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ORIGINAL ARTICLE

A 90-day adaptation to a high glycaemic diet alters postprandial lipid metabolism in non-obese horses without affecting peripheral insulin sensitivity

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Summary

High glycaemic feeds are associated with the development of insulin resistance in horses. However, studies that evaluated the effect of high glycaemic feeds used horses that either ranged in body condition from lean to obese or were fed to increase body condition over a period of months; thus, the ability of high glycaemic feeds to induce insulin resistance in lean horses has not been determined. This study evaluated the insulin sensitivity of 18 lean horses fed a 10% (LO; $n = 6$), 20% (MED; $n = 6$) or 60% (HI; $n = 6$) non-structural carbohydrate complementary feed for 90 days. Although both the MED and HI diets increased insulinaemic responses to concentrate feeding in relation to the LO diet ($p > 0.05$), neither induced insulin resistance, as assessed by glucose tolerance test, following the 90-day feeding trial. Interestingly, the post-feeding suppression of plasma non-esterified fatty acids was less pronounced in HI-fed horses ($p = 0.054$) on days 30 and 90 of the study, potentially indicating that insulin-induced suppression of adipose tissue lipolysis was reduced. As insulin-resistant animals often have elevated plasma lipid concentrations, it is possible that altered lipid metabolism is an early event in the development of insulin resistance. The effects of high glycaemic feeds that are fed for a longer duration of time, on glucose and lipid metabolism, should be investigated further.

Introduction

Insulin promotes postprandial glucose utilization by activating glucose uptake by skeletal muscle and adipose tissue. Tissue responsiveness to insulin stimulation is termed insulin sensitivity. A reduction in insulin sensitivity, or insulin resistance, is a physiological state in which normal concentrations of insulin produce a less than normal biological response (Kahn, 1978). Thus, an insulin-resistant horse would require greater pancreatic insulin secretion, resulting in greater circulating insulin concentrations, to control blood glucose following consumption of a meal. As insulin-resistant horses also exhibit increased fasting insulin concentrations (Frank et al., 2006; Treiber et al., 2006; Carter et al., 2009b), it is likely that a greater insulin concentration is required to maintain blood glucose even in the unfed state. In addition to altered glucose metabolism, increased plasma triglycerides and non-esterified fatty acids (NEFAs) are noted in insulin-resistant horses (Frank et al., 2006), indicating that insulin resistance influences lipid metabolism as well.

Grain-based complementary feeds are often fed to horses and can supply up to 50% of a horse's daily digestible energy (DE) requirement (Hudson et al.,

2001). However, consumption of feeds with starch and sugar contents of 40–60% [dry matter (DM) basis] increases postprandial glucose and insulin responses (Williams et al., 2001; Vervuert et al., 2009) and is associated with insulin resistance in conjunction with obesity (Hoffman et al., 2003; Quinn et al., 2008; Carter et al., 2009b). While obesity correlates with insulin resistance independent of diet (Treiber et al., 2006; Vick et al., 2007), studies that demonstrate a negative effect of diet on insulin sensitivity used horses across a range of body condition scores (BCS) (Hoffman et al., 2003) or horses that were fed to increase BCS (Quinn et al., 2008; Carter et al., 2009b). Thus, a separate evaluation of diet influence on insulin sensitivity is absent.

As insulin resistance predisposes horses to the debilitating hoof disease, laminitis (Treiber et al., 2006; Bailey et al., 2008; Carter et al., 2009a), it is imperative that the effect of high starch and sugar diets on insulin sensitivity be evaluated. Therefore, the primary hypothesis of this experiment was that consuming high starch and sugar feeds would cause daily elevations of postprandial insulin concentrations, and this elevated insulin would, in turn, reduce insulin sensitivity in non-obese horses, resulting in altered postprandial glucose, insulin, NEFA and triglyceride responses.

Materials and methods

Use of animals for this experiment was approved by Virginia Tech's Institutional Animal Care and Use Committee. This experiment was carried out at Virginia Tech's Middleburg Agricultural Research and Extension Center in Middleburg, VA, during the months of June (30 day acclimation period), July, August and September (90-day experimental period; Fig. 1). Mares were selected for this experiment from a larger population to have fasting insulin concentrations <20 mU/l, BCS (Henneke et al., 1983) <7.5 and age <20 years. For the selection process, horses were assessed 30 days prior to the start of the acclimation period. Following selection, mares were blocked by insulin, BCS and age into three groups. Two horses from each block were randomly assigned to one of three diets. Following this, two horses from each diet were randomly assigned to one of three drylots, such that each drylot contained 6 horses total.

Horses

Eighteen mature (age 9–18 years), non-pregnant, Thoroughbred mares previously maintained on pasture (tall fescue, Kentucky bluegrass, white clover mix) and once-daily forage balancer pellets were used for this experiment. For 30 days prior to the start of the experiment, horses were housed in drylots and acclimated to consuming their concentrate ration in individual stalls. Horses were offered their concentrate ration in two equal aliquots at 0800 and 1400 hours and allowed *ad libitum* access to water and iodized salt. Following consumption of concentrate, horses were returned to drylots and group-fed hay. Bodyweight (BW) and BCS were determined on day −30, −15, 0, 15, 30, 45, 60, 75 and 90 with BCS determined as the average of two independent scorers.

Treatments and experimental design

This experiment utilized a randomized complete block design with repeated measures. Drylot start dates were staggered to allow for completion of intensive measurements on testing days. Daily DE requirements were estimated based on NRC calculations (NRC 2007). Treatment feeds were formulated to differ in non-structural carbohydrates [NSC; the sum of starch and ethanol-soluble carbohydrate (ESC) fractions] content and consisted of 10 (LO), 20 (MED) and 60 (HI) % NSC (Table 1). All mares received LO during the 30-day adaptation period, and on day 1, mares assigned to either MED or HI were abruptly switched to their treatment feeds. Horses were offered 20% of their daily DE requirement as concentrate and 80% of their calculated DE

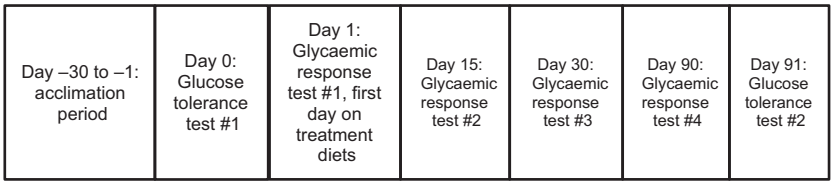

Fig. 1 Sampling time points for 90-day feeding study.

Table 1 Nutrient composition and diet ingredients of three diets designed to differ in starch and sugar content

		Treatment concentrate		
	Hay	LO	MED	HI
Nutrient (DM basis)				
DE (MCal/kg)*	2.2	2.2	2.7	3.5
Crude protein	17.1	15.0	21.3	12.4
ADF	34.2	32.7	24.4	5.3
NDF	50.1	49.1	36.4	12.4
WSC	6.7	6.8	6.0	8.4
ESC	4.7	4.9	4.2	5.8
Starch	2.2	3.0	15.4	50.6
Fat	2.3	4.9	2.9	3.4
Ash	9.6	11.6	8.4	6.0
Ingredient (%)				
Beet pulp	–	14	–	–
Dehydrated alfalfa	–	55	32.5	–
Feed lime	–	–	–	0.75
Ground barley	–	–	37.5	17.5
Ground corn	–	–	5	61.75
Molasses	–	–	–	7.5
Monosodium phosphate	–	1	–	–
Oil	–	2.5	2	–
Salt	–	0.65	0.65	0.65
Soybean meal (48%)	–	–	1.5	8.75
Soyhulls	–	23.75	17.75	–
Vitamin E	–	0.25	0.25	0.25
Vitamin/mineral pack	–	2	2	2
Yeast culture	–	0.25	0.25	0.25

DM, dry matter; DE, digestible energy; ADF, acid detergent fibre; NDF, neutral detergent fibre; WSC, water-soluble carbohydrate; ESC, ethanol-soluble carbohydrate.
*DE estimated based on nutrient composition.

requirement as hay. Hay was group fed in pens following the consumption of concentrate and included an initial 10% refusal rate. The hay refusal rate was increased to 20% halfway through the study when one mare (HI treatment) exhibited symptoms of a gastric ulcer (yawning, refusal of supplement and hay, physical examination findings). The mare received veterinary treatment (omeprazole), and hay was increased to all pens to increase the length of time that forage was available. The mare was not deemed an outlier following residual analysis, and the data were therefore not removed from the results.

Fasting blood samples

At 0700 hours, on day 1, 15, 30, 45, 60, 75 and 90, 10 ml of blood was collected into heparin-coated, EDTA-coated and uncoated collection tubes (Vacutainer; Beckton-Dickson, Franklin Lakes, NJ, USA). Feed was withheld for at least 10 h prior to sampling. Heparin- and EDTA-collected blood samples were immediately centrifuged at 2000 *g* for 10 min at 4 °C, for plasma and frozen in 1-mL aliquots at −20 °C. Blood in uncoated tubes was allowed to clot for 2 h at 4 °C prior to harvesting of serum in an identical manner to other blood samples.

Glycaemic response test

On day 1, 15, 30 and 90, mares underwent a glycaemic response test (GRT) to determine plasma glucose, insulin, triglyceride and NEFA responses to the dietary treatments and changes in response to treatment across the 90-day study period. For each GRT, mares received their normal daily morning ration of their treatment feeds. On the afternoon prior to and on the day of testing, horses were housed in individual 3.6 m^2 box stalls. On the day prior to testing, horses were fitted aseptically with 14-G jugular intravenous catheters (Abbocath, Abbott Corp., Abbott Park, IL, USA), following desensitization of the overlying skin with 2% lidocaine. Horses were maintained in stalls overnight, and feed was withheld for at least 10 h prior to testing. At 0800 hours, the normal daily ration of feed was offered and 10 ml of blood collected into heparin-coated collection tubes (Vacutainer) at −15, −1, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210 and 240 min relative to feeding. An additional 10 mL of blood was collected into EDTA-coated collection tubes (Vacutainer) at −1, 60, 120, 180 and 240 min relative to the offering of feed.

Intravenous glucose tolerance test

On day 0 and 91, intravenous glucose tolerance tests (IVGTT) were performed to determine baseline insulin sensitivity and changes across the 90-day experimental period. On the day prior to testing, horses were fitted aseptically with jugular intravenous catheters and were maintained in stalls overnight. All feed was removed at 2000 h. At 0800 hours, horses received a jugular intravenous infusion of 150 mg/kg glucose (50% dextrose; Baxter, Deerfield, IL, USA) followed by collection of 10 mL blood into heparin-coated collection tubes (Vacutainer) at −30, −15, −1, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min relative to dextrose infusion.

Plasma and serum assays

Plasma glucose (Glucose Procedure No. 16-UV, Sigma Diagnostics, St. Louis, MO, USA), triglycerides

(TG; Triglyceride GPO reagent; Sigma Diagnostics) and NEFAs (HR Series NEFA-HR(2), Wako Chemicals, Richmond, VA, USA) were determined using enzymatic assays performed by a Beckman CX5 autoanalyzer (Beckman Coulter, Fullerton, CA, USA). Insulin was determined using an RIA (Coat-A-Count Insulin; Diagnostic Products Corp., Los Angeles, CA). Intra-assay coefficient of variation (CV) was 0.68%, 5.12% and 3.79% for glucose, NEFA and TG assays respectively. Intra-assay and inter-assay CVs for insulin assays were 4.86% and 12.19% respectively.

Statistical analysis

The mixed models procedure of SAS (SAS Enterprise Guide v.4.2; SAS Institute, Cary, NC, USA) was used for all analyses. A repeated measures ANOVA with main effects of day, treatment and day-by-treatment interaction was used to assess changes in BW, BCS and fasted concentrations of insulin, glucose, TG and NEFA across the 90-day experimental period. Horse within diet was used as the repeated term, and the covariance structure was determined using the lowest AICC value. Where a main effect of diet was detected, contrast statements were used to compare MED and HI to LO. Where a main effect of day was detected, pairwise comparisons were used to determine differences between the days of the study and were corrected using either Tukey's or Dunnett's tests.

Peak glucose and insulin concentrations and area under the curve [AUC; estimated by calculating the area of each rectangle (average insulin concentration for each time period × minutes in each time period)] during the four GRTs and two IVGTTs and time to consume the concentrate meal during the GRTs were analysed for the main effects of day, treatment and the day-by-treatment interaction, using horse within treatment as the repeated term and group as a fixed effect. Simple effect differences were detected using pairwise comparisons and corrected using either Tukey's or Dunnett's tests. Insulin AUC values were $\log_{10}$ transformed to improve the normality of residuals. Plasma glucose, insulin, triglyceride and NEFA concentrations during each GRT were analysed for the main effects of treatment and time and the treatment-by-time interaction, using horse within treatment as the repeated term. Where a main effect of treatment was identified, contrast statements were used to separate treatment differences. Insulin values were $\log_{10}$ transformed to improve the normality of residuals. Plasma glucose and insulin concentrations during each IVGTT were analysed for the main effects of treatment and time and the treatment-by-time interaction, using horse within treatment as the repeated term. Differences between the MED and HI treatments and LO were identified using contrast statements.

The reduction in NEFA in response to feeding was calculated as the difference in concentration at baseline and at 240 min. The effects of treatment and time and the treatment-by-time interaction were determined for the reduction in NEFA, using horse within treatment as the repeated term.

Results

Actual NSC content of feeds differed from formulated NSC content. The LO, MED and HI diets contained 7.9, 19.6 and 56.4% of NSC respectively. Body condition scores were not different between treatment groups ($p > 0.1$; Table 2) and were not altered across the 90-day experimental period ($p > 0.6$). Bodyweights were not altered across the experimental period ($p > 0.9$; Table 2); however, BWs were lower in horses assigned to the MED treatment ($p = 0.003$). Fasting plasma concentrations of glucose, insulin, NEFA and triglycerides were not different between treatments ($p > 0.4$; Table 2), and while glucose and insulin concentrations did not change across the experimental period ($p > 0.1$; Table 2), concentrations of NEFA and triglycerides varied ($p < 0.001$; Table 2). Fasting NEFA concentrations were decreased from day 1 values on day 45, 60, 75 and 90 ($p < 0.05$; Table 2), while triglyceride concentrations were decreased from day 1 values on day 30, 45 and 90 ($p < 0.05$; Table 2).

Time to complete feed consumption was shorter for HI- than for LO-fed horses ($p < 0.05$), but did not change between GRTs ($p > 0.4$; data not shown). During GRT 1 and 2, a significant time-by-treatment interaction ($p < 0.05$; Fig. 2) existed for plasma glucose, indicating that postprandial glucose concentrations differed for at least one treatment, with HI-fed horses having the greatest glucose concentrations. However, during GRT 3 and 4, the glucose concentrations of HI-fed horses were not different from those fed MED ($p > 0.08$; Fig. 2). For all GRT, plasma insulin concentrations were greater in the MED- and HI-fed horses than in the LO-fed horses ($p < 0.05$; Fig. 2); however, insulin concentrations of MED- and HI-fed horses were not different ($p > 0.5$; Fig. 2). To compare changes across the experiment, alterations in glucose and insulin peak concentrations and AUC were determined (Table 3). While glucose AUC ($p < 0.001$), insulin AUC ($p = 0.013$),

Table 2 Fasting plasma metabolites, BW and BCS of horses fed either a 10% NSC diet (LO), 20% NSC diet (MED) or 60% NSC diet (HI) for 90 days

Day of study									p Value		
TRT	0	15	30	45	60	75	90	SEM	TRT	Day	TRT × day
BCS											
LO	6.7	6.4	6.1	6.0	6.4	6.4	6.4	0.3	0.111	0.618	0.999
MED	6.4	6.3	6.1	6.1	6.3	6.3	6.4	0.3			
HI	6.7	6.5	6.6	6.5	6.8	6.3	6.8	0.3			
BW (kg)											
LO	610	599	594	589	588	589	596	22	0.003	0.922	1.000
MED	559	553	553	552	550	549	553	22			
HI	596	580	579	577	576	574	577	22			
Glucose (mM)											
LO	5.2	5.0	5.4	5.0	5.2	4.9	5.3	0.2	0.489	0.116	0.743
MED	5.6	5.4	5.3	5.2	5.4	5.3	5.4	0.2			
HI	5.5	5.4	5.6	5.0	5.1	5.4	5.3	0.2			
Insulin (mU/l)											
LO	2.1	2.2	1.8	2.3	2.8	2.7	2.1	1.8	0.578	0.336	0.906
MED	3.6	4.3	2.7	3.1	2.2	6.3	5.0	1.8			
HI	5.6	5.1	4.8	2.4	3.3	6.9	5.1	1.8			
NEFA (mM)											
LO	0.27	0.35	0.28	0.15	0.25	0.14	0.26	0.06	0.678	<0.001	0.248
MED	0.44	0.34	0.32	0.25	0.15	0.22	0.28	0.06			
HI	0.47	0.36	0.29	0.18	0.21	0.11	0.27	0.06			
TG (mM)											
LO	0.29	0.34	0.26	0.18	0.25	0.20	0.24	0.03	0.620	<0.001	0.073
MED	0.26	0.28	0.20	0.22	0.22	0.25	0.20	0.03			
HI	0.33	0.27	0.20	0.27	0.32	0.27	0.25	0.03			

BW, bodyweight; BCS, body condition scores; NSC, non-structural carbohydrates. TRT, treatment.

peak glucose ($p = 0.003$) and peak insulin ($p = 0.007$) were increased in horses fed the higher NSC diets, the response was not altered across the course of the experiment ($p > 0.05$).

Glucose tolerance tests were used to assess changes in insulin sensitivity across the 90-day study period. Within each IVGTT, glucose and insulin responses did not differ by treatment ($p > 0.5$; Fig. S1). However, when comparing across IVGTT (Table 4), the AUC for glucose was lower ($p > 0.002$) and peak insulin concentration greater ($p > 0.002$) during the second IVGTT than during the first.

Across all GRT, meal consumption resulted in decreased plasma triglyceride concentrations ($p < 0.001$; data not shown), without an effect of treatment ($p > 0.3$). During GRT 1, meal consumption reduced NEFA concentrations ($p < 0.001$; Fig. S2) with no difference between treatments ($p > 0.1$); however, during GRT 2, 3 and 4, a significant time-by-treatment interaction existed ($p < 0.01$). Postprandial NEFA concentrations were used to assess the sensitivity of adipose tissue to insulin-induced suppression of lipolysis. On day 1 of feeding, consumption of MED ($p = 0.041$) and HI ($p = 0.008$) treatment diets reduced NEFA concentrations a greater degree than that of LO diet (Fig. 3). In horses fed LO or MED, NEFA reduction was not altered across the 90-day study period; however, NEFA reduction was blunted in the HI-fed horses during GRT 3 and 4 ($p < 0.05$).

Discussion

Insulin functions to stimulate glucose disposal in the postprandial state and also to facilitate the switch from lipid to glucose oxidation and promotion of nutrient storage as triglycerides and glycogen. Although previous investigations have primarily identified the effect of high glycaemic feeds on insulin sensitivity in conjunction with obesity (Hoffman et al., 2003; Quinn et al., 2008; Carter et al., 2009b), it is possible that high glycaemic feeds can induce insulin resistance independent of obesity. A feed is

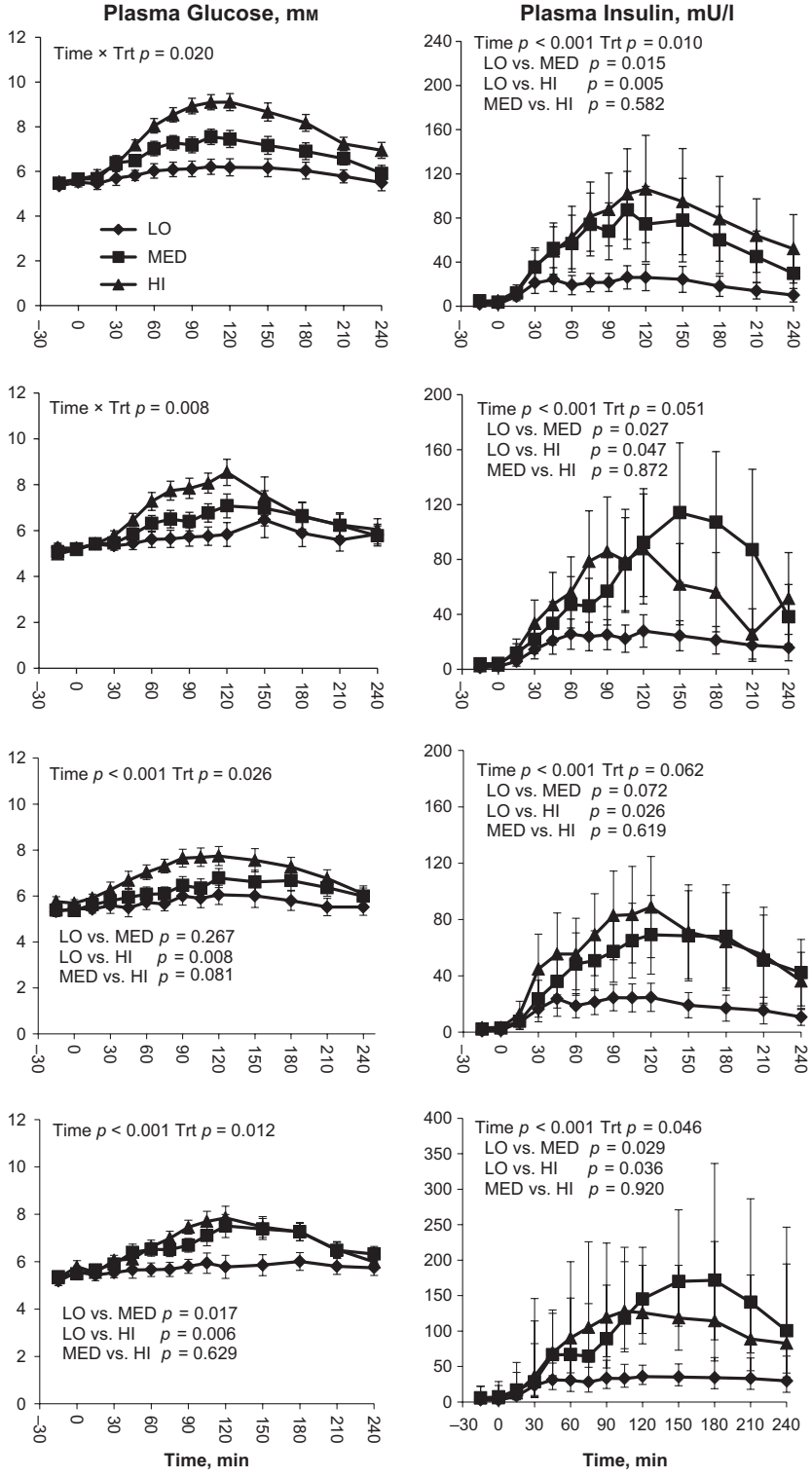

Fig. 2 Mean plasma glucose (left panels) and insulin (right panels) during four glycaemic response tests (GRT) to a diet either low [LO, 10% non-structural carbohydrates (NSC)], medium (MED, 20% NSC) or high (HI, 60% NSC) in starch and sugar content. Testing occurred on day 0 (GRT1), 15 (GRT2), 30 (GRT3) and 90 (GRT4) of dietary treatment.

Table 3 Mean (SEM) glucose and geometric mean (SEM) insulin responses to consumption of experimental supplements in horses, averaged across four time points, on day 1, 15, 30 and 90

Measurement	Treatment			p Value
	LO	MED	HI	
Glucose AUC, (mmol × min)/l	1876 (797)	4982 (797)	7797 (816)	<0.001
Insulin AUC, (mU × min)/l*	4460 (1.3)	14771 (1.3)	15396 (1.3)	0.013
Peak glucose (mM)	6.5 (0.4)	7.6 (0.4)	8.8 (0.4)	0.003
Peak insulin (mU/l)*	34.8 (1.3)	118.1 (1.3)	121.2 (1.3)	0.007

*Values were $\log_{10}$ transformed and are presented as the back-transformed mean and SEM.

Table 4 Glucose and insulin response to a glucose tolerance test either before (day 0) the beginning of the study or following (day 91) 90 days of treatment diet feeding

Day	Treatment				p Value		
	LO	MED	HI	SEM	GTT	TRT	GTT × TRT
Glucose, AUC, (mmol × min)/l							
0	6276	7406	7025	454	0.002	0.496	0.090
91	6103	5759	4972	454			
Insulin, AUC, (mU × min)/l							
0	1653	2167	3221	908.4	0.109	0.517	0.471
91	2339	3761	3367	908.4			
Peak glucose (mM)							
0	13.0	12.0	12.1	0.4	0.102	0.076	0.421
91	12.1	12.0	11.3	0.4			
Peak insulin* (mU/l)							
0	22.4	29.5	34.5	1.3	0.002	0.430	0.848
91	31.3	46.3	47.5	1.3			

*Values for peak insulin were $\log_{10}$ transformed and are presented as the back-transformed mean and SEM.

considered high glycaemic if it causes a large response in blood glucose concentrations and can range in NSC content, with typical values of 40–60% DM. For use in the present study, the NSC component was chosen to include only the starch and ESC fractions, as these fractions are digested and absorbed in the small intestine, and thus contributes directly to a rise in postprandial blood glucose. Dietary starch also dose dependently increases peak insulin concentrations and the duration of increased insulin concentrations (Vervuert et al., 2009). Therefore, a horse being offered a high-NSC feed could have higher daily insulin concentrations than if it was offered a low NSC feed. Therefore, the hypothesis of this experiment was that daily elevations of postprandial insulin concentrations, because of consuming high-NSC feeds, would reduce insulin sensitivity in non-obese horses. The main objective of this experiment was to determine alterations in postprandial glucose, insulin, NEFA and TG responses across the 90-day feeding trial in response to consumption of diets that varied in NSC content.

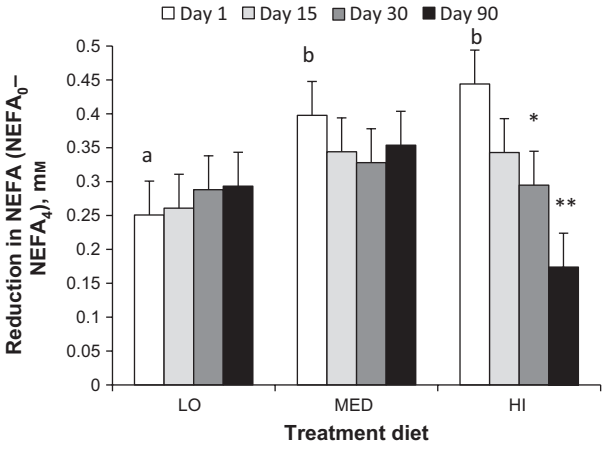

Fig. 3 Reduction in plasma non-esterified fatty acid (NEFA) concentrations (0 h NEFA – 4 h NEFA) following meal consumption on d 1 (GRT1), 15 (GRT2), 30 (GRT3) and 90 (GRT4) of a 90-day feeding trial in horses fed a concentrate consisting of 10% (LO), 20% (MED) or 60% (HI) non-structural carbohydrates. [ab]Different superscripts indicate that means differed for treatments during GRT1. *Within-treatment means differed from day 1 values $p < 0.05$. **Within-treatment means differed from day 1 values $p < 0.01$.*

In the present experiment, diets with greater NSC content resulted in increased glucose and insulin responses to meal consumption. This is in agreement with previous research showing that dietary starch inclusion increases peak plasma insulin and glucose concentrations (Vervuert et al., 2009). It was also interesting that insulin responses of MED-fed horses were equivalent to HI-fed horses, even though peak glucose concentrations for MED-fed horses were not different from LO-fed horses. It is possible that the increased protein content of the MED diet resulted in an insulin response as certain amino acids, such as arginine, stimulate insulin secretion in horses (Sticker et al., 2001).

Insulin resistance is indicated, in part, as reduced glucose disposal into insulin-sensitive tissues, such as skeletal muscle and adipose tissue. Insulin sensitivity can be estimated as the ability of insulin to clear exogenously administered glucose (Bergman et al., 1981). Estimates of insulin sensitivity can be derived through use of various dynamic sampling tests, including the euglycaemic–hyperinsulinaemic clamp (EHC), minimal model and the IVGTT. The minimal model is a time-intensive method that requires

mathematical modelling for the determination of insulin sensitivity (the capacity of insulin to promote glucose disposal), while the EHC estimates insulin sensitivity from the rate of glucose infusion required to maintain euglycaemia during concurrent constant-rate insulin infusion. The EHC is often considered the gold standard for determining insulin sensitivity; however, it requires specialized equipment and experienced personnel. Traditionally, results from the IVGTT method only included glucose concentrations, and for this reason, the test was considered to be less sensitive in the quantification of insulin sensitivity (Kronfeld et al., 2005). Our inclusion and analysis of plasma insulin increases the sensitivity of the test, with peak insulin concentrations indicating the pancreatic response to glucose infusion, and the glucose and insulin AUCs indicating how capable insulin was of returning glucose concentrations to baseline values. Ultimately, the IVGTT was chosen for its simplicity and lower cost.

In the present experiment, glucose tolerance tests were used to determine whether 90 days of consuming a high glycaemic feed reduced insulin sensitivity. Although feeds used in this study were similar in NSC content to feeds previously associated with insulin resistance (Hoffman et al., 2003), the glucose and insulin responses to exogenous glucose administration were not different between dietary treatment groups following completion of the experiment. Thus, it is possible that feeding high glycaemic feeds does not alter insulin sensitivity in lean horses. It is also possible that a longer period of time or an increased proportion of feed in the total ration could have altered our findings.

Intriguingly, however, at the end of the 90-day experimental period, all treatment groups had increased peak insulin concentrations, but no change in the insulin AUC, indicating an increased pancreatic insulin response to glucose. Simultaneously, glucose AUCs were decreased in all horses without alteration to peak glucose concentrations. This could suggest an increased glucose clearance rate in relation to baseline values, possibly resulting from the increased insulin peak. If a decreased glucose AUC had occurred independent of higher insulin, it could suggest increased tissue insulin sensitivity. Alternatively, if the increased insulin peak had occurred independent of a lower glucose AUC, it could suggest reduced insulin sensitivity. Unfortunately, the data collected from the IVGTT do not allow us to separate out the magnitude of these effects to make an assertion. The finding that these changes occurred for all animals independent of treatment could suggest a possibility that exogenous factors, such as season, affect basal insulin concentrations. Previously, seasonal effects on insulin sensitivity have been reported; however, these were in ponies grazing pasture (Treiber et al., 2006; Bailey et al., 2008) and could have been because of seasonal alterations in pasture nutrient content (Frank et al., 2010a).

As insulin sensitivity was not reduced because of feeding high-NSC diets in this experiment, our finding of unchanged fasting insulin concentrations was expected. Fasting insulin concentrations are negatively correlated with insulin sensitivity (Treiber et al., 2006; Vick et al., 2007; Carter et al., 2009b), and a concentration of 20 mIU/l is used to define insulin resistance by the American College of Veterinary Internal Medicine (Frank et al., 2010b). All of the horses had fasting insulin concentrations that remained well below this value for the duration of the experiment.

In addition to glucose metabolism, insulin regulates lipid metabolism, in part through its inhibitory action on the activity of hormone-sensitive lipase in adipose tissue (Breidenbach et al., 1999; Nishino et al., 2007). In the fasted state, the decline in circulating insulin releases inhibition on hormone-sensitive lipase, mediating the switch from glucose to lipid oxidation and resulting in increased concentrations of circulating NEFA. Production and secretion of triglycerides by the liver is mostly a substrate-driven process, whereby an increase in NEFA uptake stimulates an increase in triglycerides synthesis (Lewis et al., 1993; Viljanen et al., 2009). Insulin can also directly influence plasma triglycerides by reducing secretion from hepatocytes (Duerden and Gibbons, 1990). Thus, in the fed state, an increase in insulin concentration decreases plasma triglycerides. In the state of insulin resistance, the ability of insulin to inhibit these pathways may be reduced, and as such, obese horses may have elevated plasma triglycerides and NEFA concentrations (Frank et al., 2006; Carter et al., 2009a). However, in horses fed for weight gain over an 8-week period, the onset of insulin resistance was not concurrently associated with increased fasting lipid concentrations (Carter et al., 2009b). This suggests that a longer period of insulin resistance may be required for the development of fasting hyperlipidaemia. In the present experiment, treatment did not influence either fasting triglyceride or NEFA concentrations and was in agreement with the maintenance of insulin sensitivity.

In the postprandial state of insulin-sensitive individuals, insulin inhibits adipose tissue lipolysis and

hepatic triglyceride production and secretion, resulting in reduced plasma lipid concentrations. Further, insulin increases mRNA abundance of fatty acid transporters and lipoprotein lipase in equine skeletal muscle (Suagee et al., 2011), indicating that insulin also stimulates NEFA and triglyceride clearance from plasma. During all four GRT, meal consumption reduced plasma triglyceride concentrations, without any effect of diet, indicating that triglyceride concentrations are acutely reactive to an increase in insulin, potentially through both inhibited hepatic production and secretion and increased skeletal muscle uptake. Similarly, NEFA concentrations were suppressed 4 h following feed consumption in the first GRT for all treatments, and this suppression was greater for the MED- and HI-fed horses than for the LO-fed horses. However, during GRT 3 and 4, the suppression of NEFA concentrations at 4 h was less pronounced for horses fed the HI diet. This could indicate that adipose tissue insulin resistance developed, in spite of no apparent change in overall glucose clearance rates, an effect that is most likely due to no change in skeletal muscle insulin sensitivity. In insulin-resistant humans, insulin-induced suppression of adipose tissue lipolysis is reduced, and insulin sensitivity correlates with plasma NEFA suppression following low-dose insulin infusion (Bakir and Jarrett, 1981). It is further intriguing that the alterations in NEFA suppression were only detected in HI-fed horses, although horses fed MED and HI exhibited similar insulin responses to feed consumption. It is possible that this was because of the different nutrient profile of each ration, and this should be taken into consideration in future research.

It does not appear that a 90-day adaptation to a high glycaemic diet alters peripheral glucose disposal and therefore skeletal muscle insulin sensitivity, in lean horses, as estimated by fasting insulin concentrations and glucose and insulin responses to intravenous glucose administration. Further, glucose, insulin and triglyceride responses to diet were unchanged throughout the experiment. Intriguingly, however, insulin-induced adipose tissue lipolysis was reduced after 90 days of high-NSC feed consumption, possibly indicating that adipose tissue insulin sensitivity was altered. This could be an early stage in the development of diet-associated insulin resistance and should be investigated further.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Plasma glucose (upper panels) and insulin (lower panels) responses to an *intravenous* infusion of glucose (150 mg/kg bodyweight), prior to the start of the study (GTT1; left panels) and following 90 days of treatment diet administration (GTT2; right panels).

Fig. S2 Plasma non-esterified fatty acid (NEFA) responses during four glycemic response tests (GRT) to a diet either low (LO, 10% NSC), medium (MED, 20% NSC), or high (HI, 60% NSC) in starch and sugar content.

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References

Bailey, S. R.; Habershon-Butcher, J. L.; Ransom, K. J.; Elliott, J.; Menzies-Gow, N. J., 2008: Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. *American Journal of Veterinary Research* **69**, 122–129.

Bakir, S. M.; Jarrett, R. J., 1981: The effects of a low-dose intravenous insulin infusion upon plasma glucose and non-esterified fatty acid levels in very obese and non-obese human subjects. *Diabetologia* **20**, 592–596.

Bergman, R. N.; Phillips, L. S.; Cobelli, C., 1981: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *Journal of Clinical Investigation* **68**, 1456–1467.

Breidenbach, A.; Fuhrmann, H.; Deegen, E.; Lindholm, A.; Sallmann, H. P., 1999: Studies on equine lipid metabolism 2. Lipolytic activities of plasma and tissue lipases in large horses and ponies. *Journal of Veterinary Medicine Series A* **46**, 39–48.

Carter, R.; Treiber, K. H.; Geor, R. J.; Douglass, L. W.; Harris, P. A., 2009a: Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. *Equine Veterinary Journal* **41**, 171–178.

Carter, R. A.; McCutcheon, L. J.; George, L. A.; Smith, T. L.; Frank, N.; Geor, R. J., 2009b: Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *American Journal of Veterinary Research* **70**, 1250–1258.

Duerden, J. M.; Gibbons, G. F., 1990: Storage, mobilization and secretion of cytosolic triacylglycerol in hepatocyte cultures. The role of insulin. *Biochemistry Journal* **272**, 583–587.

Frank, N.; Elliott, S. B.; Brandt, L. E.; Keisler, D. H., 2006: Physical characteristics, blood hormone concen-

trations, and plasma lipid concentrations in obese horses with insulin resistance. *Journal of the American Veterinary Medical Association* **228**, 1383–1390.

Frank, N.; Elliott, S. B.; Chameroy, K. A.; Toth, F.; Chumbler, N. S.; McClamroch, R., 2010a: Association of season and pasture grazing with blood hormone and metabolite concentrations in horses with presumed pituitary pars intermedia dysfunction. *Journal of Veterinary Internal Medicine* **24**, 1167–1175.

Frank, N.; Geor, R. J.; Bailey, S. R.; Durham, A. E.; Johnson, P. J., 2010b: Equine metabolic syndrome. *Journal of Veterinary Internal Medicine* **24**, 467–475.

Henneke, D. R.; Potter, G. D.; Kreider, J. L.; Yeates, B. F., 1983: Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal* **15**, 371–372.

Hoffman, R. M.; Boston, R. C.; Stefanovski, D.; Kronfeld, D. S.; Harris, P. A., 2003: Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *Journal of Animal Science* **81**, 2333–2342.

Hudson, J. M.; Cohen, N. D.; Gibbs, P. G.; Thompson, J. A., 2001: Feeding practices associated with colic in horses. *Journal of the American Veterinary Medical Association* **219**, 1419–1425.

Kahn, C. R., 1978: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism* **12**, 1893–1902.

Kronfeld, D. S.; Treiber, K. H.; Geor, R. J., 2005: Comparison of nonspecific indications and quantitative methods for the assessment of insulin resistance in horses and ponies. *Journal of the American Veterinary Medical Association* **226**, 712.

Lewis, G. F.; Uffelman, K. D.; Szeto, L. W.; Steiner, G., 1993: Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes* **42**, 833–842.

Nishino, N.; Tamori, Y.; Kasuga, M., 2007: Insulin efficiently stores triglycerides in adipocytes by inhibiting lipolysis and repressing PGC-1alpha induction. *Kobe Journal of Medical Science* **53**, 99–106.

NRC, 2007: *Nutrient Requirements of Horses*, 6th rev edn. National Academies Press, Washington, DC.

Quinn, R. W.; Burk, A. O.; Hartsock, T. G.; Petersen, E. D.; Whitley, N. C.; Treiber, K. H.; Boston, R. C., 2008: Insulin sensitivity in Thoroughbred geldings: effect of weight gain, diet, and exercise on insulin sensitivity in Thoroughbred geldings. *Journal of Equine Veterinary Science* **28**, 728–738.

Sticker, L. S.; Thompson, D. L. Jr; Gentry, L. R., 2001: Pituitary hormone and insulin responses to infusion of amino acids and N-methyl-D,L-aspartate in horses. *Journal of Animal Science* **79**, 735–744.

Suagee, J. K.; Corl, B. A.; Hulver, M. W.; McCutcheon, L. J.; Geor, R. J., 2011: Effects of hyperinsulinemia on glucose and lipid transporter expression in insulin-sensitive horses. *Domestic Animal Endocrinology* **40**, 173–181.

Treiber, K. H.; Kronfeld, D. S.; Hess, T. M.; Byrd, B. M.; Splan, R. K.; Staniar, W. B., 2006: Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *Journal of the American Veterinary Medical Association* **228**, 1538–1545.

Vervuert, I.; Voigt, K.; Hollands, T.; Cuddeford, D.; Coenen, M., 2009: Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal* **182**, 67–72.

Vick, M. M.; Adams, A. A.; Murphy, B. A.; Sessions, D. R.; Horohov, D. W.; Cook, R. F.; Shelton, B. J.; Fitzgerald, B. P., 2007: Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *Journal of Animal Science* **85**, 1144–1155.

Viljanen, A. P.; Iozzo, P.; Borra, R.; Kankaanpaa, M.; Karmi, A.; Lautamaki, R.; Jarvisalo, M.; Parkkola, R.; Ronnemaa, T.; Guiducci, L.; Lehtimaki, T.; Raitakari, O. T.; Mari, A.; Nuutila, P., 2009: Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance. *Journal of Clinical Endocrinology and Metabolism* **94**, 50–55.

Williams, C. A.; Kronfeld, D. S.; Staniar, W. B.; Harris, P. A., 2001: Plasma glucose and insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber. *Journal of Animal Science* **79**, 2196–2201.