (SS), fat and fiber (FF), and commercial concentrates (CC) during an 80 km endurance race. * Significant higher CK in SS fed eliminated horses ($P < 0.001$) compared to FF, CC finishers and eliminated horses.
Logarithmus der mittleren CK-Aktivität im Plasma von Pferden, die den Distanzritt beendeten, im Vergleich zu disqualifizierten Pferden, differenziert nach dem Fütterungsregime SS: stärkereich/fettarm, FF: fett-, faserreich, CC intermediär, kommerzielles Mischfutter
** signifikant höhere CK-Aktivität für SS-gefütterte und disqualifizierte Pferde (P < 0.001) im Vergleich allen anderen Gruppierungen*

(analyzed without the rhabdomyolysis cases) had higher CK at 48 km ($P < 0.001$), and recovery ($P < 0.001$) than finishing horses (Figure 3). Eliminated horses (analyzed without the rhabdomyolysis cases) had higher AST at 48 km ($P = 0.005$), and recovery ($P < 0.001$) than finishing horses. Eliminated horses had overall higher (131.1 ± 10.2 ng/dl) cortisol ($P = 0.036$) than finishing horses (119.9 ± 4.7 ng/dl)(Figure 4). Specifically at recovery cortisol was lower ($P = 0.005$) in finishers than eliminated horses.

Increases were found in plasma glycerol, triglycerides, CK, AST, cortisol, and RISQI, and decreases in insulin and MIRG with sampling time ($P < 0.005$; Table 2).

Horses fed SS and CC had overall lower ($P = 0.019$) RISQI than FF fed horses. Especially at PRE, RISQI was higher ($P = 0.018$) in FF fed horses compared to SS and

CC horses (Figure 5). MIRG, however just had a trend to be lower ($P = 0.088$) in FF fed horses compared to SS and CC fed horses. There was a higher incidence of horses in the 5th quintile for MIRG comparing SS to FF horses ($X^2 = 7.07$, $P = 0.0078$) and comparing FF to CC horses ($X^2 = 4.55$, $P = 0.033$) at PRE, meaning higher insulin secretion in the response to glucose in SS and CC horses, compared to FF. Horses fed SS (17.7 ± 1.6 mU/L) and CC (18.6 ± 1.8 mU/L) feeds had overall higher insulin ($P = 0.042$) than FF (13.6 ± 0.9 mU/L) fed horses (Figure 6). Horses on SS diets had higher plasma CK (11516 ± 5116 IU/L) than FF (514 ± 34 IU/L) or CC (461 ± 40 IU/L) diets ($P < 0.001$). Eliminated horses on SS diet had the highest CK values compared to all other horses ($P< 0.001$; Figure 7). Horses on SS diets had higher plasma AST (669 ± 163 IU/L) than FF (273 ± 7 IU/L) or CC (280 ± 7 IU/L) diets ($P < 0.001$).

Discussion

The results of this study suggest that insulin resistance can be a determining factor in the elimination of horses during endurance races. Furthermore, supplementation with feeds high in fat and fiber can attenuate insulin resistance as shown by a higher RISQI in FF fed horses.

Two horses were eliminated with signs of rhabdomyolysis and eliminated horses had higher muscle enzymes at 27, 48 km and at recovery. Higher enzymes were also found in SS fed horses. High fat/ low carbohydrate diets have been shown to lead to lower muscle enzymes after exercise in severely affected horses with recurrent rhabdomyolysis cases (*McKenzie* et al. 2003). Such diets have been recommended to reduce rapid glycogen synthesis in horses with polysaccharide storage myopathy (*Annandale* et al. 2004). Excitable horses are also more prone to rhabdomyolysis, and they may become calmer when fat adapted (*Holland* et al. 1996).

Higher plasma cortisol levels in eliminated horses indicate that eliminated horses were more stressed than finishers. Also diets rich in starch have been shown to increase cortisol during exercise, due to increased excitement (*Slade* et al. 1975, *Crandell* et al. 1999). Cortisol increases insulin resistance (*Guyton* and *Hall* 2001), which is could be compensated for with increased insulin secretion as observed in this study.

Exercise usually enhances insulin sensitivity (*Brun* et al. 1995; *Powell* et al. 2002, *Treiber* et al. 2006). However another study found that insulin sensitivity was unchanged after exercise (*Pratt* et al. 2005). During exercise, glucose transport is driven by the Ca^{++} mediated GLUT-4 transport in addition to the insulin mediated GLUT-4 transport (*Richter* et al. 2004) leading to increases in insulin sensitivity. In all horses proxies indicated that insulin sensitivity increased with exercise throughout the race. In eliminated horses however, insulin sensitivity was overall lower (lower RISQI). No differences in plasma glucose were found despite differences in insulin sensitivity, a result of compensation by an increased insulin response (MIRG). Also the higher incidence of eliminated horses in the 5th quintile indicates higher insulin secretion to control plasma glucose and compensation for lower insulin sensitivity.

Proxies have usually been used for analysis of basal samples from resting, overnight fasted horses (Treiber et al. 2005a). The analysis of the proxies before, during, and after the race allowed comparing different physiological states during exercise, the first use of insulin resistance proxies for this purpose, as far as we know. Although different amounts in ingested feeds were consumed before the race, the adaptation to the specific feed yielded observed differences among groups. Insulin sensitivity (RISQI) was in the two lowest quintiles overall, indicating low insulin sensitivity in all horses compared to another study (*Treiber* et al. 2005b). However horses were not fasted and ingested grain during and after the race, which may have increased plasma insulin and glucose, increasing RISQI and MIRG. A controlled study would be necessary to confirm the results obtained in the present study. Higher MIRG in SS and CC horses compared to FF horses indicate that a higher insulin secretion was necessary for the clearance of glucose as compensation.

Higher insulin could lead to decreased lipolysis (*Saltiel* and *Kahn* 2001) decreasing fatty acid oxidation during exercise, and contributing to decreased performance and elimination as observed in this study.

Lower insulin sensitivity (RISQI) in SS and CC horses, and in eliminated horses indicated that insulin resistance (low RISQI) was attenuated by fat and fiber feeding and also decreased the likelihood of elimination. Higher insulin sensitivity in finishers and fat and fiber fed horses would have enabled more rapid glucose uptake by contracting muscles.

Conclusion

Fat and fiber (FF) feeding avoids insulin resistance that develops as a chronic adaptation to meals of feeds rich in starch and sugar (SS). The present results show that FF feeding avoided hyperinsulinemia and insulin insensitivity, and lowered the elimination rate of horses, compared to SS feeding. They also indicated that horses adapted to FF had lower plasma CK and cortisol, indications of muscle damage and stress, respectively. This study reinforces many previous ones that show advantages of fat adaptation for both sprinting and endurance exercise.

Literature

Annandale E. J., Valberg S. J., Mickelson J. R. and *Seaquist E. R.* (2004): Insulin sensitivity and skeletal muscle glucose transport in horses with equine polysaccharide storage myopathy. Neuromuscular Disorders, 666-674

AERC (1999): Veterinary Guidelines for judging AERC endurance competitions. The American Endurance Ride Conference, Auburn, CA

Brun J. F., Guintrand-Hugret C., Boegner C., Bouix O. and *Orsetti A.* (1995): Influence of short term submaximal exercise on parameters of glucose assimilation analyzed with the minimal model. Metabolism 44, 833-840

Crandell K. G., Pagan J. D., Harris P. A. and *Duren S. E.* (1999): A comparison of grain, oil and beet pulp as energy sources for the exercised horse. Equine Vet J 30, 485-489

Guyton and *Hall* (2001): Textbook of medical physiology. Philadelphia, PA: W. B. Saunders Company, 875

Hoffman R. M., Boston R. C., Stefanovsky D., Kronfeld D. S. and *Harris P. A.* (2003): Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J Anim Sci 81, 2333-2342

Holland J. L., Kronfeld D. S. and *Meachem T. N.* (1996): Behavior of horses is affected by soy lecithin and corn oil in the diet. J Anim Sci 74, 1252-1255

McKenzie E. C., Valberg S. J., Godden S. M., Pagan J. D., MacLeay J. M., Geor R. J. and Carlson G. P. (2003): Effect of dietary starch, fat, and bicarbonate content on exercise reponses and serum creatin kinase activity in equine recurrent rhabdomyolysis. J Vet Intern Med 17, 693-701

Powell D. M., Reedy S. E., Session D. R. and *Fitzgerald B. P.* (2002): Effect of short term exercise training on insulin sensitivity in obese and lean mares. Equine Vet J 34, 81-84.

Pratt S., Geor R. J. and *McCutcheon J.* (2005): Insulin sensitivity in the horse. Proc. XIX Equine Sci Soc Symp,120

Richter E. A., Nielsen J. A., Jorgensen S. B., Frosig C., Birk J. B. and *Wojtaszewski F. P.* (2004): Exercise signalling to glucose transport in skeletal muscle. Proc Nutr Soc 63, 211-216

Saltiel A. R. and *Kahn C. R.* (2001): Insulin signaling and the regulation of glucose and lipid metabolism. Nature 414, 799-806

Slade L. M., Lewis L. D. and *Quinn C. R.* (1975): Nutritional adaptations of horses for endurance type performance. Proceedings of the 4th Equine Nutr Physiol Symp 4, 114-128

Treiber K. H., Boston R. C., Kronfeld D. S., Staniar W. B. and *P. A. Harris* (2005a): Insulin resistance and compensation in thoroughbred weanlings adapted to high-glycemic meals. J Anim Sci 83, 2357-2364

Treiber K. H., Hess T. M., Kronfeld D. S., Boston R. C., Geor R. J., Friere M., Silva A. and *P. A. Harris* (2006): Glucose dynamics during exercise: Dietary energy sources affect minimal model parameters in trained arabian geldings during endurance exercise. Equine Vet J 36S, 631-636

Treiber K. H., Kronfeld D. S., Hess T. M., Boston R. C. and *P. A. Harris* (2005b): Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. Am J Vet Res 66, 2114-2121

Tanja M Hess
thessvet@yahoo.com
Rua Aarão Reis 115
Santa Tereza
Rio de Janeiro-RJ
Cep 20240-090
BRAZIL
thessvet@yahoo.com