Diabetes mellitus in a domesticated Spanish Mustang

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An 18-year-old Spanish Mustang mare was referred to the University of Missouri Veterinary Medical Teaching Hospital for evaluation of progressive weight loss and persistent hyperglycemia. The owners had purchased the mare 4 months prior to admission and reported a history of weight loss, intermittent diarrhea, and polydipsia of 2 months' duration. The mare's ration had consisted of 4.5 lb of grain concentrate, 2.2 L of water, and polydipsia of 2 months' duration. The mare was bright, alert, and responsive but weighed only 260 kg (572 lb). Abnormalities identified on initial examination at the teaching hospital included poor body condition (body condition score of 3 on a scale from 1 to 9), fecal staining and urine scalding of the hindquarters, and excessive hoof growth affecting all 4 feet. Signs of lameness were not evident, and the appearance of each hoof was not characteristic of laminitis. A CBC and serum biochemical profile were performed. Abnormalities included hyperfibrinogenemia (0.7 g/dL; reference range, 0.1 to 0.4 g/dL), hyperglycemia (359 mg/dL; reference range, 72 to 114 mg/dL), hyperproteinemia (8.0 g/dL; reference range, 4.9 to 6.9 g/dL), hyperglobulinemia (4.9 g/dL; reference range, 3.2 to 4.0 g/dL), hypertriglyceridemia (103 mg/dL; reference range, 4 to 50 mg/dL), high alkaline phosphatase activity (354 U/L; reference range, 109 to 315 U/L), and high γ-glutamyltransferase activity (70 U/L; reference range, 12 to 45 U/L). The day after admission, food was withheld for 12 hours and serum insulin and glucose concentrations were measured concurrently. Serum insulin concentration, determined with a commercially available radioimmunoassay (RIA), was 21 pmol/L (reference range, 30 to 300 pmol/L). A concomitantly measured serum glucose concentration was 364 mg/dL. Serum insulin and glucose concentrations were again measured on day 4 and were 54 pmol/L and 373 mg/dL, respectively.

The horse's daily consumption of water during the 6 weeks it was hospitalized ranged from 25 to 40 L/d (96 to 154 mL/kg of body weight/d [44 to 70 mL/lb/d]; reference range, 30 to 70 mL/kg/d [14 to 32 mL/lb/d]), representing marked polydipsia. Routine urinalyses consistently revealed marked glycosuria. Additional clinical abnormalities that were identified included intermittent loose feces (although diagnostic testing to determine the cause of this mild diarrhea was not undertaken) and polyphagia.

Further endocrinologic testing included determination of baseline serum triiodothyronine (T3) and T4 concentrations and an overnight dexamethasone suppression test (DST). Serum T3 (65 ng/dL; reference range, 25 to 90 ng/dL) and T4 (1.8 µg/dL; reference range, 0.5 to 3.0 µg/dL) concentrations were within reference limits, and administration of dexamethasone (10 mg, IM) resulted in an appropriate decrease in serum cortisol concentration from 4.4 to 0.4 µg/dL after 15 hours.

Because the serum insulin concentration was not high even though serum glucose concentration was markedly elevated, the horse's weight loss and polydipsia were attributed to diabetes mellitus. Alternative or contributing explanations for weight loss and polydipsia included malabsorptive gastrointestinal tract disease and diarrhea. To more completely characterize...
the horse’s condition, a series of additional diagnostic tests was undertaken. After food was withheld for 12 hours, venous blood samples were collected from this horse and 5 healthy, age-matched control horses and submitted to a commercial laboratory for determination of the glycosylated hemoglobin, specifically hemoglobin \( A_1c \) (HbA1c), fraction. Results of the HbA1c assay for this horse (2.5%) and the healthy control horses (mean \( \pm \) SD, 2.98 \( \pm \) 0.73%) did not exceed the threshold (7%) suggested for therapeutic intervention in human diabetic patients.\(^{2,3}\) Plasma concentration of C-peptide, measured with a commercially available RIA,\(^4\) in this horse (0.18 pmol/mL) was substantially lower than concentration in the 5 healthy control horses (mean \( \pm \) SD, 0.64 \( \pm \) 0.17 pmol/mL) and the lower reference limit for healthy humans (reference range, 0.22 to 1.08 pmol/mL).

A continuous glucose monitoring system\(^5\) was used to assess the horse’s response to glucose administration and an orally administered hypoglycemic agent. The continuous glucose monitoring system records the concentration of glucose in interstitial fluid (at an SC location) every 5 minutes for 48 hours. Use of this system in horses has previously been validated.\(^4\) All measurements were performed after food had been withheld for 12 hours; a minimum of 1 week was allowed to elapse between tests.

Use of the continuous glucose monitoring system confirmed that the concentration of glucose in the horse’s interstitial fluid was persistently high (Figure 1). An IV glucose tolerance test was performed by administering 50% glucose solution (0.5 g/kg [0.23 g/lb], IV), as described.\(^5\) The resulting interstitial fluid glucose concentration exceeded the maximum detection limit of the sensor (400 mg/dL) for 45 minutes, and the glucose concentration remained above the baseline value for 5 hours (Figure 2).

The horse was treated with a combination of glyburide and metformin hydrochloride\(^6\) (glyburide, 5 mg; metformin, 500 mg), which is used to treat diabetes mellitus in humans. Interstitial fluid glucose concentration was within reference limits for plasma glucose concentration (72 to 114 mg/dL) 4.5 hours after administration of glyburide and metformin (Figure 2). Unfortunately, the horse dislodged the glucose monitoring system sensor approximately 5.5 hours after drug administration, and additional data were not available.

A modified insulin tolerance test was performed as described\(^7\) to evaluate the effect of insulin on plasma glucose concentration in the horse. Briefly, a 16-gauge polyethylene catheter was inserted in a jugular vein to facilitate collection of blood samples and minimize stress. Following collection of a blood sample for determination of baseline plasma glucose concentration, a low dose of regular insulin\(^8\) was administered (0.4 U/kg [0.18 U/lb], IV) and plasma glucose concentration was measured every 30 minutes for 180 minutes. The horse was observed carefully for signs of hypoglycemia. The blood glucose concentration decreased from a baseline concentration of 303 mg/dL to a concentration of 235 mg/dL 3 hours after insulin administration, representing a 22% decrease. The hypoglycemic response to insulin administration in this horse was considered modest and slow, compared with the response in healthy horses, in which a rapid and substantial reduction in plasma glucose concentration is recorded.\(^9\)

The horse was euthanatized, and a postmortem examination was performed. Gross abnormalities were not identified. Representative tissue specimens were fixed in neutral-buffered 10% formalin, stained with H&E, and examined by means of light microscopy. The pituitary gland, pancreas, liver, representative areas of the alimentary tract, and hoof lamellar interface were carefully examined. Clinically important histologic lesions were not identified in these tissues. Representative sections of the pancreas were submitted to a commercial laboratory\(^10\) for immunohistochemical staining with a murine monoclonal antibody against insulin to highlight insulin-producing \( \beta \) cells in the islets of Langerhans. Islets in sections from this horse were characterized by marked attenuation of insulin-positive cells, compared with islets in sections from age-matched control horses, with staining generally limited to the periphery of the islets (Figure 3).

Diabetes mellitus resulting from a lack of insulin or insulin action is apparently rare in equids.\(^2,11\) Although diabetes mellitus resulting from chronic pancreatitis,\(^12\) ovarian neoplasia,\(^13\) or pregnancy has been identified in horses, most reports\(^12-14\) of diabetes melli-
Horses have described insulin resistance attributable to hyperadrenocorticism resulting from pituitary pars intermedia dysfunction. Of the few reports of horses with true diabetes mellitus that have been published in the veterinary literature, only described plasma insulin concentrations. In each of those horses, reported insulin concentrations were high and secondary or type 2 (non–insulin-dependent) diabetes mellitus was identified. In contrast, the horse described in the present report had inappropriately low insulin and C-peptide concentrations.

In contrast with diabetes mellitus, insulin resistance is common in adult horses and has been referred to as peripheral Cushing’s syndrome and equine metabolic syndrome. Hyperadrenocorticism and obesity represent 2 of the most common causes of insulin resistance in genetically susceptible individuals. Hyperadrenocorticism was disqualified as the cause of insulin resistance in this horse on the basis of results of the DST and postmortem examination. Spanish Mustangs have only recently been domesticated and have been recognized as having inherited “thrifty genes” that permit highly efficient use of their dietary intake, leading to heightened ability to endure periods of harsh environmental conditions. When these horses are fed grain-rich rations that broadly exceed their metabolic requirements and that provide calories in a concentrated form (a diet far removed from their natural diet), they quickly become obese.

Superfluous fat tissue is not, as previously believed, simply a benign repository of stored energy. Adipocytes represent an important source of numerous diverse hormones (adipokines) that play a role in regulating body mass and body composition. The accretion of adiposity is attended by production of excessive

Figure 2—Interstitial fluid glucose concentration in a horse with diabetes mellitus following IV administration of glucose (A) and following oral administration of a combination of glyburide and metformin (B). Horizontal lines represent the upper and lower limits of the plasma glucose concentration reference range.

Figure 3—Photomicrographs of sections of the pancreas from a horse with diabetes mellitus (A) and a healthy, age-matched control horse (B). Sections were stained immunohistochemically to identify β cells in the islet of Langerhans. Notice the marked attenuation of the number of β cells in the diabetic horse; β cells that were evident were located at the periphery of the islet. Bar = 100 μm.
quantities of endocrine signals, including leptin, resistin, adiponectin, mineralocorticoid-releasing factors, free fatty acids, and certain pro-inflammatory cytokines (eg, tumor necrosis factor-α and interleukin-6).21-22 Omental adipocytes also possess the enzyme 11β-hydroxysteroid dehydrogenase-1, which converts circulating inactive cortisone to the physiologically active glucocorticoid cortisol.23,24 Many adipose-derived endocrine signals, including cortisol, directly inhibit the action of insulin and cause insulin resistance.

It is possible that the horse described in the present report had had insulin resistance for some time and that diabetes mellitus developed as a consequence of heightened β-cell stimulation over time (pancreatic β-cell exhaustion).25,26 Although the horse had not been obese when purchased, the new owners had been feeding a glycemic ration that may have contributed to the development of diabetes mellitus. Development of diabetes mellitus in this instance would have been similar to the situation in humans in which type 2 (non–insulin-dependent) diabetes mellitus develops in sedentary, obese individuals after the age of 40 years.27,28 Although insulin resistance is a common problem in equids, most affected horses do not develop overt diabetes mellitus. Horses with insulin resistance are often obese and at risk for the development of laminitis.29 A diagnosis of prediabetic insulin resistance in horses is supported by demonstration of fasting hyperinsulinemia with normal or slightly high blood glucose concentration and glucose intolerance, as determined with an IV glucose tolerance test. Possibly, the lower risk for diabetes mellitus in horses, compared with humans, is a result of their shorter longevity or the fact that equine rations typically contain little fat.

Although glucose concentration in this horse did not return to the baseline concentration until 5 hours after administration of a glucose bolus, it was not possible to conclude with certainty that the horse in fact had insulin resistance. In healthy horses, the IV glucose tolerance test is characterized by a return of glucose concentration to baseline values within 90 minutes,5 but it was not possible to conclude with certainty that the horse in fact had insulin resistance. After administration of a glucose bolus, it was not possible to conclude with certainty that the horse in fact had insulin resistance. In healthy horses, the IV glucose tolerance test is characterized by a return of glucose concentration to baseline values within 90 minutes, but glucose intolerance in this horse could have been attributed to either insufficient insulin or insufficient insulin effect. In this horse, the fact that plasma insulin concentration was low despite the persistent hyperglycemia suggested that production of insulin was reduced. Intravenous administration of insulin caused only a slight decrease in the plasma glucose concentration in this horse, giving the impression that the action of insulin was impaired as well (insulin insensitivity).9 However, there is inherent risk in assessing the response to a single dose of insulin in a previously untreated diabetic individual. It may require several days of insulin treatment before the effectiveness of exogenous insulin administration can be judged because protracted, untreated, insulin-dependent diabetes mellitus is associated with substantial metabolic stress and resulting homeostatic mechanisms (eg, increased cortisol secretion) may initially counteract insulin's effects.9

We administered a single dose of a combination of glyburide and metformin, 2 agents with complementary mechanisms of action that are used to improve glycemic control in human patients with type 2 diabetes.25,26 to the horse described in the present report and found that glucose concentration decreased. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity.27,28 Glyburide apparently lowers blood glucose concentration by stimulating the release of insulin from the pancreas, an effect dependent on functioning β cells in the pancreatic islets.29 Given that few β cells were seen in pancreatic islets from this horse, it seems likely that the decrease in glucose concentration was a result of metformin's action to improve the effectiveness of insulin at its receptor targets. This suggests that metformin should be further investigated for the treatment of insulin resistance in horses. On the other hand, it is possible that an increase in insulin secretion in response to glyburide and metformin administration contributed to the reduction in glucose concentration. Collection of serum samples for measurement of insulin and C-peptide concentrations following administration of glyburide and metformin would have helped to resolve this issue.

The islets of Langerhans are normally composed of a heterogeneous cell population comprising at least 7 different cell types.30 The distribution patterns of the various cell types are intimately related to islet microcirculation, and insulin-producing β cells normally occupy a central location.31 The numbers of various hormone-containing cells in the islets in humans have been found to be relatively constant irrespective of their location within the pancreas. For healthy control horses described in the present report, immunohistochemical staining revealed that β cells occurred homogeneously throughout the islets and that the immunohistochemical appearance of the islets was constant throughout the sections of pancreas that were examined. However, in the horse with diabetes mellitus, persisting β cells were clearly restricted to the periphery of the islet. Although use of immunohistochemical staining for characterization of equine islets has not been previously reported, transmission electron microscopy has been used to examine the islets of a horse with diabetes mellitus.32 In that horse, which also had primary insulin resistance, a reduced number of β cells limited to the periphery of the islets was noted.10 Results of histologic examination of pancreatic samples from 3 other horses with diabetes mellitus have been reported. In 1 report,11 no histologic abnormalities were seen, and in the other 2, chronic pancreatitis and atrophy were identified.12,13

Endogenous insulin secretion is best assessed by measuring concentration of C-peptide, which is co-secreted with insulin in a 1:1 molar ratio but which, unlike insulin, experiences little first-pass clearance by the liver.27,28 Measurement of C-peptide concentration under standardized conditions provides a sensitive, well-accepted, and clinically validated assessment of β-cell function.32,33 Moreover, attenuated secretion of C-peptide contributes to the pathophysiologic consequences of diabetes mellitus independent of the results of failed insulin secretion. The plasma concentration of C-peptide in this horse was much lower than that of healthy, age-matched control horses and less than the lower reference limit for humans, suggesting that measurement of C-peptide concentration may be a useful method of evaluating β-cell function in equids.

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Measurement of the glycosylated hemoglobin fraction has been widely accepted as a useful index for the treatment of diabetes mellitus in humans because the test can be performed at any time of day without special patient preparation and is more convenient for patients and physicians than glucose tolerance tests and measurement of plasma glucose concentration in fasted patients. A high glycosylated hemoglobin fraction reliably identifies a large proportion of individuals with undiagnosed diabetes mellitus who are at risk for developing diabetic complications. In light of the fact that marked hyperglycemia had been evident for at least several weeks, we were surprised that this horse’s glycosylated hemoglobin concentration was not high. However, the HbA1c assay uses an ion-exchange, high-performance liquid chromatographic approach that has not been validated for use in horses. In addition, the fact that glomerular basement membranes appeared histologically normal in this horse suggests that hyperglycemia might not have been present for enough time to cause substantial hemoglobin glycosylation. Over time, chronic hyperglycemia may lead to thickening of the glomerular basement membranes (Kimmelstiel-Wilson glomerulosclerosis).

The present report indicates that, albeit rare, pancreatic β-cell failure may contribute to the development of diabetes mellitus in horses. Insulin resistance, characterized by a normal or slightly high blood glucose concentration and hyperinsulinemia; laminitis; and pituitary pars intermedia dysfunction are common in obese horses. Whether pancreatic β-cell failure arises as a result of chronic insulin resistance in horses is deserving of further investigation. Further investigation regarding the relationship between insulin resistance, diabetes mellitus, and pituitary pars intermedia dysfunction is clearly warranted.

References