Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance

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Objective—To compare obese horses with insulin resistance (IR) with nonobese horses and determine whether blood resting glucose, insulin, leptin, and lipid concentrations differed between groups and were correlated with combined glucose-insulin test (CGIT) results.

Animals—7 obese adult horses with IR (OB-IR group) and 5 nonobese mares.

Procedures—Physical measurements were taken, and blood samples were collected after horses had acclimated to the hospital for 3 days. Response to insulin was assessed by use of the CGIT, and maintenance of plasma glucose concentrations greater than the preinjection value for ≥45 minutes was used to define IR. Area under the curve values for glucose (AUCg) and insulin (AUCi) concentrations were calculated.

Results—Morgan, Paso Fino, Quarter Horse, and Tennessee Walking Horse breeds were represented in the OB-IR group. Mean neck circumference and BCS differed significantly between groups and were positively correlated with AUC values. Resting insulin and leptin concentrations were 6 and 14 times as high, respectively, in the OB-IR group, compared with the nonobese group, and were significantly correlated with AUCg and AUCi. Plasma nonesterified fatty acid, very low-density lipoprotein, and high-density lipoprotein-cholesterol (HDL-C) concentrations were significantly higher (86%, 104%, and 29%, respectively) in OB-IR horses, and HDLC concentrations were positively correlated with AUC values.

Conclusions and Clinical Relevance—Measurements of neck circumference and resting insulin and leptin concentrations can be used to screen obese horses for IR. Dyslipidemia is associated with IR in obese horses. (J Am Vet Med Assoc 2006;228:1383–1390)

Obesity is a major health concern in humans because of its association with IR, type 2 diabetes mellitus, and coronary heart disease.1 Insulin resistance has also been associated with obesity in horses,3 and this condition may be of particular concern because of the putative link between laminitis and glucose metabolism.3 This link is supported by anecdotal observations that obese horses are more likely to develop laminitis and that dietary changes such as grazing on lush pasture can trigger episodes of laminitis. Other support is provided by in vitro evidence that hoof tissues have a high requirement for glucose.1 Pass et al3 reported that hoof explants undergo epidermal-dermal separation readily when glucose concentrations are low in the medium. It has also been reported that ponies with laminitis are less responsive to insulin than healthy ponies4 and that ponies with both obesity and laminitis are more insulin resistant than those that are just obese.5 Laminitis has also been associated with PPID, and IR often accompanies this condition.6,7

Obesity, hyperinsulinemia, and laminitis sometimes develop concurrently in horses without detectable PPID, and this group of disorders has been referred to as syndrome X or equine metabolic syndrome.8,9 The latter term is used most commonly but may not be appropriate because metabolic syndrome is defined by the presence of IR or type 2 diabetes mellitus as well as at least 2 of 4 additional risk factors, including obesity or visceral adiposity, dyslipidemia, microalbuminemia, or arterial hypertension.10 To the authors’ knowledge, microalbuminemia and hypertension have not been reported in obese horses, but blood lipid concentrations have been measured in ponies and donkeys with IR, and many of them were obese.11,12 Plasma triglyceride and total cholesterol concentrations do not differ between clinically normal and hyperinsulinemic ponies,13 but a positive correlation (r = 0.55; P = 0.002; n = 31) exists between plasma insulin and triglyceride concentrations in donkeys with naturally occurring hyperlipidemia.12

When methods of assessing IR in horses and ponies were recently reviewed, available tests were divided into those that provide nonspecific indications of IR and those that give specific quantitative measurements of insulin sensitivity.13 The CGIT used in the...
The present study was not discussed, but this test should be included in the first category because it does not provide values for insulin sensitivity, glucose effectiveness, or endogenous insulin secretion. However, delayed return of blood glucose concentrations to the preinjection concentrations can be attributed to IR when the CGIT is used because glucose concentrations should rapidly decrease in response to exogenous insulin administration. The CGIT was also used in the present study because it is easily interpreted, has low cost, and is readily applicable to clinical practice.

In this study, obese horses were selected on the basis of BCS and IR was defined by CGIT results. The purpose of the study reported here was to compare obese horses with IR with nonobese horses to determine whether blood resting glucose, insulin, leptin, and lipid concentrations differed between groups and were correlated with CGIT results. We also wanted to identify variables that predicted CGIT responses when only a single measurement is available and determine whether obesity, IR, and dyslipidemia occur concurrently in horses.

Materials and Methods

Horses—Client-owned horses were admitted to the University of Tennessee Large Animal Hospital and remained there for a 7-day evaluation period. Horses were evaluated between July 2003 and July 2004. Seven obese horses (5 mares, 2 geldings) with CGIT results indicative of IR (OB-IR group) were compared with 5 nonobese mares with a BCS of ≥7 on a 1 to 9 scale. Insulin resistance was arbitrarily defined by plasma glucose concentrations that remained greater than the preinjection concentration for ≥45 minutes during the CGIT (ie, attenuation of the negative phase). The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee, and signed owner consent was obtained for each horse.

Experimental design—Horses were admitted on day 0, and weight, height at the withers, girth, and neck circumference were measured. Physical examinations, CBC, and serum biochemical analyses were performed. An adipose tissue biopsy specimen was also collected on day 0, and flunixin meglumine was administered orally for the next 3 days. Each horse was then housed in a 3.7 X 3.7-m stall within the teaching hospital, provided with mixed timothy-orchard grass hay and water for ad libitum intake, and acclimated to the environment for 3 days.

Blood samples were collected for resting hormone and blood lipid measurements via jugular venipuncture between 8 AM and 9 AM on the fourth day. An IV catheter was placed, and a CGIT was performed the next day. Horses were screened for PPID by use of the combined DEX-TRH suppression test on subsequent days, with the exception of 3 horses from the OB-IR group that were excluded at the owners' request. Resting plasma ACTH concentrations were measured in these horses instead. The OB-IR horses were discharged 1 week after admission.

Neck circumference measurements—Horses were restrained so that the head and neck were maintained in a normal upright position, and the distance along a straight line from the poll to the cranial aspect of the withers was measured with a measuring tape (Figure 1). Neck circumference was measured perpendicular to this line at 0.25, 0.50, and 0.75 of the distance from the withers to the poll with a measuring tape (Figure 1). Mean neck circumference values were calculated.

CGIT—Horses were provided with ad libitum grass hay and water before and during the procedure. A 14-gauge polypropylene catheter was inserted into the left jugular vein, and an injection cap and infusion set (length, 30 cm; internal diameter, 0.14 cm) were attached. The CGIT was performed as described. Briefly, glucose (150 mg/kg) was infused as a 50% dextrose solution through the infusion line and catheter, followed by heparinized saline (0.9% NaCl) solution and regular insulin (0.1 U/kg). Blood samples were collected via the catheter at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135, and 150 minutes after insulin injection. At each time point, 3 mL of blood was withdrawn from the infusion line and discarded. A 6-mL blood sample was collected, followed by infusion of 5 mL of heparinized saline solution. Blood was transferred into tubes containing sodium fluoride and potassium oxalate that were immediately cooled on ice and refrigerated or transferred into glass tubes containing no anticoagulant that were left at 21°C for 1 hour to allow clot formation. Serum or plasma was subsequently harvested after low-speed centrifugation (1,000 X g) at 4°C for 20 minutes and stored at –20°C until further analysis.

Isolation and quantification of lipoproteins—Plasma lipoprotein fractions were isolated by use of the sequential ultracentrifugation method of Havel et al. Briefly, blood was centrifuged (1,000 X g) at 4°C for 20 minutes to separate plasma from chilled blood samples. Six-milliliter plasma samples were placed in a fixed-angle rotor for ultracentrifugation at 112,000 X g for 18 hours at 10°C. A 1-mL fraction of plasma (density < 1.006 g/mL) was isolated from each tube. This fraction was referred to as VLDL. Triglyceride, PL, and total cholesterol components of VLDL were measured with enzymatic colorimetric reagents in an automated discrete analyzer. Lipoprotein lipase, phospholipase D, and cholesterol oxidase, respectively, were the principal reagents of the triglyceride.

Figure 1—Illustration of a procedure used to measure mean neck circumference in horses. a = 0.25 of the distance from poll to withers b = 0.50 of the distance from poll to withers. c = 0.75 of the distance from poll to withers. Reprinted with permission.
was injected IV. After collection of another blood sample at 3

tions were measured in serum by use of a radioimmunoassay

PL, and total cholesterol assays. Protein content of VLDL was analyzed by use of bovine serum albumin standards and a spectrophotometer in accordance with a modified method of the Lowry procedure. Plasma VLDL concentrations were calculated by summing concentrations of lipid (triglyceride, total cholesterol, PL) and protein components.

Low-density lipoprotein was isolated from the remaining plasma by ultracentrifugation under the same conditions after addition of potassium bromide to raise the plasma density to 1.063 g/mL. A 1.063 g/mL solution of potassium bromide prepared in EDTA saline solution was also added to each sample to increase the volume to 6 mL. After ultracentrifugation, a 1-mL fraction of plasma (density < 1.063 g/mL) was isolated from each tube and dialyzed overnight in 0.5M ammonium bicarbonate solution to remove dissolved salts. Postdialysis volumes were recorded for each sample, and duplicate samples were processed; this fraction was referred to as LDL. Compositional analysis of dialyzed LDL samples was performed as described. Plasma LDL concentrations were adjusted to account for addition of potassium bromide solution and volume expansion during dialysis. High-density lipoprotein was isolated and quantified by use of the same methods as described for LDL, with the exception that the plasma density was increased to 1.21 g/mL. Equine VLDL, LDL, and HDL have established density limits of < 1.006, 1.019 to 1.063, and 1.063 to 1.21 g/mL, respectively.

Measurement of plasma glucose and lipid concentrations—Glucose concentrations were measured by use of a colorimetric assay on an automated discrete analyzer. Concentrations of plasma triglyceride and total cholesterol were measured by use of enzymatic colorimetric reagents and the automated discrete analyzer. Plasma NEFA concentrations were measured by use of an in vitro enzymatic colorimetric test kit on a microtiter plate reader. For all lipid, protein, and glucose measurements, duplicate assays were performed and intra-assay coefficients of variation < 5% were required for acceptance of results.

Screening for PPID—A DEX-TRH test was performed in 4 of 7 horses in the OB-IR group and all of the horses in the NO group according to the method of Eiler et al. Briefly, a baseline blood sample was collected, and 40 g of DEX/kg was injected IV. After collection of another blood sample at 3 hours, 12 mg of TRH was injected IV and blood samples were collected at 3.5, 4, and 24 hours after DEX injection.

Measurement of blood hormone concentrations—Serum or EDTA plasma was obtained via low-speed centrifugation (1,000 x g) at 4°C for 20 minutes. Insulin concentrations were measured in serum by use of a radioimmunoassay kit validated for equine insulin. Serum samples were analyzed for leptin with double-antibody radioimmunoassay procedures validated for equine leptin. Plasma cortisol concentrations were measured by use of a radioimmunoassay kit validated for equine cortisol.

Measurement of plasma ACTH concentrations—Assay of equine ACTH was performed on EDTA plasma by use of a commercially available immunoradiometric assay for ACTH. Endogenous plasma ACTH concentrations were measured in blood collected from 3 OB-IR horses that did not undergo combined DEX-TRH testing. For all hormone assays, duplicate measurements were performed and an intra-assay coefficient of variance < 10% was required for acceptance of results.

Statistical analysis—The AUCg and AUCi concentrations were calculated from total concentrations by use of the trapezoidal method and computer software. Groups were compared by use of the Mann-Whitney U test after distributions were examined and Shapiro-Wilk tests were performed, and median, range, mean, and SD values were calculated. Correlations among variables were examined by calculating Spearman correlation coefficients. Statistical tests were performed with computer software. Significance was defined at a value of P < 0.05.

Results

No abnormalities were detected via physical examination, and results of CBC and serum biochemical tests were within reference ranges for all horses. Four of 7 horses in the OB-IR group had a history of chronic laminitis, and abnormal growth rings were seen on hooves, but these horses were not lame at the time of examination. Groups differed significantly with respect to BCS, girth, and neck circumference, but did not differ in weight or height (Table 1).

In the OB-IR group, plasma glucose concentrations were greater than baseline concentrations for ≥ 45 minutes in 1 horse, 75 minutes in 2 horses, 90 minutes in 1 horse, 120 minutes in 1 horse, 135 minutes in 1 horse, and > 150 minutes in 1 horse (Figure 2). In contrast, plasma glucose concentrations returned to a value less than the baseline value within 25 minutes in 5 horses in the NO group.

Compared with the NO group, median AUCg and AUCi values for CGIT curves were 68% and 81% higher, respectively, in the OB-IR group (Table 1). Resting

Table 1—Measured variables (median [range]) in 5 nonobese horses and 7 obese horses with IR (OB-IR).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonobese (n = 5)</th>
<th>OB-IR (7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>473 (444–476)</td>
<td>518 (426–610)</td>
<td>0.223</td>
</tr>
<tr>
<td>BCS (scale, 1–9)</td>
<td>6 (4–9)</td>
<td>7 (7–9)</td>
<td>0.004</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>145 (142–158)</td>
<td>151 (143–155)*</td>
<td>0.927</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>179 (172–183)</td>
<td>189 (162–205)*</td>
<td>0.011</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>87.7 (85.3–95.7)</td>
<td>105.8 (94.3–110.3)*</td>
<td>0.013</td>
</tr>
<tr>
<td>Plasma leptin (ng/mL)</td>
<td>0.8 (0.5–11.9)</td>
<td>11.0 (1.0–30.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Resting plasma glucose (mg/dL)</td>
<td>66.9 (49.6–74.1)</td>
<td>83.9 (76.0–97.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Resting serum insulin (µIU/mL)</td>
<td>8.1 (6.4–21.1)</td>
<td>50.5 (17.0–93.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>Resting glucose-to-insulin ratio</td>
<td>6.4 (3.2–11.6)</td>
<td>1.7 (0.9–5.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>AUCg (x 10² mg·dL–1·min–1)</td>
<td>9.2 (7.7–10.1)</td>
<td>15.5 (13.1–22.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>AUCi (x 10³ µU·mL–1·min–1)</td>
<td>12.8 (10.6–13.2)</td>
<td>23.2 (18.1–36.6)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Includes only 6 observations.
serum insulin and plasma leptin concentrations were 6 times \((P = 0.007)\) and 14 times \((P = 0.028)\) greater, respectively, in the OB-IR group, but ranges overlapped. These single measurements of insulin \((r = 0.76; P = 0.004)\) and leptin \((r = 0.75; P = 0.005)\) were positively correlated with BCS. Compared with NO horses, plasma NEFA, VLDL, and HDL-cholesterol concentrations were 86%, 104%, and 29% greater, respectively, in OB-IR horses (Table 2).

Variables significantly correlated with both \(\text{AUC}_{\text{g}}\) and \(\text{AUC}_{\text{i}}\) included BCS, neck circumference, leptin concentration, glucose concentration, insulin concentration, glucose-to-insulin ratio, and HDL-cholesterol (Table 3). In this study, \(\text{AUC}_{\text{g}}\) and \(\text{AUC}_{\text{i}}\) were most closely correlated with BCS \((r = 0.84; P < 0.001)\) and neck circumference \((r = 0.88; P < 0.001)\), respectively.

Plasma cortisol concentrations were < 10 ng/mL at 24 hours after DEX administration in all of the horses evaluated by use of the DEX-TRH test, with the exception of 1 OB-IR horse that had a concentration of 10.7 ng/mL. For baseline and 30-minute post-TRH injection plasma cortisol concentrations, 1 horse from each group had responses (74% and 150%) that exceeded the 66% cutoff established for unaffected horses. However, both horses had typical suppression at 24 hours in response to administration of DEX. Median plasma ACTH concentrations for the 3 OB-IR horses tested ranged from 2.5 to 3.7 pmol/L, and these values were less than the 10 pmol/L upper limit of the reference range provided by the laboratory.

Discussion

A small population of obese horses with IR was assembled for this study, and these horses had significantly greater resting glucose, insulin, and leptin concentrations than healthy nonobese horses. High VLDL, VLDL-triglyceride, and HDL-cholesterol concentrations were also detected in OB-IR horses, suggesting that obesity, IR, and dyslipidemia occur concurrently in certain horses.

Mares predominated in the OB-IR group, but anecdotal reports and results of other studies suggest that geldings are equally affected by obesity. Although various breeds were represented in the OB-IR group, clients consistently referred to their horses as “easy keepers” because they appeared to require fewer calo-

Table 2—Plasma lipid and lipoprotein concentrations (median [range]) in 5 nonobese horses and 7 obese horses with IR.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonobese (n = 5)</th>
<th>OB-IR (n = 7)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA (µmol/L)</td>
<td>197.1 (151.5–268.3)</td>
<td>366.5 (187.8–634.0)</td>
<td>0.028</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>75.3 (73.0–88.1)</td>
<td>78.6 (60.4–86.0)</td>
<td>0.570</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>16.9 (11.0–22.6)</td>
<td>34.6 (13.8–134.8)</td>
<td>0.123</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>24.4 (19.8–34.6)</td>
<td>49.7 (29.8–162.0)</td>
<td>0.012</td>
</tr>
<tr>
<td>VLDL-triglyceride (mg/dL)</td>
<td>15.6 (11.2–22.2)</td>
<td>30.6 (17.4–97.1)</td>
<td>0.019</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>30.3 (13.1–46.8)</td>
<td>30.9 (16.6–54.6)</td>
<td>0.465</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>11.8 (5.2–18.3)</td>
<td>13.5 (6.8–22.4)</td>
<td>0.223</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>139.7 (106.4–169.4)</td>
<td>110.8 (96.4–160.9)</td>
<td>0.166</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>27.5 (24.8–34.2)</td>
<td>35.4 (32.2–52.3)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 3—Variables significantly correlated with plasma \(\text{AUC}_{\text{g}}\) and serum \(\text{AUC}_{\text{i}}\) values for CGITs in horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of horses</th>
<th>(\text{AUC}_{\text{g}})</th>
<th>(\text{AUC}_{\text{i}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(r)</td>
<td>(P)</td>
</tr>
<tr>
<td>BCS</td>
<td>12</td>
<td>0.84</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Neck circumference</td>
<td>11</td>
<td>0.71</td>
<td>0.015</td>
</tr>
<tr>
<td>Resting leptin concen-</td>
<td>12</td>
<td>0.76</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting insulin concen-</td>
<td>12</td>
<td>0.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting glucose concen-</td>
<td>12</td>
<td>0.86</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting glucose-to-insu-</td>
<td>12</td>
<td>–0.62</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol concen-</td>
<td>12</td>
<td>0.60</td>
<td>0.039</td>
</tr>
</tbody>
</table>
ries than most horses to maintain body weight. It has been suggested that some horses are genetically predisposed to obesity because of adaptations to survival on poorer quality forages.29 According to this theory, consumption of concentrated feeds or grazing on rich pastures might therefore promote obesity in susceptible horses. Genetic and environmental factors are likely to be important in the development of obesity in horses, and it is interesting that all of the OB-IR horses in this study were 10 years of age or greater, which suggests that time is required for environmental factors to alter glucose metabolism.

Because it is likely that diet contributes substantially to the development of obesity, the diet consumed by horses prior to admission may have been a confounding factor in this study. No attempt was made to control the diet of horses prior to their arrival, so horses had access to pasture and were consuming hay from various sources and sometimes a small quantity of a concentrate feed as a treat. Hoffman et al detected the effects of diet on response to insulin by determining that horses are less responsive to insulin after consuming a diet composed primarily of starch and sugar instead of fat and fiber. The adipose biopsy procedure performed on day 0 may have also been a source of variability, but this was discounted because all horses underwent the same procedure and CGIT results for nonobese horses were consistent with those from another study.28 Effects of flunixin meglumine on response to insulin have not been investigated, but administration of phenylbutazone for 5 days did not affect glucose tolerance or insulin secretion in geldings.25

Obesity was defined by BCS in this study with the scale developed by Henneke et al.17 Alternative measures of obesity include cadaver dissection,26 rump-fat thickness,18,26 body mass index,27 and estimation of total body water by use of bioelectric impedance analysis or indicator dilution.28 Of these procedures, BCS and rump-fat thickness are the easiest to perform. A BCS is assigned after visual appraisal and palpation of fat at 6 sites on the body, including the neck, withers, back, tailhead, ribs, and area caudal to the shoulder.19 Rump-fat thickness is measured via B-mode ultrasonography at a site approximately 5 cm lateral from the midline at the center of the pelvic bone, and percentage fat is calculated with an established formula.29

Mean neck circumference was examined to assess regional adiposity because many OB-IR horses have enlargement of adipose tissues at this location, which is sometimes referred to as a cresty neck. In humans, regional adiposity develops within the abdomen and an enlarged waist circumference has been identified as a risk factor for metabolic syndrome.1 Of the physical measurements collected in this study, mean neck circumference was most closely correlated with AUCg, which suggested that enlargement of the neck is a risk factor for IR in horses.

The CGIT was selected for use in this study because of its simplicity and low cost. The frequent-sample IV glucose tolerance test and the euglycemic-hyperinsulinemic clamp test may have been better choices because both provide specific quantitative measures of insulin sensitivity,61 but these tests are technically more challenging and therefore less applicable to clinical practice. These tests also require more frequent sampling and are therefore more expensive. In this study, the time required for blood glucose concentrations to enter the negative phase was used to define IR. This definition is easily applied, and only blood glucose measurements are required. Use of the baseline measurement as a reference point also allows handheld glucometers to be used because variability between glucometers does not affect results. This procedure is also applicable to ambulatory practice because the CGIT can be abbreviated to 60 minutes.

Higher resting serum insulin concentrations and lower resting glucose-to-insulin ratios were detected in horses from the OB-IR group, compared with nonobese horses, suggesting that these measures may be useful screening tests for IR. Hyperinsulinemia is a feature of IR in humans and occurs when insulin secretion from the pancreas increases to compensate for reduced response to insulin.30 In the present study, resting serum insulin concentrations and glucose-to-insulin ratios were closely correlated with AUCg and AUCi, suggesting that the same compensatory responses occur in horses. However, it should be noted that a resting serum insulin concentration of 17.0 μU/mL was detected in 1 OB-IR horse, and this value was within the range detected for the nonobese group. This finding therefore underscores the importance of performing challenge tests such as the CGIT when evaluating horses with suspected IR.

Resting glucose concentrations were positively correlated with AUCg values, indicating that blood glucose concentrations increased as IR progressed. However, it is doubtful that resting blood glucose concentrations will serve as a useful measure of response to insulin in clinical practice because of the confounding effects of stress. Administration of epinephrine62 or dexamethasone12,32 increases blood glucose concentrations in horses, and stressful procedures such as an endoscopic examination can markedly alter CGIT results.14

In this study, horses were acclimated to the hospital environment for 3 days before the first blood samples were collected and mixed timothy-orchard grass hay was fed ad libitum before and during the CGIT to reduce stress. Horses were not tested until 3 days after their arrival because transportation increases blood cortisol concentrations and induces hyperglycemia and hyperinsulinemia in donkeys.33 A 3-day acclimation period was selected for the present study on the basis of previous experiences with the CGIT34 and because blood glucose and insulin concentrations returned to baseline concentrations within 22 hours in donkeys that were transported.33 Grass hay was fed to horses because this feed was assumed to have a low glycemic index.34 The glycemic index of the mixed timothy-orchard grass hay fed to horses in this study was not determined, but Bermuda hay has a mean glycemic index of 23%, compared with oats (100%).34 Hay fed to horses in the present study was not analyzed, but the mean nonstructural carbohydrate content of hay purchased from the same farm was 12.8% of dry matter when evaluated as part of another study35 performed during the same time period (January to October). The
A decision to permit hay consumption during testing was also influenced by the observation that obese horses become particularly agitated when deprived of feed.

An association between obesity and IR has been reported in horses[2,6,7] and ponies.[3,4] When Hoffman et al[2] studied 3 obese Thoroughbred geldings by use of the frequently-sampled IV glucose tolerance test, mean insulin sensitivity was < 20% of the value in nonobese geldings. Obesity and lack of exercise are primary risk factors for IR in humans, and the risk of developing type 2 diabetes mellitus increases with the severity of obesity.[3] Blood NEFA concentrations increase with obesity and lack of physical activity in humans because adipose tissues reach their maximum capacity for fat storage and the inhibitory effects of insulin on hormone-responsive lipase are reduced.[3] As a result, the influx of fatty acids into tissues (including those of skeletal muscle) increases, which causes diacylglycerols to accumulate in cells.[3] This phenomenon is sometimes referred to as lipotoxicity because intracellular lipids disrupt insulin receptor signaling in myocytes or β-cell function in the pancreas.[9,10] In the study reported here, plasma NEFA concentrations were greater in OB-IR horses than in NO horses and were significantly correlated with AUCg values.

Plasma concentrations of VLDL and VLDL-triglyceride were significantly greater in OB-IR horses than in NO horses, and the median VLDL concentration (49.7 mg/dL) was also greater than mean concentrations of 28.5 and 19.1 mg/dL detected in adult nonobese mares.[4,6] Increased uptake of fatty acids by the liver should increase the availability of triglyceride for VLDL synthesis and stimulate production of this lipoprotein.[1] Oversecretion of triglyceride-rich VLDL particles is associated with abdominal obesity in humans and attributed to an increase in hepatic fatty acid uptake.[1] Increased VLDL production and synthesis of triglyceride-rich VLDLs have also been associated with feed deprivation and hyperlipemia in horses, which are conditions that develop in response to increased mobilization of NEFA from adipose stores.[8,11]

Hypertriglyceridemia (≥ 150 mg/dL) and low HDL-cholesterol concentrations are 2 criteria used to define metabolic syndrome in humans.[10] These variables are associated because triglyceride carried by VLDLs and chylomicrons is exchanged for cholesterol when these triglyceride-rich lipoproteins interact with HDL in the blood.[4,11] Consequently, as VLDL concentrations increase, interactions with HDL increase, and HDL-cholesterol concentrations decrease in humans.[3] However, VLDL and HDL-cholesterol concentrations were greater in OB-IR horses than in NO horses, which differs from responses detected in humans.[3] This finding may be explained by the fact that transfer of cholesterol from HDL to VLDL is catalyzed by cholesterol ester transfer protein in humans, whereas there is a near absence of cholesterol ester transfer protein activity in equine blood.[4,11] Higher plasma HDL-cholesterol concentrations have been associated with increased lipoprotein lipase activity in horses fed high-fat diets,[4,11] which suggests that the higher HDL-cholesterol concentrations detected in OB-IR horses may be attributable to an increase in lipoprotein lipase activity. A positive correlation between plasma HDL and triglyceride concentrations has also been detected in Shetland ponies.[44]

Results of the present study provide evidence of an association between hyperleptinemia and IR in obese horses. Resting plasma leptin concentrations ranged from 1.0 to 30.5 ng/mL in OB-IR horses and were positively correlated with AUCg and AUCi values. Leptin is a protein hormone synthesized by adipose tissues that is secreted in greater quantities when the body is in a positive energy balance.[6] Buff et al.[6] determined that serum leptin concentrations were positively correlated (r = 0.64, P < 0.001) with BCS in a herd of 71 Quarter Horses, which indicates that blood leptin concentrations reflect body-fat mass in horses. Plasma leptin concentrations and BCS were also positively correlated in the present study. Hyperleptinemia has also been associated with glucose metabolism disturbances in horses.[5,6] Cartmill et al.[6] identified obese horses with higher serum leptin concentrations and found that glucose tolerance test results were abnormal in these horses. When obese (BCS ≥ 7.5) horses were divided into low-leptin (< 5 ng/mL) and high-leptin (7 to 20 ng/mL) groups, significantly greater insulin responses to IV glucose infusion were detected in the high-leptin group.[5,6]

Results of the present study indicate that resting serum insulin and leptin concentrations are useful screening tests for IR in horses, but other factors including time of day and season must be considered when interpreting these values. Gordon and McKeever[46] determined that glucose and insulin responses to feeding are higher in the morning, and Fitzgerald and McManus[47] determined that serum leptin concentrations were highest in mature mares during the late summer and early fall and lowest during the winter months in Kentucky. When the same group measured serum insulin concentrations in obese mares across a 12-month period, mean insulin concentrations were also higher during the summer and early fall.[6] Because most of the horses included in the present study were evaluated during the summer, concentrations of these hormones may have been higher than at other times of the year.

Horses included in this study were screened for PPID because this endocrinopathy has been associated with IR in horses.[7] Three of 9 horses had abnormal responses when combined DEX-TRH tests were performed. However, no clinical signs of PPID were observed, with the exception of laminitis, which has been associated with this endocrinopathy in horses,[7] and the 2 components of the test did not agree in any of the horses tested. These may have been false-positive responses, and the time of year that tests were performed may have influenced results. This issue has not been examined extensively, but Donaldson et al.[6] recently determined that false-positive results are more likely to occur when DEX suppression tests are performed in the month of September. Alternatively, horses with positive test results in the present study may have had early or mild PPID that affected only 1 component of the test.
In the present study, examination of a small population of OB-IR horses revealed that mean neck circumference should be measured in addition to BCS to identify horses at risk for IR and that single measurements of blood insulin and leptin concentrations are useful indicators of IR in obese horses. Horses concurrently affected by obesity, IR, and dyslipidemia were identified.

b. IRL Wyatt, Abbott Laboratories, North Chicago, Ill.
c. Dextrose 50% injection, Abbott Laboratories, North Chicago, Ill.
d. Hormadur R, Eli Lilly & Co, Indianapolis, Ind.
e. Beckman Instruments Inc, Fullerton, Calif.
f. Wako Chemicals USA, Richmond, Va.
g. Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
h. UV-160, Shimadzu, Kyoto, Japan.
i. Glucose, Roche Diagnostic Systems Inc, Somerville, NJ.
j. Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
k. Dexamethasone solution, Pro Labs Ltd, St Joseph, Mo.
l. ELx800 Microplate Reader, Bio-Tek, Winooski, Vt.
m. Coat-A-Count Cortisol, Diagnostic Products Corp, Los Angeles, Calif.

References


Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Correlation of magnetic resonance images with anatomic features of the equine tarsus
Rafael Latorre et al

Objective—To correlate anatomic features of the equine tarsus identified in plastinated sections with images obtained via magnetic resonance imaging (MRI).

Animals—4 horses.

Procedures—MRI (1.5-Tesla magnet) of the tarsus was performed on the pelvic limbs of 4 clinically normal horses following euthanasia. After imaging, tarsocural joint spaces and vasculature were injected with colored latex. Sagittal and transverse sections of the tarsus were plastinated to facilitate interpretation of MRI images.

Results—Relevant anatomic structures were identified and labeled on the plastinated tissue slices and corresponding MRI images. Results indicated high correlations between MRI findings and those of plastinated sections.

Conclusions and Clinical Relevance—The data obtained provided certain reference standards for normal anatomic structure sizes and positions in the equine tarsus. This information may aid future physiologic or clinical studies of this joint. (Am J Vet Res 2006;67:756–761)