Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies

Kibby H. Treiber, MS; David S. Kronfeld, PhD, DSc, MVSc; Tanja M. Hess, DVM, PhD; Bridgett M. Byrd, MS; Rebecca K. Splan, PhD; W. Burton Staniar, PhD

Objective—To evaluate genetic and metabolic predispositions and nutritional risk factors for development of pasture-associated laminitis in ponies.

Design—Observational cohort study.

Animals—160 ponies.

Procedures—A previous diagnosis of laminitis was used to differentiate 54 ponies (PL group) from 106 nonlaminitic ponies (NL group). Pedigree analysis was used to determine a mode of inheritance for ponies with a previous diagnosis of laminitis. In early March, ponies were weighed and scored for body condition and basal venous blood samples were obtained. Plasma was analyzed for glucose, insulin, triglycerides, nonesterified fatty acids, and cortisol concentrations. Basal proxies for insulin sensitivity (reciprocal of the square root of insulin [RISQI]) and insulin secretory response (modified insulin-to-glucose ratio [MIRG]) were calculated. Observations were repeated in May, when some ponies had signs of clinical laminitis.

Results—A previous diagnosis of laminitis was consistent with the expected inheritance of a dominant major gene or genes with reduced penetrance. A prelaminitic metabolic profile was defined on the basis of body condition, plasma triglyceride concentration, RISQI, and MIRG. Meeting ≥ 3 of these criteria differentiated PL from NL group ponies with a total predictive power of 78%. Determination of prelaminitic metabolic syndrome in March predicted 11 of 13 cases of clinical laminitis observed in May when pasture starch concentration was high.

Conclusions and Clinical Relevance—Prelaminitic metabolic syndrome in apparently healthy ponies is comparable to metabolic syndromes in humans and is the first such set of risk factors to be supported by data in equids. Prelaminitic metabolic syndrome identifies ponies requiring special management, such as avoiding high starch intake that exacerbates insulin resistance. (J Am Vet Med Assoc 2006;228:1538–1545)

Survival of species evolving in nutritionally sparse environments, including hunter-gatherer humans and rugged pony breeds, was probably facilitated by thrifty genes and insulin resistance.1 Since the agricultural revolution, grains and improved pastures have supplied abundant soluble carbohydrates to humans and horses. High carbohydrate diets exacerbate insulin resistance,2,3 transforming the evolutionary advantage of insulin resistance to a predisposition for certain diseases, notably type 2 diabetes mellitus1 and coronary heart disease2 in humans and—for so far somewhat speculatively—laminitis in equids.6,7

Pasture-associated laminitis accounts for 54% of cases of equine laminitis for which the initial cause is identifiable. Other initial causes include grain overload (8%) and miscellaneous feeding problems; diarrhea and colic; and complications of injury, obesity, and pregnancy (each < 5%).8 Most research associated with laminitis in horses has used experimental models yielding results that pertain mainly to pathogenic events in the acute stage, when separation of the hoof wall from the pedal bone is beginning.9,10 Once separation occurs, there is little opportunity for effective intervention.11

Preceding the acute stage of laminitis is a prodromal or developmental stage of many hours during which trigger factors, possibly including exotoxins, endotoxins, amines, or inflammatory cytokines, are circulating and presumably causing latent vasoactive, structural, and metabolic abnormalities.12 By this stage, effective intervention may not be possible. Intervention to avoid the release of triggers may prove more efficacious. In the opinion of the authors, however, the most likely opportunities for intervention reside in identifying predisposing conditions in ponies and avoiding environmental, mainly nutritional risk factors to preempt the disease.

In humans, 2 metabolic syndromes have been defined that predict an increased risk of disease. One pertains to type 2 diabetes mellitus, with a diagnostic definition requiring insulin resistance or glucose intolerance, and any 2 of high triglycerides or high density lipoprotein-cholesterol concentrations, hypertension, or microalbuminemia.4 The other pertains to coronary heart disease, with a diagnostic definition including any 3 of high glucose, triglycerides, or high density lipoprotein-cholesterol concentrations; hypertension; or abdominal obesity.5 Extensive scientific evidence has been collected to identify these risk factors and define quantitative cutoff values to characterize each predisposition. Because these meta-

ABBREVIATIONS

- RISQI: Reciprocal of the square root of insulin
- MIRG: Modified insulin-to-glucose ratio

From the Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0308. Supported in part by the late Paul Mellon, John Lee Pratt Graduate Fellowship Program in Animal Nutrition, Bernice Barbour Foundation, and Virginia Horse Industry Board.


Address correspondence to Ms. Treiber.
bolic syndromes are predictive, they are meaningful only in the diagnosis of apparently healthy individuals. An equine metabolic syndrome has been proposed as a form of mild to moderate laminitis characterized by consistent fasting hyperinsulinemia and hyperglycemia and obesity in most, but not all, cases. The existence of this syndrome has not been substantiated by published data that differentiate horses characterized with equine metabolic syndrome from other horses with mild to moderate laminitis or from healthy horses. In contrast, the study reported here derives a prelaminitic metabolic syndrome from substantial observations on apparently healthy ponies.

The original World Health Organization definition of a prediabetic metabolic syndrome stipulated the use of the euglycemic-hyperinsulinemic clamp to assess insulin resistance. Specific quantitative methods for assessing insulin resistance, such as the clamp and the minimal model, are more precise than 1-sample proxy tests; however, they are technically complex and expensive. Proxies or surrogates have been developed for screening purposes and have been advocated in studies of metabolic syndrome in humans.

In humans, the concept of a metabolic syndrome as a set of risk factors was advanced originally as syndrome X, the expression of a genetic predisposition, which was exacerbated by a high carbohydrate diet. Results of pedigree analysis of families of 22 affected children have since suggested that metabolic syndrome in humans is inherited as an autosomal dominant trait.

The purpose of the study reported here was to evaluate genetic and metabolic predispositions and nutritional risk factors for development of pasture-associated laminitis in ponies.

Materials and Methods

Ponies—A herd of 160 pure- and crossbred Welsh and Dartmoor ponies was maintained on approximately 130 acres of mixed grass and legume pasture at geographic coordinates 39°N and 78°W in northern Virginia. Ponies were kept in separate herds (15 to 60 individuals each) of stallions, colts, and fillies, pregnant broodmares, barren broodmares, or other. Ponies were rotated to different pastures approximately monthly; pasture rotation varied among herds. One hundred two ponies (64%) were females, 24 (15%) were sexually intact males, and 34 (22%) were neutered males. Previous episodes of laminitis were confirmed by a veterinarian after observation of classic diverging rings on the hoof wall with spaces between rings wider at the heel than at the toe. All ponies with rings were confirmed to have had laminitis by use of farm records of previous diagnosis of laminitis by a veterinarian. During the first week of March, those ponies were allocated to a previous laminitis (PL; n = 54) group. The remaining ponies were considered as a control nonlaminitic (NL; 106) group.

In the last week of May 2004, 137 (87 in the NL group and 50 in the PL group) ponies were observed again; 4 ponies from the PL group and 19 ponies from the NL group sampled in March were not accessible for resampling. Thirteen ponies from the PL group had classic initial clinical signs of laminitis (reluctance to move, bounding digital pulses, and increased temperature of the hoof surface) in May and were reallocated to a third (clinical laminitis [CL]) group.

Procedures and sample collection—On each observation day (March 4 to 10 and May 17 to 25, 2004), approximately 30 ponies were gathered from the pasture at 7:00 AM. Ponies were weighed on an electronic scale, and body condition was assessed on a scale from 1 to 9. Blood samples were collected via jugular venipuncture between 8:00 and 10:00 AM.

Blood samples were immediately transferred to evacuated blood collection tubes containing heparin and placed in ice water for < 30 minutes until centrifuged at 3,000 g for 10 minutes. Plasma was stored at −20°C. Concentrations of glucose, triglycerides, and nonesterified fatty acids were assayed enzymatically by use of commercial kits. Plasma insulin concentration was determined by use of a radioimmunoassay validated for equine insulin. Plasma cortisol concentration was determined by a radioimmunoassay validated for equine cortisol. The intra-assay coefficient of variation of duplicate samples was < 1% for glucose, 5% for insulin, 5% for cortisol, 3% for nonesterified fatty acids, and 5% for triglyceride concentrations.

Proxies for insulin sensitivity (RISQI) and pancreatic β-cell response (MIRG) as assessed by the minimal model of glucose-insulin dynamics were calculated from basal plasma concentrations of glucose (mg/dL) and insulin (mU/L) as follows: RISQI = 1/basal insulin concentration and MIRG = [800 – 0.3 × (basal insulin concentration – 50)]/[basal glucose concentration – 30].

Pastures were sampled by separating each 5- to 30-acre field into quadrants according to geographic orientation, relative elevation, and slope. Within each quadrant, forage samples were randomly collected every 10 m by clipping forage plants at a height of no < 2.5 cm from the ground. A composite sample from each pasture was preserved immediately in liquid nitrogen and stored at −80°C. Four samples from March and 4 from May were submitted for proximate analysis of simple sugars and starch by a Dairy Herd Improvement Association laboratory.

Pedigrees for ponies in PL and NL groups were traced to common ancestors (5 to 10 generations) and analyzed by use of computer software to investigate whether relationships among ponies, combined with detection of PL, was consistent with simple models of inheritance.

Statistical analysis—Data are reported as means ± SE unless otherwise stated; values of P < 0.05 were considered significant. Statistical analysis was performed by use of computer software. Outliers were identified by a Grubbs test. Data for March were compared by use of 2-sample t tests. Comparisons among NL, PL, and CL groups in May were performed by use of ANOVA with Bonferroni multiple comparisons. Comparisons of variables between samples collected in May and March samples were performed by use of ANOVA. Body condition was compared between sexes by ANOVA and across age by linear regression and Pearson product moment correlation.

Individual criteria were chosen on the basis of their ability to differentiate PL- from NL-group ponies. Predictive power of each criterion was determined by the appropriate categorization of PL- and NL-group ponies. Categories included true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). Sensitivity was calculated as TP/(TP + FN), specificity was calculated as TN/(TN + FP), and total predictive power was calculated as (TP + TN)/(TP + FP + TN + FN). The diagnostic criteria in the prelaminitic metabolic syndrome were obtained by cut-point analysis according to the following formula: M = ws + (1 - w) × p, where w is the percentage of the population with laminitis, s is the sensitivity of the cutoff value, and p is the specificity of the cutoff value. The cutoff value was defined by a local maximum of M in which sensitivity and specificity were > 60%. Diagnostic values of criteria were compared by use of receiver operating characteristic curves. The statistical power was > 0.80 for each criterion and for the prelaminitic metabolic syndrome.
Results
At the time of sampling, all ponies were in apparently good health and no lameness was observed except for the 13 ponies in the CL group in May that had uncomplicated laminitis assessed at day 1 or 2 of clinical signs. Ages of ponies ranged from 1 to 27 years. Mean age of ponies in the NL group was 5.7 ± 0.6 years (range, 1 to 27 years), mean age of ponies in the PL group was 12.6 ± 1.2 years (range, 5 to 32 years), and mean age of ponies in the CL group was 14.4 ± 2.1 years (range, 5 to 26 years).

Thirty-one mares sampled in March were pregnant (7 in the NL group and 24 in the PL group). Twenty-three of those resampled in May had foaled (4 in the NL and 19 in the PL groups), and 1 pony in the PL group was still pregnant in May.

Pregnancy did not affect measured variables except increase circulating triglyceride concentrations in mares in the PL group (P < 0.001), but not in mares in the NL group (P = 0.78). Lactation increased RISQI (P < 0.001) and decreased MIRG (P = 0.002) and triglyceride concentrations (P < 0.001) in ponies in PL and NL groups.

Laminitis was expressed in 34% of all ponies, and the prevalence of laminitis was 8-fold lower (P < 0.001) in mature stallions (1/18 [6%]) than that in females (53/102 [52%]). Laminitis was not observed in any of the 34 geldings, but those geldings may not have been representative because they were young (age range, 2 to 7 years) and thus may not have had an opportunity to express the PL phenotype. Observed prevalence of laminitis was consistent with expected prevalence derived from the action of a major gene or genes expressed dominantly, but with reduced penetrance attributable to sex-mediated factors, age of onset, and further epigenetic factors. Nearly all ponies in the PL group were progeny of females in the PL group when status of both ponies was known. All female offspring of the 1 stallion in the PL group were also in the PL group.

In March, ponies in the PL group had significantly (P < 0.001) higher body condition scores (6.4 ± 0.1) than ponies in the NL group (5.8 ± 0.1). Generally, ponies in the PL group had “cresty necks” (ie, large adipose tissue pads of varying sizes along the upper lines of the cervical region and nuchal ligament) as well as localized adipose tissue accumulation on the shoulder, on upper portions of ribs, and at the tail head. The body condition score was weakly correlated with age (r ≤ 0.16; P = 0.049) but not different between sexes (P = 0.12). Hirsutism was observed in only 4 pony mares (3 in the PL group and 1 in the NL group) approximately 17 to 30 years old and was not associated with laminitis (odds ratio, 2.5; P = 0.61).

Overall, there was a significant (P < 0.001) increase in body condition score from 6.1 ± 0.1 in March to 6.3 ± 0.1 in May. This increase was attributable to a significant (P < 0.001) increase of 0.3 in body condition score in NL-group ponies, whereas an increase of 0.1 in body condition score in PL-group ponies was not significant (P = 0.24). However, in May, ponies in the PL group maintained significantly (P = 0.001) higher absolute body condition scores than ponies in the NL group.

Hormones and metabolites—In March, the plasma concentration of insulin was significantly (P < 0.001) higher in PL-group ponies (21.6 ± 2.2 mU/L) than in NL-group ponies (10.7 ± 0.8 mU/L; Figure 1). The plasma concentration of triglycerides was significantly higher in the absence of laminitis (P < 0.001).

Figure 1—Metabolic profiles of nonlaminitic ponies (NL group), ponies with a previous history of pasture-associated laminitis (PL group), and ponies with clinical laminitis (CL group, May only) in March (A) and May (B). Means for PL- and CL-group ponies are given relative to corresponding means in NL-group ponies in March, which were assigned a value of 100%. Letters indicate significant (P < 0.05) differences among groups for each parameter. *Significantly (P < 0.05) different from parameter in NL- and PL-group ponies in March. X = Hyperglycemic ponies in the CL group that were outliers.
(P < 0.001) higher in PL-group ponies (97.2 ± 10.7 mg/dL) than in NL-group ponies (52.3 ± 3.6 mg/dL). The plasma cortisol concentration was significantly (P < 0.001) lower in PL-group ponies (5.3 ± 0.3 µg/dL) than in NL-group ponies (6.8 ± 0.2 µg/dL). Plasma concentrations of glucose (PL group, 93.7 ± 1.3 mg/dL; NL group, 93.5 ± 0.8 mg/dL) and nonesterified fatty acids (PL group, 0.33 ± 0.04 mEq/L; NL group, 0.38 ± 0.02 mEq/L) were not significantly different (P = 0.13 and 0.11, respectively) between the 2 groups.

In May, plasma insulin concentrations were significantly (P < 0.001) higher in ponies in the NL group (103.7 ± 24.0 mU/L), compared with ponies in the NL (12.0 ± 1.1 mU/L) or PL (21.5 ± 3.2 mU/L) group (Figure 1). The plasma concentration of insulin was also significantly (P = 0.030) higher in ponies in the PL group than in the NL group. The plasma triglyceride concentrations was significantly higher in ponies in the CL group (12.0 ± 2.6 mg/dL), compared with ponies in the PL (42.3 ± 4.2 mg/dL; P = 0.011) or NL (40.1 ± 2.1 mg/dL; P = 0.002) group; however, it was not significantly (P = 0.59) different between the NL and PL groups. Plasma concentrations of cortisol, nonesterified fatty acids, and glucose did not differ (P > 0.05) among any groups. However, glucose values for 2 hyperglycemic ponies (plasma glucose concentrations, 146 and 191 mg/dL) in the CL group were statistical outliers.

From March to May, plasma triglyceride concentrations decreased significantly (P < 0.001) by 62% in ponies in the PL group and 27% in ponies in the NL group to similar values (PL, 42.3 ± 4.2 mg/dL; NL, 39.9 ± 2.1 mg/dL). Plasma concentrations of nonesterified fatty acids decreased significantly (P < 0.001) by 80% in ponies in the NL and PL groups. Conversely, plasma cortisol concentrations increased significantly (P < 0.001) by 59% in NL and PL-group ponies to similar values (PL, 9.8 ± 0.6 µg/dL; NL, 10.4 ± 0.4 µg/dL).

In March, RISQI was significantly (P < 0.001) lower in ponies in the PL group (0.25 ± 0.001 [mU/L]²⁻¹) than in ponies in the NL group (0.37 ± 0.01 [mU/L]²⁻¹). The MIRG was significantly (P < 0.001) higher in ponies in the PL group (7.29 ± 0.33 mUinsulin/[10L•mgglucose]) than in ponies in the NL group (4.97 ± 0.23 mUinsulin/[10L•mgglucose]) (Figure 1).

In May, RISQI was significantly (P < 0.001) lower in ponies in the CL group (0.12 ± 0.01 [mU/L]²⁻¹), compared with ponies in the NL (0.42 ± 0.03 [mU/L]²⁻¹) or PL (0.33 ± 0.04 [mU/L]²⁻¹) group. The RISQI was also significantly (P = 0.040) lower in ponies in the PL group, compared with the NL group. The MIRG was significantly (P < 0.003) higher in ponies in the CL group (11.3 ± 0.8 mUinsulin/[10L•mgglucose]), compared with ponies in the NL (5.2 ± 0.3 mUinsulin/[10L•mgglucose]) or PL (7.3 ± 0.7 mUinsulin/[10L•mgglucose]) group. The MIRG was also significantly (P = 0.007) higher in ponies in the PL group, compared with ponies in the NL group. For all ponies, from March to May, RISQI increased significantly (P = 0.017) by 22%.

On the basis of metabolic differences detected between ponies in the PL and NL groups, we statistically derived criteria for prelaminitic metabolic syndrome in apparently healthy ponies that have 3 or more of the following characteristics: insulin resistance (RISQI < 0.32 [mU/L]²⁻¹), compensatory β-cell secretory response (MIRG > 5.6 mUinsulin/[10L•mgglucose]), hypertriglyceridemia (triglyceride concentration > 57.0 mg/dL), or obesity (body condition score > 6.0 with localized fat deposits on neck and tailhead).

Individual criteria had predictive powers of ≥70% with sensitivity and specificity each ≥64%. The area under each criterion’s receiver operating characteristic curve was between 0.72 and 0.77, and there was no significant (P = 0.67) difference between criteria. Three or more criteria were chosen as the cutoff to identify ponies with prelaminitic metabolic syndrome because this provided the highest total predictive power (78%) and balanced sensitivity (74%) and specificity (79%) to provide the overall best accuracy in differentiating ponies at increased risk from those not at risk (Figure 2). Of ponies sampled in May, 11 clinical cases of pasture-associated laminitis developed in 35 ponies with and 2 of 82 ponies without prelaminitic metabolic syndrome. An odds ratio was calculated as follows: odds ratio = (11/44)/(2/80) = 10.0 (90% confidence interval, 2.4 to 34.1; P < 0.001).

In March, pastures consisted primarily of tall fescue (Festuca arundinacea), orchard grass (Dactylis glomerata), Kentucky bluegrass (Poa pratensis), and white clover (Trifolium repens) with few weeds. Pastures were generally grazed down to approximately 5 cm, and availability of the seemingly dormant forage was minimal. In contrast, spring growth was evident in May when large forage plants, especially clover, were flourishing and general height had increased to approximately 12 cm. Clover was sparse in March but abundant in May. Pasture starch content was significantly (P = 0.039) higher in May (7.8 ± 0.6% of dry matter) than in March (4.2 ± 1.0%).

**Discussion**

To the authors’ knowledge, the study reported here is the first to apply specific proxies of insulin resistance to a nonhuman population to develop a data-based...
nonhuman metabolic syndrome that is a predisposition for disease. Determination of a possible genetic predisposition for laminitis is notable, especially with relevance to a phenotype in the form of a characteristic metabolic profile. To the authors’ knowledge, for the first time, insulin resistance has been characterized in laminitis in ponies by a specific method, with compensated insulin resistance in apparently healthy ponies in which pasture-associated laminitis had been previously diagnosed and exaggerated compensated insulin resistance in ponies with clinical laminitis on day 1 or 2 of the disease. To the authors’ knowledge, results of our study are also the first to indicate an association between an increase in pasture starch content and the new development of laminitis.

Insulin resistance develops with chronic adaptation to meals of grain and molasses, 1 probably from the cumulative effects of repeated large fluctuations in glycemia and insulinemia after such meals. Results of our study suggested that insulin resistance may also develop as a chronic adaptation to gradually increasing starch content in spring pasture. Furthermore, an abrupt intake of a large amount of starch may mimic the starch overload model and lead to rapid fermentation in the cecum with production of trigger factors, which could contribute to insulin resistance and laminitis. 9

The actual increase of starch intake from March to May is probably underestimated by pasture proximate analysis. More pasture, particularly clover, was available in May, and ponies and horses have a preference for legumes over grasses. 24,25

Although fructan in pasture grass has been proposed as a causative factor for laminitis, 26 no significant (P = 0.16) difference in pasture contents of water-soluble carbohydrate, the proximate fraction containing fructan in pasture, was detected between March and May. Moreover, no association between pasture fructan and laminitis, such as that detected in the study reported here for starch, has been reported.

Insulin resistance is a thrifty pattern of metabolism that spares glucose use and conserves energy. 26 When the expression of a particular gene is associated with insulin resistance, that gene may also be regarded as thrifty. 9 Many such thrifty genes have been identified in humans, 27 and a dominant mode of inheritance has been suggested for metabolic syndrome. 27 Admittedly, a predisposition for laminitis in ponies would be expected to be polygenic, rather than the effect of a single or small number of genes. However, results from this study support a mode of inheritance consistent with segregation of a major dominant gene or genes especially evident in females. Molecular characterization of such a major gene could provide a diagnostic screening test for the predisposition of laminitis.

Reported sex differences in the incidence of laminitis have been inconsistent among studies. 3,28,35 In our study, laminitis was detected in only 1 male; however, all ponies in the PL group could be traced back to the single male ancestor. A possible model of inheritance may include nonaffected carrier males.

Partial suppression of the PL genotype also suggests an epigenetic threshold for expression. 27 In particular, age, obesity, or an increase in dietary fructan or legume starch available in spring pasture is likely an epigenetic trigger for the phenotypic expression of an underlying genetic predisposition and the appearance of clinical laminitis. 59 Dietary triggers suggest an opportunity for intervention by dietetics and feeding management.

Obesity is associated with increased insulin resistance in horses. 35 In general, adipose tissue secretes proinflammatory adipocytokines, which influence insulin sensitivity. 35 Adipose tissue also influences the balance of circulating concentrations of nonesterified fatty acids and triglycerides, which tend to increase insulin resistance. 35,36 In humans, localized adipose tissue such as intra-abdominal fat has been particularly implicated in providing endocrine signals that disrupt glucose and insulin signalling. 35,37 Specific fat deposits in ponies previously diagnosed with laminitis, including the cresty neck, may represent similar metabolically active adipose tissue. 35

Hyperinsulinemia tends to sustain insulin-mediated glucose disposal, compensating for insulin insensitivity. By inference, hyperinsulinemia has been considered an indication of insulin insensitivity, albeit nonspecific and ambiguous. 13 Euglycemia in all but 2 ponies (outliers) in the CL group indicates that hyperinsulinemia adequately compensates for tissue insulin insensitivity.

Another form of compensation, an increased use of lipids as an energy substrate, is suggested by detection of hypertriglyceridemia in PL-group ponies, particularly during pregnancy when energy demand was increased. Plasma concentrations of nonesterified fatty acids were not increased and almost certainly attributable to rapid conversion to triglycerides by the liver. 29 Hypertriglyceridemia can have adverse effects on the hepatic, renal, and cardiovascular systems, although these were not apparent in ponies in our study.

Changes detected in plasma concentrations of triglycerides and nonesterified fatty acids between March and May were compatible with a change from the use of stored fat as a major energy source during winter to increased use of soluble carbohydrates available in spring pastures. This metabolic switch over would tend to exacerbate insulin resistance, increasing the risk of laminitis in the spring.

In our study, metabolic changes detected during lactation were consistent with results of other studies 36,41 in horses and other species. These changes suggest that the risk of laminitis would be decreased during lactation.

Cortisol is considered a thrifty hormone, stimulating gluconeogenesis and exacerbating insulin resistance to conserve glucose. 42,43 The hypothalamic-pituitary-adrenal axis regulates cortisol secretion, with posterior pituitary dysfunction manifesting in horses often as hyperinsulinemia and localized fat deposition. 44 Similar to that observed in PL-group ponies in this study. However, unlike horses with posterior pituitary dysfunction, the PL-group ponies in this study had typical basal plasma concentrations of cortisol and glucose, many were young, and hirsuitism was not associated with PL. Similar inconsistencies from the classic clini-
cal signs of posterior pituitary dysfunction have been detected in another study\(^\text{10}\) evaluating ponies with a history of laminitis. Nevertheless, endocrine changes in the hypothalamic-pituitary axis, such as altered ACTH secretion\(^\text{10}\) or peripheral hypercorticism,\(^\text{1}\) have not been ruled out as possible factors of a thrifty disposition and increased risk of laminitis.\(^\text{3}\)

Statistically derived proxies or surrogates of parameters of insulin resistance have been used extensively in human public health; however, to the authors’ knowledge, this is the first direct application of such proxies or surrogates in an equine population. In our study, simple proxies enabled the assessment of insulin resistance in 160 ponies.

Subsequently, the minimal model was performed on 14 of the ponies described here, and lower insulin sensitivity with increased β-cell responsiveness was observed in PL-group ponies, compared with NL-group ponies.\(^\text{39}\) Such results from a specific quantitative evaluation of the dynamic glucose and insulin system corroborate the estimation of insulin sensitivity and increased β-cell responsiveness parameters by use of RISQI and MIRG in the study reported here.

An RISQI in the lowest quintile of apparently healthy horses indicates the presence of insulin resistance in PL-group ponies.\(^\text{31}\) An MIRG in the highest quintile of apparently healthy horses characterizes the insulin resistance in PL-group ponies as compensated by increased β-cell secretory response.\(^\text{41}\) Effective compensation was further indicated by euglycemia in PL-group ponies.

In May, proxies continued to indicate compensated insulin resistance in PL-group ponies and exaggerated compensation in CL-group ponies. However, 4 of the 13 CL-group ponies and 1 PL-group pony had negative MIRG results because of extremely high basal insulin concentrations (> 157 mU/L) and were considered outliers (P < 0.08) for the aforementioned comparisons. Low MIRG indicates an inadequate insulin response to glucose stimulation and can occur when basal insulin secretion is low or extremely high. Low MIRG suggests an increased risk of hyperglycemia. Accordingly, the 2 hyperglycemic ponies in the CL group were among these outlier ponies with low MIRG. Those 2 ponies had failed compensation for insulin resistance.

Results of the study reported here indicated that ponies predisposed to pasture-associated laminitis are metabolically distinct from ponies not at risk. This metabolic distinction is associated with insulin resistance, compensatory hyperinsulinemia, and disturbed glucose and fat metabolism, all of which resonate with risk factors of metabolic syndromes in humans.\(^\text{33}\)

Evaluation of these differences from basal morning blood samples permitted determination of a prelaminitic metabolic syndrome that identifies ponies at high risk for developing laminitis. Determination of prelaminitic metabolic syndrome was used to predict development of clinical cases of pasture-associated laminitis in 11 of 13 ponies in May. An odds ratio of 10 and a total predictive power of 78% were quantitative assessments of the value of using prelaminitic metabolic syndrome to identify ponies requiring special management. Prelaminitic status may also influence economic decisions pertaining to pony breeding and trade. We have observed that qualities present in PL-group ponies have resulted in preference in the show ring, as breeding stock, and at sale.

Within the design of the study reported here, it was not possible to entirely rule out the possibility that the metabolic differences detected resulted from the original episode of laminitis. However, the authors know of no such precedent because ponies did not have signs of pain, stress, or other chronic stimuli attributable to a previous episode of laminitis. One 16-year-old mare had had a single severe episode of laminitis secondary to an infection in 1998 and was notably lame. This mare fulfilled only 1 criterion of the prelaminitic metabolic syndrome.

Other reports\(^\text{3,58}\) have addressed insulin insensitivity in laminitis but not the roles of increased pancreatic β-cell insulin secretion and fatty acid utilization. Results of the study reported here indicated that a genetic predisposition for laminitis was expressed partly by chronic compensated insulin resistance, hypertriglyceridemia, and characteristic fat deposition in ponies. During active laminitis, hyperinsulinemia and hypertriglyceridemia were exaggerated, with failed compensation in 2 CL-group ponies resulting in a diabeticlike state. Digestion of starch or fructan may also contribute to the release of additional trigger factors (eg, exotoxins, endotoxins, or amines), contributing to hoof failure directly,\(^\text{43,44}\) and via exacerbation of preexisting insulin resistance.

Laminitis in horses is generally considered to involve a vascular component,\(^\text{46,49}\) reflecting current concepts of diabetes mellitus and cardiovascular disease in humans. Insulin is a vasoregulatory hormone, invoking vasodilation via nitric oxide through signaling pathways similar to those of insulin-mediated glucose metabolism as detected in human cell cultures.\(^\text{50}\) Insulin resistance is therefore expected to decrease the vasodilatory effects of insulin. In addition, concomitant hyperinsulinemia may signal other factors that result in vasoconstriction or endothelial damage, including cytokines, growth factors, neurohormones, and endothelin-1.\(^\text{51-53}\)

Results of an in vitro study\(^\text{54}\) in equids indicate that glucose deprivation of hoof-to-bone connective tissue results in separation. Insulin resistance can compromise glucose availability to insulin-dependent tissues. Keratinocytes of the equine lamina contain insulin-responsive glucose transporters and may therefore be partially regulated by insulin.\(^\text{55}\)

Findings of the study reported here indicated that insulin resistance was a major metabolic and hormonal predisposing condition for laminitis. Ponies identified as metabolically and perhaps genotypically predisposed to laminitis can benefit from special management to avoid laminitis. Avoiding factors that contribute to obesity and insulin resistance, such as the moderation of dietary carbohydrates, particularly starch, may decrease changes in systemic insulin signaling and reduce the risk of developing laminitis.

a. BD Vacutainer evacuated blood collection tube, Fisher Health Care, Chicago, Ill.

c. Glucose Procedure #16-UV, Sigma Diagnostics, St Louis, Mo.
d. Triglyceride GPO reagent, Sigma Diagnostics, St Louis, Mo.
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