Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis

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Objective—To determine whether pasture, and specifically the addition of fructan carbohydrate to the diet, induces exaggerated changes in serum insulin concentration in laminitis-predisposed (LP) ponies, compared with ponies with no history of the condition, and also to determine insulin responses to the dexamethasone suppression test.

Design—Prospective study.

Animals—10 LP and 11 control adult nonobese mixed-breed ponies.

Procedures—Insulin-modified IV glucose tolerance tests were performed (5 ponies/group). In diet studies, ponies were kept on pasture and then changed to a hay diet (10 ponies/group). Second, ponies were maintained on a basal hay diet (4 weeks) before being fed a hay diet supplemented with inulin (3 g/kg/d [1.4 g/lb/d]). Serum insulin and plasma glucose concentrations were analyzed before and after dietary changes. Serum cortisol and insulin concentrations were also measured in a standard dexamethasone suppression test.

Results—The LP ponies were insulin resistant (median insulin sensitivity of 0.27×10^4 $L \bullet min^{-1} \bullet mU^{-1}$ in LP ponies, compared with 0.64×10^4 $L \bullet min^{-1} \bullet mU^{-1}$ in control ponies). Median insulin concentration in LP ponies was significantly greater than that in control ponies at pasture, decreased in response to feeding hay, and was markedly increased (5.5-fold) following the feeding of inulin with hay. The LP ponies had a greater increase in serum insulin concentration at 19 hours after dexamethasone administration (median, 222.9 mU/L), compared with control ponies (45.6 mU/L).

Conclusions and Clinical Relevance—Nonobese ponies predisposed to develop laminitis had compensated insulin resistance, and this phenotype was revealed by feeding plant fructan carbohydrate or by dexamethasone administration. (*J Am Vet Med Assoc* 2007;231:1365–1373)

In populations of ponies at pasture, certain ponies may be predisposed to laminitis, whereas others appear to be more resistant. Identifying ponies at risk for this condition may allow appropriate preventive countermeasures to be instigated.[1] Ponies with recurrent laminitis have been observed to be insulin resistant,[2,3] and more recently, it has been postulated that this is part of a 'prelaminitic metabolic syndrome,' which may be analogous to the metabolic syndrome observed in humans.[4,5] In an inbred closed herd of Welsh and Dartmoor ponies, insulin resistance has been shown by use of basal proxies for SI[6] and also with the insulin-modified FSIGT test.[7] If insulin resistance is also a significant factor conferring increased risk of laminitis in the wider pony population, then identifying these ponies while they are without clinical signs of laminitis may enable countermeasures to be used to prevent the onset of acute episodes.[1,5] Prelaminitic metabolic syndrome in the wider outbred population is likely to involve more than a single gene polymorphism, potentially leading to complex variations in phenotype; therefore, it is important to investigate outbred populations as well as closed herds.

The peak incidence of pasture-associated laminitis tends to occur during the spring and summer months,[8,9] and this is thought to be associated with pasture carbohydrate content.[10] Fructans, a group of fructo-oligosaccharides of varying molecular size and branching structure, are produced as a storage carbohydrate

Abbreviations

SI	Insulin sensitivity
FSIGT	Frequently sampled IV glucose tolerance
PLMS	Prelaminitic metabolic syndrome
LP	Laminitis predisposed
PPID	Pituitary pars intermedia dysfunction
AIRg	Acute insulin response to glucose
DM	Of dry matter

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in grasses, and concentrations increase under climatic conditions favoring photosynthesis over growth. Periods of high pasture nonstructural carbohydrate content (which includes simple sugars, starches, and fructans) appear to correlate with peak periods of laminitis incidence.[10] Most mammals are thought not to have the necessary enzymes to digest fructans directly within the small intestine; however, in horses, a proportion of dietary fructans may undergo acid hydrolysis or bacterial breakdown to fructose in the proximal portion of the gastrointestinal tract.[10,11] Fructose has recently been implicated in the metabolic syndrome of humans, where its metabolism may contribute to insulin resistance and hypertension.[12]

Increased carbohydrate consumption exacerbates insulin resistance in horses.[13] However, effects of pasture and fructan carbohydrates on insulin resistance in clinically normal unaffected ponies and those predisposed to laminitis have yet to be assessed. Obesity is a further factor that may be related to insulin resistance and can increase laminitis risk.[7,14] Whether insulin resistance in PLMS is a primary event or occurs secondary to obesity is also unclear.

Basal hyperinsulinemia is not a consistently good indicator of insulin resistance in ponies with PLMS; therefore, proxy markers of insulin resistance, derived from basal insulin and glucose concentrations, have been determined in order to improve predictive ability.[15] Predicting laminitis predisposition by use of these proxies in an inbred population, where a major gene was thought to be responsible for this tendency, gave a moderate predictive power of 78%, although the power of this method in the wider outbred population may be limited by greater interindividual variability. A more discriminating means of determining insulin resistance in ponies may be to use a dynamic challenge test; for example, such ponies may have increased insulin secretion in response to glucose.[16] The gold-standard methods of determining insulin resistance are the euglycemic, hyperinsulinemic clamp method and the FSIGT test,[17] but these are impractical outside of the experimental setting.

The purpose of the study reported here was to examine an outbred group of nonobese ponies of mixed breeds typical of the UK population to determine whether pasture, and specifically the addition of fructan carbohydrate to the diet, exacerbates hyperinsulinemia in LP ponies, compared with control (unaffected) ponies. In addition, the dexamethasone suppression test, used to screen ponies for PPID, was examined to determine whether it might have potential to be used as a dynamic test for insulin secretory response.

Materials and Methods

Animals—Twenty-one adult nonobese mixed native-breed ponies (14 mares and 7 geldings; mean ± SD age, 15.1 ± 4.7 years) were used in this study. Mean body weight was 332 ± 51 kg (730.4 ± 112.2 lb), and the mean body condition score, assessed by the methods of Henneke et al,[18] was 5.3 ± 0.8 (on a scale of 1 to 9). Ponies had been present in the Royal Veterinary College research herd for 5 to 10 years and had no previous history of obesity. Ten of the ponies had ≥ 1 episode of acute pasture-associated laminitis in the previous 2 years, but none had clinical signs of laminitis in the 3 months leading up to the study (LP ponies); the remaining 11 ponies had not had any signs of laminitis in at least the previous 3 years (control ponies). No significant differences were found in age, weight, or body condition score between groups. Episodes of laminitis had been diagnosed by experienced equine veterinarians, and other causes of lameness, such as arthritis, were ruled out. Ponies with ongoing lameness or evidence of chronic laminitis were not included in the study. Both groups of ponies were intermingled in the same herd and were kept at pasture all year round, with no feeding of concentrates.

Minimal model analysis of SI—Insulin sensitivity was assessed in 5 ponies from both groups, which then formed the core for the other studies described here. Mean age in control ponies was 13.8 ± 4.2 years, and mean weight was 366 ± 48 kg (805.2 ± 105.6 lb). Mean age in LP ponies was 14.1 ± 4.4 years, and mean weight was 323 ± 38 kg (710.6 ± 83.6 lb). The insulin-modified FSIGT test was used with minimal model analysis of the glucose and insulin curves. This is currently considered to be one of the most accurate methods for determining SI and has been described by others.[7,13,19] These tests were performed in May, ponies were brought in the day before the test so that bilateral jugular venous catheters could be placed, and the ponies were individually housed overnight (with ad libitum hay and water) to minimize stress. The modified FSIGT test was initiated at 9:00 AM with a bolus of glucose (300 mg/kg [136.4 mg/lb])[a] rapidly administered (within 2 minutes) through the right jugular catheter. Twenty minutes after the glucose bolus, an insulin bolus[b] (20 mU/kg [9.1 mU/lb]) was rapidly administered (within 30 seconds) through the same catheter. Thirty-six venous samples (10 mL) were collected from the left jugular catheter during the 6-hour FSIGT test. Basal blood samples were taken 60, 45, and 0 minutes before the glucose dose. Blood samples were drawn at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes after the glucose bolus. Blood samples were immediately transferred to heparinized sample tubes[c] and placed in ice until centrifuged (3,000 × *g* for 10 minutes). Plasma was removed within 30 minutes of collection and frozen at −80°C (−112°F) prior to assay for glucose and insulin.

The glucose and insulin curves were interpreted according to the minimal model of glucose and insulin dynamics.[20] The application of this method in horses has been described previously by Treiber et al.[7] Glucose effectiveness was calculated as the fractional disposal rate of glucose (min^{-1}), which is the capacity of the cells to take up glucose without insulin mediation. Insulin sensitivity ($L \bullet min^{-1} \bullet mU^{-1}$) was calculated as the ratio of the delivery of insulin to the interstitium (ie, P3) and the disposal of insulin from the interstitial fluid (ie, P2) and represents the efficiency of insulin to accelerate glucose uptake by cells. Responsiveness of beta cells to the glucose load was described by the AIRg ($mU/L \bullet min^{-1}$), which is the increase in plasma insulin above basal concentration integrated from 0 to 10 min-

utes after the glucose dose.[21] The product of AIRg and SI determines the disposition index or the appropriateness of the beta cell response relative to the degree of insulin resistance in the tissue. The minimal model was applied to the data by use of a software program, as previously described.[22] This software iteratively fits the minimal model equations to the glucose observations, finding optimal values for glucose effectiveness, P2, and P3 (and, by substitution, SI = P3/P2).[22]

First study on feeding grass versus hay—Ten ponies from each group were kept at pasture for at least 2 months leading up to mid-September, when they were housed in groups and fed a hay diet. The pasture consisted of a mixed sward, including some clover; samples of the pasture were taken 1 day before the ponies were brought in by use of the standard technique of walking the field in a large W pattern and obtaining samples every 10 meters.[23] Samples were then frozen at −80°C until carbohydrate analysis could be performed.[24] Ponies were then brought in from the field to be housed in groups (groups of 10; 5 from each group) in an open barn and fed mature Timothy hay (known to contain a low amount of fructan carbohydrate) ad libitum. Fructans and other carbohydrates were measured in the hay at the same time that the grass was analyzed.[24] Blood samples obtained by jugular venipuncture were obtained as the ponies were removed from the pasture (at-pasture blood samples), and samples were again taken 1, 2, 5, and 7 days after the start of the hay diet (all samples taken at approx 10:00 AM) for glucose and insulin analysis. Food was not withheld from ponies prior to obtaining blood samples; therefore, hydrolysable carbohydrates in the pasture or hay may have influenced serum insulin and glucose concentrations. However, it was felt that concentrations obtained after grazing were more applicable to the natural situation.

Second study on feeding inulin—In a second study performed during March, 6 LP ponies and 5 control ponies were used. Ponies were individually stabled and fed hay ad libitum for 2 weeks, with hay consumption being recorded for each pony. Then inulin (a commercially available form of fructan carbohydrate; 3 g/kg [1.4 g/lb])[d] was included into the diet, split into 3 daily meals, and dusted onto a high-protein dried grass[e] (15% protein). The dried grass was given to provide a protein source that would mimic that present in lush grass and was fed as a third of the forage ration; the remainder was provided as hay. The amount of inulin in the diet was safely below the amount expected to cause laminitis, on the basis of published data from another group of investigators using raftilose.[25] Blood samples were taken (3 hours after feeding) for insulin, glucose, and triglyceride analysis, prior to and 48 hours after the inclusion of inulin in the diet.

Dexamethasone suppression tests—A 19-hour overnight dexamethasone suppression test was performed on all of the ponies, according to standard protocols.[26,27] This was undertaken in August, and 0.04 mg/kg (0.018 mg/lb) of dexamethasone[f] was administered IM between 4:00 PM and 5:00 PM. The dose of dexamethasone was calculated after body weight was estimated by girth circumference.[28] Blood samples obtained by jugular venipuncture were taken before and 19 hours after dexamethasone administration, and serum was prepared and stored at −80°C until analysis. Plasma cortisol concentrations > 25 nmol/L (1.0 µg/dL), 19 hours after administration of dexamethasone, were considered abnormal.

Samples—Blood was collected into tubes[c] containing either heparin or fluoride oxalate anticoagulant and also into plain tubes for serum collection. These tubes were incubated in a 37°C (98.6°F) water bath for 30 minutes and then centrifuged at 3,000 × *g* for 10 minutes, and the serum separated. Tubes containing heparin or fluoride oxalate were centrifuged at 3,000 × *g* for 10 minutes, and the plasma separated. All samples were then stored at −80°C until the assays were performed. Glucose concentrations were measured from plasma that contained fluoride oxalate, and triglyceride concentrations were measured in heparinized plasma samples[g] by the Clinical Pathology Laboratory at the Royal Veterinary College. Serum insulin concentrations were determined by use of a radioimmunoassay.[h]

Statistical analysis—Values from blood biochemical analysis are expressed as medians. Plasma triglyceride, plasma glucose, and serum insulin concentrations were log transformed prior to analysis by a 2-way ANOVA and Bonferroni post hoc tests. Values obtained while on the hay diet were compared with those associated with inulin feeding or pasture feeding, and results for LP and control ponies were similarly compared. The insulin response to dexamethasone administration was also analyzed by a 2-way ANOVA. Fold changes and absolute changes in serum insulin concentrations were compared by use of an unpaired Student *t* test. Indices derived from minimal model analysis of FSIGT test data were expressed as median and range, and values for control and LP ponies were compared by use of the Mann-Whitney test. Analysis was performed by use of computer software.[i] Values of $P < 0.05$ were considered significant.

Results

Minimal model analysis of SI—Median (range) plasma glucose concentrations increased from a baseline of 5.5 mmol/L (5.3 to 5.8 mmol/L) to a peak of 20.6 mmol/L (13.6 to 23.4 mmol/L) after glucose infusion in control ponies. No significant differences were found between groups in terms of basal plasma glucose concentrations (5.6 mmol/L; 5.3 to 7.6 mmol/L), peak plasma glucose concentration (21.0 mmol/L; 14.5 to 28.2 mmol/L), or area under the curve (**Figure 1**).

Median (range) values for AIRg, however, were significantly ($P = 0.008$) greater in LP ponies (1,597.0 mU/L•min^{-1}; 1,150.0 to 2,060.0 mU/L•min^{-1}), compared with control ponies (994.3 mU/L•min^{-1}; 93.2 to 1,126.0 mU/L•min^{-1}); the area under the insulin curve was also significantly increased for LP ponies, compared with control ponies. Insulin sensitivity was significantly ($P = 0.016$) lower in LP ponies (0.27×10^4 L•min^{-1}•mU^{-1}; 0.10×10^4 to 0.43×10^4 L•min^{-1}•mU^{-1}) than in control ponies (0.64×10^4 L•min^{-1}•mU^{-1}; 0.41×10^4 to 1.26×10^4 L•min^{-1}•mU^{-1}), confirming that LP

ponies were insulin resistant, relative to control ponies. The disposition index (arbitrary units) was unchanged between control (453.0; 259.6 to 709.0) and LP ponies (410.2; 184.6 to 726.9).

First study on feeding grass versus hay—Ponies in the first study included 10 ponies from both groups. The LP ponies comprised 5 mares and 5 geldings with a mean ± SD weight of 331 ± 69 kg (728.2 ± 151.8 lb) and age of 16.4 ± 4.3 years. Control ponies comprised 7 mares and 3 geldings with a weight of 358 ± 57 kg (787.6 ± 125.4 lb) and age of 16.0 ± 5.8 years.

Diet analysis

The fructan content of the September pasture was 138 g/kg DM (including trisaccharides and those with > 3 degrees of polymerization). Di- and monosaccharides comprised 44 g/kg DM; thus, the total water soluble carbohydrate was 182 g/kg DM. In comparison, the Timothy hay had a fructan content of 34 g/kg DM and a total water-soluble carbohydrate of 66 g/kg DM. Because ponies were managed as a herd at pasture and then housed in groups, fructan (and water-soluble carbohydrate) consumption of individual ponies could not be calculated.

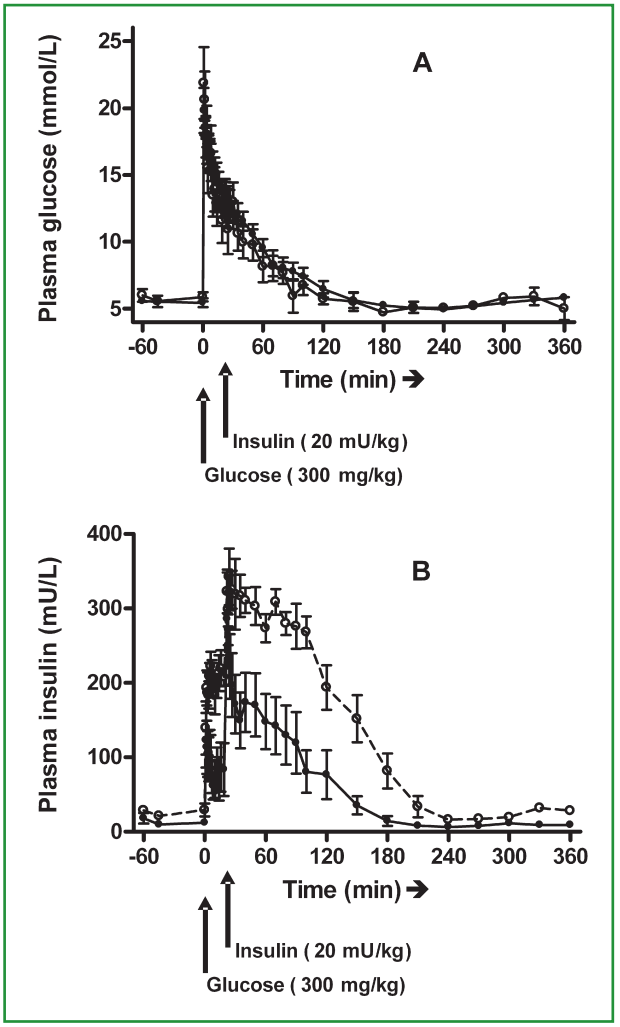

Figure 1—Plasma glucose (A) and insulin (B) concentrations from the FSIGT test, performed on 5 control ponies (black circles; solid line) and 5 LP ponies (white circles; dashed line). Glucose (300 mg/kg [136.4 mg/lb]) and insulin (20 mU/kg [9.1 mU/lb]) were administered at 0 and 20 minutes, respectively, as indicated by the arrows.

Insulin concentrations

Median (interquartile range) serum insulin concentrations in both groups of ponies on grass and hay diets were determined (**Figure 2**). In control ponies, serum insulin concentrations did not change significantly with the transition between grass (11.5 mU/L [4.6 to 25.1 mU/L]) and hay (12.1 mU/L [4.3 to 21.5 mU/L]). On the grass diet, the serum insulin concentration in LP ponies (23.8 mU/L [14.0 to 41.1 mU/L]) was significantly (P = 0.05) greater than in control ponies. Furthermore, in LP ponies, serum insulin concentrations decreased after the hay diet was introduced, and by day 7 on the hay diet, serum insulin concentrations were significantly (P = 0.05) decreased to 15.6 mU/L (12.2 to 34.2 mU/L) such that they were no longer significantly different from control ponies. The laboratory reference range for serum insulin concentration was 5.5 to 36.0 mU/L.

Glucose and triglycerides

No significant changes in median (interquartile range) plasma glucose concentrations were observed in either group following the transition from grass to hay. Also no differences were found between LP and control ponies. Plasma glucose concentrations in control po-

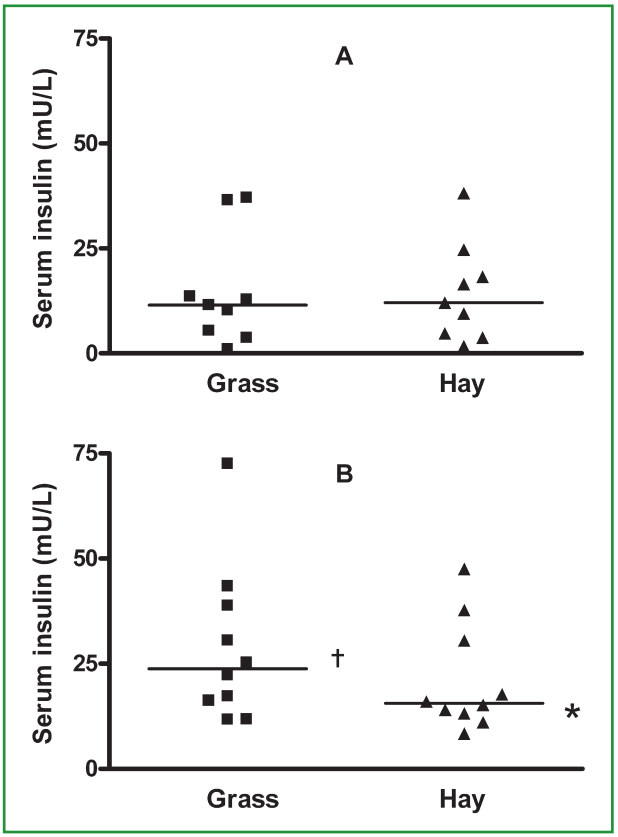

Figure 2—Serum insulin concentrations in control ponies (A) and LP ponies (B) on a grass diet and 7 days after a change to a Timothy hay diet. Lines represent median value. *Significant ($P < 0.05$) difference between hay versus grass. †Significant ($P < 0.05$) difference between LP ponies versus control ponies.

nies were 4.6 mmol/L (4.4 to 5.5 mmol/L) on the grass diet and 5.3 mmol/L (4.7 to 5.5 mmol/L) on the hay diet (day 7). In LP ponies, plasma glucose concentrations were 4.6 mmol/L (4.4 to 5.1 mmol/L) on the grass diet and 4.7 mmol/L (4.4 to 5.1 mmol/L) on the hay diet. The laboratory reference range for glucose was 5.0 to 7.0 mmol/L.

Similarly, no effects of change in diet on median (interquartile range) plasma triglyceride concentrations were found. Plasma triglyceride concentrations in control ponies were 0.40 mmol/L (0.36 to 0.71 mmol/L) on the grass diet and 0.43 mmol/L (0.34 to 0.94 mmol/L) on the hay diet. In LP ponies, the plasma triglyceride concentrations were 0.40 mmol/L (0.28 to 0.63 mmol/L) on the grass diet and 0.62 mmol/L (0.40 to 0.84 mmol/L) on the hay diet. The laboratory reference range for plasma triglycerides was 0.17 to 0.46 mmol/L.

Second study on feeding inulin—Ponies in the second study included 6 LP ponies and 5 control ponies (a total of 4 mares and 7 geldings). Mean body condition score was 5.9 in control ponies and 5.8 in the LP ponies. Mean ± SD age of all ponies in this study was 13.1 ± 0.9 years and body weight was 337 ± 36 kg (741.4 ± 79.2 lb; no significant differences between groups).

Diet analysis

Calculations of total fructan consumption for each pony, on the basis of forage analysis, while on the basal hay diet gave a mean ± SEM value of 4.1 ± 0.2 g/kg/d (1.86 ± 0.091 g/lb/d), which increased to 6.1 ± 0.2 g/kg/d (2.77 ± 0.091 g/lb/d) with the inclusion of inulin (3 g/kg) and dried grass. No pony in this study showed any signs of lameness or laminitis.

Insulin concentrations

Median (interquartile range) serum insulin concentrations on both diets were determined (**Figure 3**). In control ponies, serum insulin concentrations increased (2.0 ± 0.3-fold change) between the basal hay diet (14.4 mU/L [10.7 to 79.1 mU/L]) and at 48 hours of the hay diet supplemented with inulin (37.9 mU/L [24.6 to 106.2 mU/L]), but this apparent change was not significant. There was 1 outlier in the control group, which had a consistently high serum insulin concentration in this study. However, in LP ponies, although their serum insulin concentrations were not significantly different from control ponies while on the basal hay diet (44.7 mU/L [13.4 to 118.8 mU/L]; 3 LP ponies with values within reference range limits), insulin concentrations increased significantly ($P = 0.001$) following the addition of fructan carbohydrates (137.0 mU/L [53.0 to 533.5 mU/L] at 48 hours). The relative increase in serum insulin concentration following the diet change was significantly ($P < 0.001$) greater in LP ponies (5.5 ± 2.2-fold change), compared with control ponies (2.0 ± 0.3-fold change).

Glucose and triglycerides

No significant changes in median (interquartile range) plasma glucose or triglyceride concentrations were observed in either group following the addition of fructan carbohydrates to the diet. Plasma glucose concentrations in control ponies were 5.3 mmol/L (5.1 to 5.4 mmol/L) on the basal hay diet and 4.9 mmol/L (4.8 to 5.2 mmol/L) on the hay diet supplemented with inulin. In LP ponies, the plasma glucose concentrations were 5.2 mmol/L (5.0 to 5.4 mmol/L) on the basal hay diet and 5.9 mmol/L (5.0 to 6.4 mmol/L) when the diet was supplemented with inulin.

Median (interquartile range) plasma triglyceride concentrations in control ponies were 0.36 mmol/L (0.26 to 0.54 mmol/L) on the hay diet and 0.36 mmol/L (0.33 to 0.39 mmol/L) on the hay diet supplemented with inulin. In LP ponies, the plasma triglyceride concentrations were 0.50 mmol/L (0.19 to 1.06 mmol/L) on the hay diet and 0.58 mmol/L (0.42 to 0.79 mmol/L) when the diet was supplemented with inulin (no significant difference in values between diets).

Dexamethasone suppression tests—Median (interquartile range) basal serum cortisol concentrations were 59.4 nmol/L (45.5 to 96.1 nmol/L) in control ponies and 67.3 nmol/L (59.5 to 80.4 nmol/L) in LP ponies

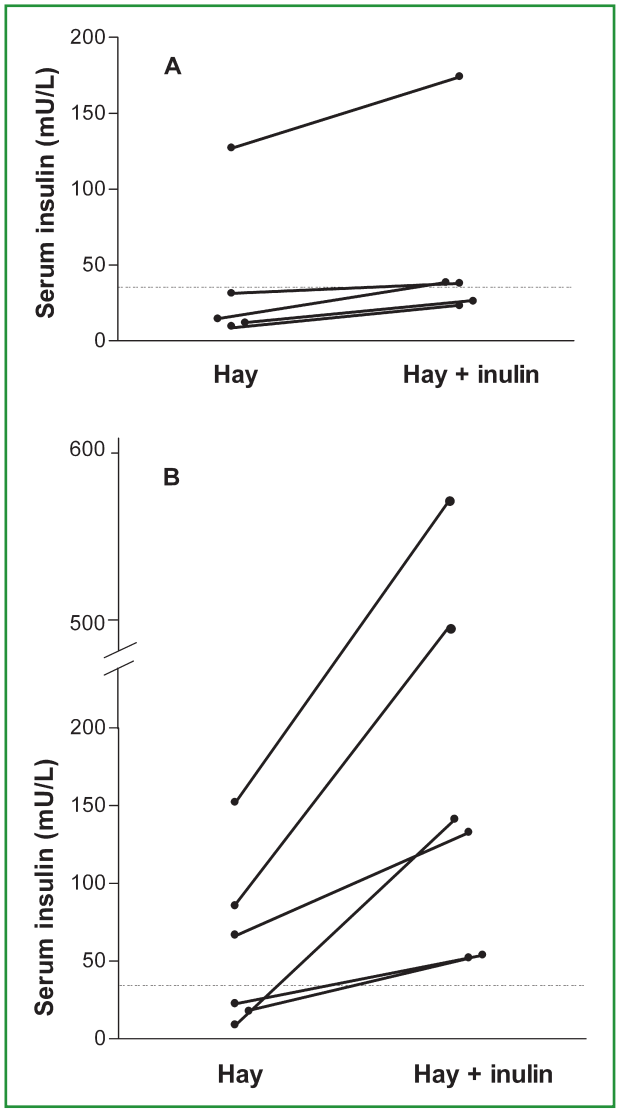

Figure 3—Serum insulin concentrations in control ponies (A) and LP ponies (B) on the hay diet and 48 hours after the inclusion of inulin (3 g/kg) in the hay diet. Dashed lines indicate upper limit of laboratory reference range (36.0 mU/L).

(no significant difference was found between groups). All of the ponies used in the present study had serum cortisol concentrations of < 25 nmol/L at 19 hours after the administration of dexamethasone (**Figure 4**).

When analyzing the serum insulin concentrations in the same samples, results of the dexamethasone suppression tests revealed an abnormal insulin response in the insulin-resistant LP ponies (**Figure 5**). Median (interquartile range) basal serum insulin values were 8.2 mU/L (5.6 to 16.9 mU/L) in control ponies and 38.7 mU/L (22.4 to 137.0 mU/L) in LP ponies; however, 5 of 9 LP ponies had basal serum insulin concentrations within the reference range (5.5 to 36.0 mU/L). After dexamethasone administration, LP ponies had a significantly exaggerated increase in serum insulin concentration, compared with control ponies. All control ponies had postdexamethasone serum insulin concentrations of < 75 mU/L (45.6 mU/L; 22.7 to 65.9 mU/L; significantly [$P = 0.001$] different from before dexamethasone administration). However, LP ponies had postdexamethasone serum insulin concentrations of 222.9 mU/L (102.1 to 367.7 mU/L; significantly [$P = 0.001$] different from before dexamethasone administration). The lowest serum insulin concentration after dexamethasone administration in LP ponies was 83.6 mU/L, and the highest was 488.3 mU/L. The difference between serum insulin concentrations before and after dexamethasone administration was significantly ($P < 0.001$) greater in LP ponies (161.9 ± 30.9 mU/L) than in control ponies (34.0 ± 6.3 mU/L), although the fold change was not significantly different between groups (5.4- and 4.1-fold increases for control and LP ponies, respectively).

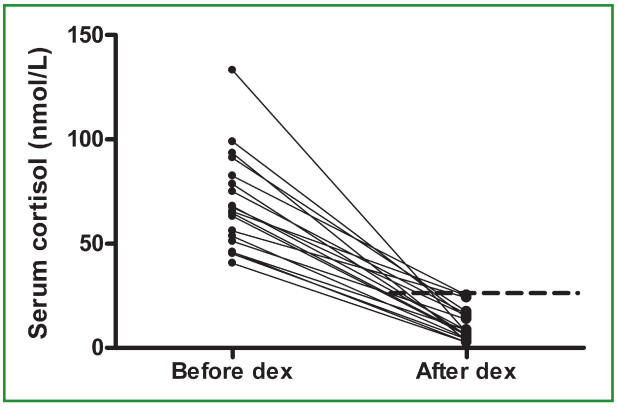

Figure 4—Serum cortisol concentrations in control and LP ponies before and 19 hours after the administration of dexamethasone (dex; 0.04 mg/kg). Dashed line indicates 25 nmol/L.

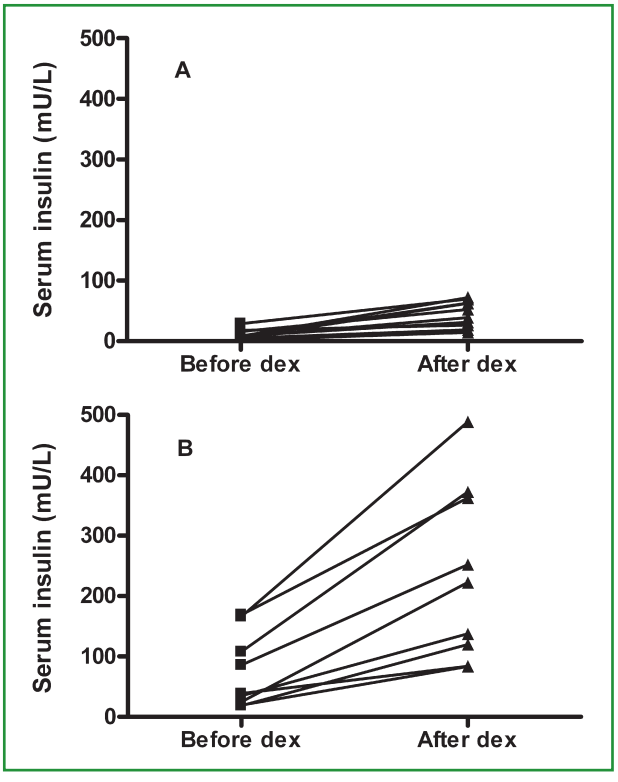

Figure 5—Serum insulin concentrations in control ponies (A) and LP ponies (B) before (squares) and 19 hours after (triangles) the administration of dexamethasone (dex; 0.04 mg/kg [0.018 mg/lb]).

Discussion

These data indicated that the ponies that were predisposed to laminitis were insulin resistant and had exaggerated production of insulin in response to fructan carbohydrates and corticosteroids. A small increase in the median insulin concentration was observed in these ponies at pasture, and this increase could be mimicked by increasing fructan carbohydrates in the diet. However, basal insulin concentrations are of limited predictive value when screening for this phenotype, even when ponies are at pasture, because concentrations tend to fall within the laboratory reference range limits. The exaggerated insulin response caused by fructan feeding, or in response to the administration of dexamethasone in a standard dexamethasone suppression test, could be of practical use to indicate the metabolic abnormalities characteristic of insulin resistance as part of PLMS.

The control and LP ponies used in this study were kept together on the same premises under the same management conditions. It has been shown that obesity can cause insulin resistance in ponies and horses[14,29]; therefore, obese ponies (condition score > 7/9) were excluded from this study so that the influence of obesity on SI could be minimized. Ponies used in the present investigation were of mixed native UK breeds (New Forest, Welsh Mountain, Dartmoor, and mixed breed). Both mares and geldings were used, although no differences in any of the blood variables or metabolic responses were observed between the sexes in the present study. None of the mares were pregnant, although theoretically, SI might have been affected by reproductive hormone changes in these ponies.

The euglycemic-hyperinsulinemic clamp technique and the insulin-modified, FSIGT test with minimal model analysis are the most accurate methods for determining SI in horses.[17,30] Data from the insulin-modified IV glucose tolerance tests in the present study show that the ponies that were predisposed to laminitis were able to control their plasma glucose concentrations to the same degree as the control animals. However, to achieve this glucose clearance, the secretion of insulin from the pancreatic beta cells was increased relative to the control group. Furthermore,

insulin clearance from the circulation was reduced, and the minimal model analysis of the data produced a value of SI in LP ponies that was less than half that of control ponies. Comparing the values obtained in the present study with those of Treiber et al,[7] these workers also found a significant reduction in the SI index in LP ponies. However, their SI values were lower than ours in both groups; this may be explained by the fact that the ponies in that study were all considered overweight (body condition score 6 to 8 out of 9). The AIRg values in the Treiber study were also increased in the laminitis-prone group, which is consistent with the findings of the present study.

The mechanistic link between insulin resistance and laminitis has yet to be determined. In the metabolic syndrome of humans, insulin resistance is associated with hypertension caused by endothelial dysfunction.[31] This leads to the increased risk of cardiovascular events, such as heart attacks, strokes, and peripheral vascular disease.[32] It is possible that endothelial dysfunction increases the likelihood of digital vasospasm and tissue ischemia in laminitis-prone ponies[33] or that insulin resistance impairs the insulin-dependent uptake of glucose into laminar epithelial cells.[34] Much current research in the human literature is focussing on increased fructose consumption playing a key role in the development of metabolic syndrome.[35] This may well be relevant to the situation in horses and ponies grazing fructan-rich pastures, since it has been suggested recently that there may be considerable breakdown of fructan-type carbohydrates by bacteria in the stomach and small intestine of horses, releasing fructose.[11,j] Fructose is rapidly metabolized in the liver, serving as a relatively unregulated source of acetyl coenzyme A. This leads to hepatic triglyceride synthesis and insulin resistance[35]; models of the metabolic syndrome can readily be induced in several animals by feeding high-fructose diets.[12,36]

The indication that fructan carbohydrates may contribute to hyperinsulinemia in some ponies is a novel finding, since it was thought until recently that prececal fermentation and acid hydrolysis of these carbohydrates were negligible and that they passed into the equine cecum unchanged to be fermented by hindgut bacteria.[10] Furthermore, although starch and sugar are known to have a high glycemic index in horses and can induce insulin resistance,[13,37,38] fructose, the major breakdown component of fructans, tends to cause less of an insulin response, compared with glucose.[39] The extent to which fructose metabolism differs between horses, ponies, and other animals is unclear. Whether ponies with PLMS are able to absorb more fructose or metabolize it more efficiently than other ponies remains to be determined.

In both of the present dietary studies, most of the ponies that were predisposed to laminitis had normal basal insulin concentrations while on the basal hay diet. However, when out at pasture, which had a greater fructan content than hay, these ponies as a group had significantly increased serum insulin concentrations, compared with control ponies. Furthermore, when the total fructan content in the diet was increased in a controlled manner from 4.1 to 6.1 g/kg/d, serum insulin concentrations increased above the reference range in all ponies, some having a marked rise. It should be mentioned that the addition of dried grass with a lower fructan content than the hay meant that the overall fructan amount in the diet was only increased by 2 g/kg, although the free inulin (3 g/kg) may have been more easily broken down than that within the forage. This group of ponies, however, did not become hyperglycemic at any stage. This indicates that fructan carbohydrates could be a major factor in the development of hyperinsulinemia in LP ponies at pasture, particularly at times of increased fructan production in grass.

The dietary addition of inulin appears to amplify the differences in insulin dysregulation seen between normal and laminitis-prone ponies. These data support the putative link between insulin resistance and laminitis in ponies [2,40] and may be useful in understanding and identifying this syndrome. This effect of a small amount of inulin on plasma insulin may be of practical use in the identification of ponies suspected of being insulin resistant and therefore predisposed to laminitis. A similar exaggerated insulin response has been recorded in insulin-resistant ponies after oral glucose administration.[16] Basal insulin measurements alone may not be sufficient to determine insulin resistance, particularly where diets have a low glycemic index, and also considering the normal diurnal fluctuations in insulin concentrations.[41] Even at pasture in September, 7 of 10 LP ponies had insulin concentrations of < 36.0 mU/L. Also, in other studies involving inbred LP ponies[6] or outbred populations, although the groups of ponies predisposed to laminitis were hyperinsulinemic as a population, there was considerable overlap, compared with the control populations. Therefore, the use of proxy indices derived from insulin and glucose concentrations only afforded a moderate predictability of laminitis risk (78% in the inbred herd with a genetic predisposition to laminitis).

Thus, the response to the feeding of inulin might be useful as a practical dynamic test to highlight the exaggerated insulin response, which is characteristic of insulin resistance associated with PLMS in ponies. However, it should be borne in mind that other conditions may also lead to insulin resistance, such as obesity and PPID. Both of these conditions are associated with insulin resistance and the increased incidence of laminitis.[14,42] The production of cytokines such as tumor necrosis factor-α has recently been postulated to be the cause of insulin resistance related to obesity in horses.[43] The fact that the ponies used in this study were not obese would indicate that insulin resistance in PLMS is a primary phenomenon and is not merely secondary to obesity. However, obesity is also frequently observed in ponies with PLMS.[7] In horses, carbohydrate challenges may affect not only insulin concentrations but also hormones controlling appetite, such as leptin and ghrelin[44]; therefore, it is interesting to speculate that these pathways may also be affected excessively in ponies with PLMS.

Equine pars intermedia dysfunction has been associated with increased incidence of acute laminitis and is a potential cause of insulin resistance,[41,45] as a result of the antagonistic effects of cortisol on the ac-

tions of insulin.[46] Although the currently used diagnostic tests for PPID, including the dexamethasone suppression test, yield many false-positive results at certain times of the year[47] and may not be highly sensitive, the dexamethasone suppression tests in the present study gave some indication that PPID was unlikely to be a significant cause of insulin resistance in the LP ponies. Furthermore, the measurement of insulin in these samples introduced the possibility of using this common procedure as a dynamic test that may be useful in the presumptive diagnosis of PLMS in the field. Chronic administration of dexamethasone (21 days) has been shown to cause profound insulin resistance in horses,[48] though the change in serum insulin concentrations resulting from the standard 19-hour dexamethasone suppression test has not been reported in ponies or horses to the authors' knowledge. Insulin concentrations were measured before and after dexamethasone administration in 2 insulin-resistant ponies and 2 healthy ponies in 1 previous study,[16] but this was combined with the effects of fasting for 120 hours, which markedly reduced insulin production.The findings of the current study, where there was a profoundly exaggerated insulin response to dexamethasone in the LP ponies, could be explored further to see whether this could be developed as a convenient dynamic diagnostic test for the abnormal beta cell response in PLMS, while at the same time providing some indication of PPID status. Further validation would be necessary to determine appropriate cutoff values, sensitivity, and accuracy. It should be mentioned, however, that ponies that progress to uncompensated insulin resistance (pancreatic insufficiency) may not have such an exaggerated insulin response, although in such cases, hyperglycemia might be observed.

There may be a substantial number of ponies in the wider population that are insulin resistant and have clinical histories of chronic or recurrent laminitis but that do not have PPID. In a study by Reeves et al,[46] out of 8 ponies that were insulin resistant and suspected of having PPID, 7 had normal responses to the combined dexamethasone suppression test and thyrotropin-releasing hormone stimulation test. All of these ponies had a basal hyperinsulinemia. Results of that study and the present study indicate that PLMS is a distinct entity and is unlikely to represent an early stage of PPID, even though PPID may be quite common in the pony population and is positively correlated with age.[47] However, the possibility remains that the exaggerated increase in serum insulin concentrations following dexamethasone administration might be an early indication of PPID that cannot be detected by examining cortisol concentrations.

Therefore, we conclude from these findings that outbred mixed-breed ponies predisposed to laminitis have a compensated insulin resistance, which may not be apparent when resting plasma insulin concentrations are measured, particularly when fed diets of a low glycemic index. However, in these individuals, dietary fructans and glucocorticoids may be capable of unmasking an exaggerated insulin response. The potential link between dietary fructans and plasma insulin concentrations in control and insulin-resistant ponies is worthy of further study, and these data may be useful in developing practical screening methods for identifying animals at risk of laminitis so that appropriate dietary countermeasures can be instigated.

a. Dextrose solution 40%, Dales Pharmaceuticals Ltd, Skipton, North Yorkshire, England.
b. Humulin S, 100 U/mL, Eli Lilly and Co, Indianapolis, Ind.
c. Vacutainer, Becton-Dickinson, Oxford, England.
d. Orafti Group, Tienen, Belgium.
e. Readigrass, Spillers Effem Equine Ltd, Milton Keynes, Buckinghamshire, England.
f. Colvasone, Norbrook Laboratories Ltd, Carlisle, Cumbria, England.
g. ILab 600 machine, Instrumentation Laboratory (UK) Ltd, Warrington, England.
h. Coat-A-Count insulin, Diagnostic Products Corp, Los Angeles, Calif.
i. GraphPad Prism, version 4.00 for Windows, GraphPad Software Inc, San Diego, Calif.
j. Ince J, Longland AC, IGER, Aberystwyth, Wales, UK: Personal communication, 2006.

References

1. Harris PA, Bailey SR, Elliott J, et al. Countermeasures for pasture-associated laminitis in ponies and horses. *J Nutr* 2006;136(suppl 7):2114S–2121S.
2. Coffman JR, Colles CM. Insulin tolerance in laminitic ponies. *Can J Comp Med* 1983;47:347–351.
3. Field JR, Jeffcott LB. Equine laminitis—another hypothesis for pathogenesis. *Med Hypotheses* 1989;30:203–210.
4. Johnson PJ. The equine metabolic syndrome peripheral Cushing's syndrome. *Vet Clin North Am Equine Pract* 2002;18:271–293.
5. Kronfeld DS, Treiber KH, Hess TM, et al. Metabolic syndrome in healthy ponies facilitates nutritional countermeasures against pasture laminitis. *J Nutr* 2006;136:2090S–2093S.
6. Treiber KH, Kronfeld DS, Hess TM, et al. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J Am Vet Med Assoc* 2006;228:1538–1545.
7. Treiber KH, Hess TM, Kronfeld DS, et al. Insulin resistance and compensation in laminitis-predisposed ponies characterized by the Minimal Model. Proceedings of the Equine Nutrition Symposium, Hannover. *Pferdeheilkunde* 2005;21:91–92.
8. Kane AJ, Traub-Dargatz J, Losinger WC, et al. The occurrence and causes of lameness and laminitis in the U.S. horse population, in *Proceedings*. 46th Annu Conv Am Assoc Equine Pract 2000;277–280.
9. Katz LM, De Brauwere N, Elliott J, et al. A retrospective epidemiological study of laminitis in one region of the UK, in *Proceedings*. 40th Br Equine Vet Assoc Cong 2001;199.
10. Longland AC, Byrd BM. Pasture nonstructural carbohydrates and equine laminitis. *J Nutr* 2006;136:2099S–2102S.
11. Coenen M, Mößeler A, Vervuert I. Fermentative gases in breath indicate that inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *J Nutr* 2006;136:2108S–2110S.
12. Nakagawa T, Tuttle KR, Short RA, et al. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clin Pract Nephrol* 2005;1:80–86.
13. Treiber KH, Boston RC, Kronfeld DS, et al. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycaemic meals. *J Anim Sci* 2005;83:2357–2364.
14. Frank N, Elliott SB, Brandt LE, et al. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J Am Vet Med Assoc* 2006;228:1383–1390.
15. Treiber KH, Kronfeld DS, Hess TM, et al. 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* 2005;66:2114–2121.
16. Freestone JF, Wolfsheimer KJ, Ford RB, et al. Triglyceride, insulin and cortisol responses of ponies to fasting and dexamethasone administration. *J Vet Intern Med* 1991;5:15–22.
17. Pratt SE, Geor RJ, McCutcheon LJ. Repeatability of two meth-

ods for assessment of insulin sensitivity and glucose dynamics in horses. *J Vet Intern Med* 2005;19:883–888.
18. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 1983;15:371–372.
19. Kronfeld DS, Treiber KH, Geor RJ. Comparison of nonspecific indications and quantitative methods for the assessment of insulin resistance in horses and ponies. *J Am Vet Med Assoc* 2005;226:712–719.
20. Bergman RN. Toward physiological understanding of glucose tolerance: minimal-model approach. *Diabetes* 1989;38:1512–1527.
21. Bergman RN. The minimal model: yesterday, today, and tomorrow. In: Bergman RC, Lovejoy JC, eds. *The minimal model approach and determinants of glucose tolerance*. Baton Rouge, La: Louisiana State University Press, 1997;3–50.
22. Boston RC, Stefanovski D, Moate PJ, et al. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther* 2003;5:1003–1015.
23. Fryer HC, Smith EF, Holland LA, et al. A comparison of two grass sampling methods for digestibility trials conducted on pasture. *J Range Manage* 1959;12:306–308.
24. Amiard V, Morvan-Bertrand A, Billard JP, et al. Fructans, but not the sucrosyl-galactosides, raffinose and loliose, are affected by drought stress in perennial ryegrass. *Plant Physiol* 2003;132:2218–2229.
25. French KR, Pollitt CC. Equine laminitis: loss of hemidesmosomes in hoof secondary epidermal lamellae correlates to dose in an oligofructose induction model: an ultrastructural study. *Equine Vet J* 2004;36:230–235.
26. Messer NT. How to diagnose equine pituitary pars intermedia dysfunction, in *Proceedings*. 45th Annu Conv Am Assoc Equine Pract 1999;45:145–147.
27. Dybdal NO, Hargreaves KM, Madigan JE, et al. Diagnostic testing for pituitary pars intermedia dysfunction in horses. *J Am Vet Med Assoc* 1994;204:627–632.
28. Gee H, Harris P. Condition scoring and weight estimation: practical tools, in *Proceedings*. 1st BEVA-Waltham Nutr Symp Pract Nutr for All 2005;15–24.
29. Jeffcott LB, Field JR, McLean JG, et al. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. *Equine Vet J* 1986;18:97–101.
30. Rijnen KEPM, van der Kolk JH. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies (Erratum published in *Am J Vet Res* 2004;65:362). *Am J Vet Res* 2003;64:1260–1264.
31. Rask-Madsen C, King GL. Mechanisms of disease: endothelial dysfunction in insulin resistance and diabetes. *Nat Clin Pract Endocrinol Metab* 2007;3:46–56.
32. Reaven GM. Insulin resistance, the insulin resistance syndrome, and cardiovascular disease. *Panminerva Med* 2005;47:201–210.
33. Bailey SR, Marr CM, Elliott J. Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet J* 2004;167:129–142.
34. Pass MA, Pollitt S, Pollitt CC. Decreased glucose metabolism causes separation of hoof lamellae in vitro: a trigger for laminitis? *Equine Vet J Suppl* 1998;26:133–138.
35. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)* 2005;2:5.
36. Lewis GF, Uffelman K, Naples M, et al. Intestinal lipoprotein overproduction, a newly recognized component of insulin resistance, is ameliorated by the insulin sensitizer rosiglitazone: studies in the fructose-fed Syrian golden hamster. *Endocrinology* 2005;146:247–255.
37. Vervuert I, Coenen M. Glycaemic index of feeds for horses. Proceedings of the Equine Nutrition Symposium, Hannover. *Pferdeheilkunde* 2005;21:79–82.
38. Stull CL, Rodiek AV. Responses of blood glucose, insulin and cortisol concentrations to common equine diets. *J Nutr* 1988;118:206–213.
39. Bullimore SR, Pagan JD, Harris PA, et al. Carbohydrate supplementation of horses during endurance exercise: comparison of fructose and glucose. *J Nutr* 2000;130:1760–1765.
40. Treiber KH, Kronfeld DS, Geor RJ. Insulin resistance in equids: possible role in laminitis. *J Nutr* 2006;136:2094S–2098S.
41. McGowan CM, Frost R, Pfeiffer DU, et al. Serum insulin concentrations in horses with equine Cushing's syndrome: response to a cortisol inhibitor and prognostic value. *Equine Vet J* 2004;36:295–298.
42. Love S. Equine Cushing's disease. *Br Vet J* 1993;149:139–153.
43. Vick MM, Adams AA, Murphy BA, et al. Relationship among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *J Anim Sci* 2007;85:1144–1155.
44. Gordon ME, McKeever KH. Oral and intravenous carbohydrate challenges decrease active ghrelin concentrations and alter hormones related to control of energy metabolism in horses. *J Anim Sci* 2006;84:1682–1690.
45. Donaldson MT, Jorgensen AJ, Beech J. Evaluation of suspected pituitary pars intermedia dysfunction in horses with laminitis. *J Am Vet Med Assoc* 2004;224:1123–1127.
46. Reeves HJ, Lees R, McGowan CM. Measurement of basal serum insulin concentration in the diagnosis of Cushing's disease in ponies. *Vet Rec* 2001;149:449–452.
47. Donaldson MT, McDonnell SM, Schanbacher BJ, et al. Variation in plasma adrenocorticotropic hormone concentration and dexamethasone suppression test results with season, age and sex in healthy ponies and horses. *J Vet Intern Med* 2005;19:217–222.
48. Tiley HA, Geor RJ, McCutcheon LJ. Effects of dexamethasone on glucose dynamics and insulin sensitivity in Standardbred horses. *J Vet Intern Med* 2006;20:799.