Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses

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Objective—To determine the effects of diet-induced weight gain on glucose and insulin dynamics and plasma hormone and lipid concentrations in horses.

Animals—13 adult geldings.

Procedures—Horses were fed 200% of their digestible energy requirements for maintenance for 16 weeks to induce weight gain. Frequently sampled IV glucose tolerance tests were performed before and after weight gain to evaluate glucose and insulin dynamics. Adiposity (assessed via condition scoring, morphometric measurements, and subcutaneous fat depth) and plasma concentrations of insulin, glucose, nonesterified fatty acids, triglycerides, and leptin were measured on a weekly or biweekly basis.

Results—Mean ± SD body weight increased by 20% from 440 ± 44 kg to 526 ± 53 kg, and body condition score (scale 1 to 9) increased from 6 ± 1 to 8 ± 1. Plasma glucose, triglyceride, and nonesterified fatty acid concentrations were similar before and after weight gain. Leptin and insulin concentrations increased with weight gain. Mean ± SD insulin sensitivity decreased by 71 ± 28%, accompanied by a 408 ± 201% increase in acute insulin response to glucose, which resulted in similar disposition index before and after weight gain.

Conclusions and Clinical Relevance—Diet-induced weight gain in horses occurred concurrently with decreased insulin sensitivity that was effectively compensated for by an increase in insulin secretory response. Obesity resulted in hyperinsulinemia and hyperleptinemia, compared with baseline values, but no changes in lipid concentrations were apparent. Preventing obesity is a potential strategy to help avoid insulin resistance, hyperinsulinemia, and hyperleptinemia in horses. (Am J Vet Res 2009;70:1250–1258)

Obesity is a growing health concern for equids because of its increasing prevalence and association with altered metabolic function and disease. Obesity has been associated with insulin resistance in horses and ponies, and insulin resistance in the presence of obesity has been associated with altered reproductive activity in mares and an increased risk of and predisposition for pasture-associated laminitis. A so-called equine metabolic syndrome has been described as a grouping of metabolic abnormalities in equids that may include insulin resistance, obesity, dyslipidemia, and chronic laminitis. Additionally, increased risk for pasture-associated laminitis in ponies has been associated with generalized obesity, regional accumulation of neck crest adipose tissue, hyperinsulinemia, and hyper-

**Abbreviations**

- AIRg: Acute insulin response to glucose
- BCS: Body condition score
- CNS: Cresty neck score
- DE: Digestible energy
- DI: Disposition index
- FSIGTT: Frequently sampled IV glucose tolerance test
- NEFA: Nonesterified fatty acid
- Sg: Glucose effectiveness calculated via minimal model analysis
- SI: Insulin sensitivity calculated via minimal model analysis
leptinemia. Although these factors are often observed simultaneously, it is unknown whether obesity is the primary cause of or contributes to metabolic abnormalities or whether these abnormalities are inherent characteristics of the animals.

In previous studies comparing groups of lean and obese horses, the obese horses had lower insulin sensitivity and high circulating concentrations of insulin, glucose, leptin, and NEFAs. Additionally, across a range of body conditions, percentage body fat and BCS are associated with insulin sensitivity and circulating concentrations of insulin and leptin. Previous studies in horses used cross-sectional analysis with regard to comparing metabolic variables of horses at different amounts of adiposity. However, induction of obesity in a longitudinal study would permit intraindividual comparison of variables before and after weight gain. Such a study design would limit the influence of interindividual variation, to determine metabolic alterations with small changes in body condition (< 2 unit mean BCS change) and differentiate between metabolic abnormalities inherent to an individual horse from those induced by an increase in adiposity. Additionally, evaluating horses during a mean body condition change from moderate (BCS < 7 [scale, 1 to 9]) to overweight (7 ≤ BCS < 8) to obese (BCS ≥ 8) would be most appropriate to determine metabolic abnormalities during the development of obesity and most applicable to horses at risk of imminently developing obesity.

The study reported here was designed to test the hypothesis that diet-induced weight gain in horses decreases insulin sensitivity and increases plasma concentrations of insulin, glucose, NEFAs, triglyceride, and leptin. Specifically, the purpose of the study was to identify changes in adiposity, minimal model parameters of glucose and insulin dynamics, and basal concentrations of insulin, glucose, NEFAs, triglyceride, and leptin as adiposity increases and an overweight or obese state is achieved through overfeeding.

**Materials and Methods**

**Horses**—Thirteen Arabian or Arabian cross geldings ranging in age from 8 to 20 years from the Virginia Tech Middleburg Agricultural Research and Extension Center’s research herd were evaluated during the study period (June 2006 to January 2007). Initial mean (range) body weight was 448 kg (381 to 570 kg), and horses were in moderate condition (mean BCS, 6 [range, 5 to 8]). Prior to study initiation, all horses were maintained on pasture as a single group for > 6 months. During the study, horses were maintained as a single group on a drylot. The experimental protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee.

To exclude the possibility of pituitary pars intermedia dysfunctions, dexamethasone suppression tests were performed according to the protocol of Donaldson et al. During week 2, dexamethasone was administered IM (40 μg/kg of body weight) at 2 pm. Blood samples for measurement of plasma cortisol concentration were collected via jugular venipuncture into evacuated sodium heparin collection tubes immediately before and 19 hours after dexamethasone administration.

**Experimental design**—In a longitudinal study lasting 30 weeks, all 13 horses were concurrently exposed to the protocol’s dietary treatments and sampling procedures. During period 1 (weeks 0 to 3), horses were maintained on a forage diet consisting of mixed grass-legume hay (Figure 1) and fed 104% (range, 82% to 122%) of their maintenance DE requirements on the basis of body weight during the first week of the period. During period 2 (weeks 4 to 7), horses were maintained on a high concentrate and hay diet and fed 110% (range, 87% to 126%) of their maintenance DE requirements on the basis of body weight during the first week of the period. During periods 3 and 4 (weeks 8 to 24), horses were fed approximately twice their DE requirements for maintenance of a high concentrate and hay diet to induce weight gain. Dividing the weight gain section of the study into 2 periods enabled the adjustment of feed intake on the basis of an increase in body weight between periods 3 and 4. During period 3, horses were fed a mean of 197% (range, 157% to 216%) of their maintenance DE requirements on the basis of body weight during the first week of the period, which decreased to 179% (range, 143% to 195%) during the final week of the period. During period 4, horses were fed a mean of 193% (range, 156% to 211%) of their maintenance DE requirements on the basis of body weight during the first week of the period, which decreased to 183% (range, 145% to 199%) during the final week of the period. During period 5 (weeks 25 to 30), horses were maintained at an overweight or obese state (BCS ≥ 7) on a forage diet. During period 5, horses were fed a mean of 133% (range, 106% to 146%) of their maintenance DE requirements on the basis of body weight during the first week of the period.

The high concentrate and hay diet consisted of approximately 60% of DE requirements from a concentrate feed (commercial sweet feed), 20% from chopped alfalfa forage, and 20% from a mixed grass-legume hay (Table 1). Horses were fed 3 times daily at 7 AM, 2 PM, and 7 PM. Concentrate feed and chopped alfalfa forage were fed individually in stalls, with concentrate feed and alfalfa fed in separate buckets. Horses were given approximately 1 hour to consume each meal, and any unconsumed feed was weighed daily. Hay was weighed out daily and group fed in...
the drylot on the ground in multiple rations, with estimated hay wastage recorded. Multiple batches of hay were fed throughout the study and individually sampled for nutrient composition.

Basal blood samples were collected the first day of each week (weeks 1 to 30) between 7 AM and 9 AM hours. Body weights were measured the first day of each week, and adiposity measurements were evaluated biweekly. Horses were allocated into 3 groups and subjected to the insulin-modified FSIGTT procedure on the first, second, or third day of weeks 3, 24, and 30, and to the regular FSIGTT procedure on the first, second, or third day of weeks 7 and 16, corresponding to the last week of each period.

As part of a companion study investigating changes in inflammatory cytokine expression during diet-induced obesity, tissue biopsies were performed during the fourth or fifth day of weeks 3, 7, 16, 24, and 30 on the second or third day after each horse underwent FSIGTT procedures. Muscle tissue samples were collected percutaneously from the middle glutal muscle by use of a Bergstrom needle biopsy technique, and adipose tissue samples were collected from the subcutaneous adipose tissue of the nuchal crest through a surgical incision. Horses were maintained in stalls for 4 or 5 days and administered 2 g of phenylbutazone/d, PO, for 3 days after tissue biopsies were performed.

Measures of adiposity—Girth and abdominal circumferences, height (at the wither), neck crest height, and neck circumference at 25%, 50%, and 75% of neck length were measured as described. Intra-assay coefficients of variation for morphometric measurements were < 3% for triplicate measurements in each of 3 horses. Four evaluators independently graded BCS from 1 to 9 and neck crest adiposity as CNS from 0 to 10. Scores were rated to the nearest whole- or half-score increment, and the mean of the 4 scores for each horse was used for data analysis. Intraclass correlation coefficients for the reliability of the mean scores were 0.92 for BCS and 0.81 for CNS.

Measurements of subcutaneous fat thickness were performed via B-mode ultrasonography over the rump, 5 cm lateral from the dorsal midline at the center of the pelvic bone, shoulder, three quarters the distance from the dorsal midline to a point one third the distance from the point of the shoulder to the point of the hip; ribs, two thirds the distance from the point of the shoulder to the point of hip between the twelfth and thirteenth ribs; and back, one quarter the distance from the dorsal midline to the point where the rib measurement was taken. Intra- and interassay coefficients of variation for ultrasonographic measurements were < 6% for triplicate measurements in each of 3 horses.

Basal blood sampling—On the first day of each week prior to daily feeding, blood samples were collected between 7 AM and 9 AM via jugular venipuncture into 10-mL evacuated tubes containing sodium heparin or potassium EDTA as an anticoagulant.

FSIGTT—On days of FSIGTT procedures, horses were removed from the drylot at 7 AM hours, catheters were inserted into a jugular vein, and horses were placed in stalls for the duration of the testing procedure. No feed or hay was offered prior to or during the testing procedure on FSIGTT days; however, horses had continual access to water. Thirty minutes after catheter placement, the FSIGTT procedure was initiated as described. Briefly, a glucose bolus (50% [wt/vol] dextrose solution) of 0.3 g/kg of body weight was administered rapidly (within 2 minutes) through the catheter, followed after 20 minutes by rapid administration (within 10 seconds) of an insulin bolus of 20 mU/kg of body weight (insulin-modified FSIGTT; periods 1, 4, and 5) or approximately 10 mL of saline (0.9% NaCl) solution (regular FSIGTT; periods 2 and 3). Blood samples were collected at ~20 (between 8:30 AM and 9:00 AM), ~5, 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, 100, 120, 150, 180, 210, and 240 minutes with respect to completion of glucose administration. Samples were immediately placed into 10-mL evacuated tubes containing sodium heparin and kept on ice until centrifugation.

Analysis of samples—Plasma was separated via centrifugation (3,000 × g) within 30 minutes of sample collection, separated into 1-mL aliquots, and stored at −80°C until analysis. Plasma glucose and insulin (in heparin) concentrations were measured in weekly basal samples and in FSIGTT samples. Plasma triglyceride, NEFAs, and leptin (in EDTA) concentrations were measured in a biweekly subset of the basal blood samples. Plasma glucose, triglyceride, and NEFA concentrations were assayed enzymatically by use of commercial kits and an automated analyzer. Plasma insulin, leptin, and cortisol concentrations were measured by use of commercial radioimmunoassays validated for use in equine plasma. All analyses were performed in duplicate. Intra-assay coefficients of variation were 0.43%, 2.7%, 4.0%, 5.4%, 9.6%, and 3.9% for glucose, triglyceride, NEFAs, insulin, leptin, and cortisol, respectively. Mean interassay coefficients of variation were 4.6% for glucose and 6.4% for insulin. All other analyses were performed in a single assay run for each analysis.

The minimal model of glucose and insulin dynamics was applied to glucose and insulin data for each FSIGTT by use of commercially available software and described methods. The model was used to calculate values for SI, Sg, AIRg, and DI.

Statistical analysis—As a result of laboratory error, insulin was not administered during FSIGTT procedures during periods 2 and 3; therefore, results from these periods were not included in the analysis. The Shapiro-Wilk test was used to test for normality of variables within each week or period. A Grubbs test (α = 0.01) was used to determine outliers within each week or period, which were subsequently removed from analyses. Five insulin, 1 glucose, 2 triglyceride, 1 NEFA, 1 SI, and 1 DI values were removed prior to analyses. A 2-sample paired t-test was used to compare cortisol measurements before and after dexamethasone administration. The effect of week on adiposity and basal blood variables was assessed via repeated-measures ANOVA by use of the Huynh-Feldt ε correction factor to adjust for sphericity, with Fisher-Hayter pairwise comparisons made between weeks. Mean values during weeks 1 to 3 (period 1) were calculated, then compared with sub-
sequents weeks by use of Fisher-Hayter pairwise comparisons. For minimal model parameters, the effect of period was assessed via repeated-measures ANOVA by use of the Huynh-Feldt correction factor to adjust for sphericity, with Fisher-Hayter pairwise comparisons made between periods. Pearson correlation coefficients were calculated by use of mean pre–weight gain values (mean of weeks 1 to 7 for adiposity measure; period 1 for minimal model parameters), post–weight gain values (mean of weeks 24 to 30 for adiposity measures; period 5 for minimal model parameters), or the difference between pre– and post–weight gain values. Values of $P < 0.05$ were considered significant. Data are reported as mean ± SEM unless stated otherwise. Statistical analyses were conducted by use of a computer software program.³⁶

**Results**

**Horses**—No horses were excluded on the basis of results of dexamethasone suppression testing to assess the possibility of pituitary pars intermedia dysfunction because plasma cortisol concentration decreased ($P < 0.001$) from resting concentrations ($4.1 \pm 0.3 \mu g/dL$) to $< 0.5 \mu g/dL$ in all horses 19 hours after dexamethasone administration.

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Table 1.—Estimated dietary intake per horse on a daily basis and calculated dietary composition during each period in a study of 13 horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Component</th>
<th>Period</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (kg/d)</td>
<td>Mixed grass-legume hay</td>
<td>8</td>
<td>1.8</td>
<td>2.7</td>
<td>3.2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chopped alfalfa forage</td>
<td>0</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentrate (sweet feed)</td>
<td>0</td>
<td>3.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nutrient intake (% of dry-matter intake)</td>
<td>Crude protein</td>
<td>12.3</td>
<td>16.4</td>
<td>16.3</td>
<td>16.2</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid detergent fiber</td>
<td>38.0</td>
<td>20.5</td>
<td>19.1</td>
<td>19.4</td>
<td>31.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutral detergent fiber</td>
<td>60.1</td>
<td>30.9</td>
<td>29.5</td>
<td>29.9</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water-soluble carbohydrates</td>
<td>5.8</td>
<td>7.6</td>
<td>7.4</td>
<td>7.4</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>1.8</td>
<td>25.2</td>
<td>27.3</td>
<td>27.1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ether extract</td>
<td>2.7</td>
<td>5.4</td>
<td>5.6</td>
<td>5.5</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>DE intake</td>
<td>Mcal/d</td>
<td>15.5</td>
<td>15.8</td>
<td>29.5</td>
<td>32.1</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>Percentage of requirements*</td>
<td>104</td>
<td>110</td>
<td>197</td>
<td>193</td>
<td>133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Percentage of daily DE requirements that were fulfilled by estimated DE intake. Required DE = 33.3 kcal/kg of body weight, where body weight is the mean group body weight for the first week of each period.

Table 2.—Adiposity measurements (mean ± SD [range]) during the final week of each period and overall change from pre–weight gain to post–weight gain in 13 horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period 1 (week 3)</th>
<th>Period 2 (week 7)</th>
<th>Period 3 (week 16)</th>
<th>Period 4 (week 24)</th>
<th>Period 5 (week 30)</th>
<th>Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>440 ± 44a</td>
<td>435 ± 42a</td>
<td>494 ± 45a</td>
<td>528 ± 48a</td>
<td>526 ± 53a</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>BCS</td>
<td>3.9 (3.8 to 4.0)</td>
<td>3.9 (3.8 to 4.0)</td>
<td>3.9 (3.8 to 4.0)</td>
<td>3.9 (3.8 to 4.0)</td>
<td>3.9 (3.8 to 4.0)</td>
<td>3.9 (3.8 to 4.0)</td>
</tr>
<tr>
<td>Girth circumference (cm)</td>
<td>179 ± 7</td>
<td>179 ± 7</td>
<td>179 ± 7</td>
<td>179 ± 7</td>
<td>179 ± 7</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>196 ± 8</td>
<td>196 ± 8</td>
<td>208 ± 7</td>
<td>217 ± 8</td>
<td>216 ± 9</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>CNS</td>
<td>2.2 ± 0.5</td>
<td>2.1 ± 0.6</td>
<td>2.9 ± 0.6</td>
<td>3.2 ± 0.4</td>
<td>3.1 ± 0.5</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>Mean neck circumference (cm)</td>
<td>91 ± 3.9</td>
<td>91 ± 4</td>
<td>96 ± 4</td>
<td>97 ± 4</td>
<td>98 ± 3</td>
<td>6.2 ± 1.8</td>
</tr>
</tbody>
</table>

*Difference between the mean of weeks 1 to 7 (pre–weight gain) and weeks 24 to 30 (post–weight gain).
†Calculated as percentage fat = 6.22 + 5.07x, where x is subcutaneous rump fat thickness in cm.¹³

Means in the same row with different superscripts differ significantly ($P < 0.05$) in pairwise comparisons.
Three horses had mild signs of laminitis during the study, including bilateral forelimb lameness (Obel grade, 1 to 2), prominent digital pulses, and increased hoof temperature. Phenylbutazone (2 g/d, PO) was administered to those horses as needed for pain relief. Clinical signs of laminitis began during weeks 18, 20, and 22 and were resolved by week 27. According to results of the Grubbs test for outliers within each week or period, these horses did not have outlying values for any variables during the study, except a high ($P < 0.01$) value for triglyceride concentration during week 14 (1 horse) and a high value for NEFA concentration during week 5 (a different horse), which were subsequently removed from data analysis.

Adiposity—All measurements of adiposity differed ($P < 0.001$) by week, increasing to values greater than ($P < 0.05$) period 1 and 2 values (before weight gain) by the end of period 3 (Figure 1; Table 2). The only exception was subcutaneous fat thickness over the rib, which did not differ by week ($P = 0.11$). Adiposity measurements were not significantly different within the pre–weight gain period (weeks 1 to 7) and within the post–weight gain period (weeks 24 to 30). Therefore, measurements were pooled within pre– or post–weight gain periods to calculate the overall change in adiposity measurements. Overall, there was a 20% increase in body weight, 160% increase in rump subcutaneous fat thickness, almost 2-unit increase in BCS, and 1 unit increase in CNS. By use of the nearest BCS integer value for obesity classification, all 13 horses were overweight or obese (BCS $\geq 7$) and 10 horses were obese (BCS $\geq 8$) during the post–weight gain period.

Basal blood variables—Insulin concentration differed by week ($P < 0.001$), increasing to values significantly ($P < 0.05$) greater than period 1 concentrations during period 4 and in week 30 (Figure 2). Mean weekly concentrations of insulin ranged from 3.5 $\pm$ 0.3 mU/L (week 2) to 61.7 $\pm$ 15.7 mU/L (week 23) across the study. Glucose concentration differed by week ($P < 0.001$); however, period 1 concentrations were not dif-
different than subsequent weeks and no consistent changes were apparent between weeks. Mean weekly concentrations of glucose ranged from 88.1 ± 0.9 mg/dL (week 6) to 96.2 ± 1.0 mg/dL (week 30) across the study. Leptin concentration differed by week (P < 0.001), increasing (P = 0.01) consistently from period 1 concentrations during weeks 10 to 24. Additionally, leptin concentrations during weeks 26 and 28 were lower (P < 0.05) than during week 24.

Triglyceride concentration differed by week (P < 0.001), with 5 of the 8 measured values during periods 3 and 4 being lower (P < 0.05) than period 1 concentrations (Figure 3). Mean concentrations of triglyceride ranged from 19.4 ± 1.6 mg/dL (week 26) to 39.2 ± 3.5 mg/dL (week 28) across the study. Concentrations of NEFA differed by week (P < 0.001). Concentrations were lower (P = 0.01) than period 1 values during periods 2, 3, and 4. Mean concentrations of NEFAs ranged from 0.24 ± 0.02 mEq/L (week 28) to 0.34 ± 0.03 mEq/L (week 30) during periods 1 and 5 and from 0.05 ± 0.01 mEq/L (week 14) to 0.19 ± 0.02 mEq/L (week 24) during periods 2, 3, and 4.

Glucose and insulin dynamics—Mean SI was lower (P < 0.05) in periods 4 and 5, compared with period 1 (Figure 4; Table 3). Mean AIRg was higher (P < 0.05) in periods 4 and 5, compared with period 1. Mean DI and Sg were similar (P > 0.05) among periods 1, 4, and 5.

Period 1 SI values were negatively correlated to the change (period 5 value – period 1 value) in SI values (r = –0.99 [P < 0.001]). Change in subcutaneous shoulder fat thickness was negatively associated with change in AIRg (r = –0.61 [P = 0.025]). No other significant correlations between overall changes or pre– and post–weight gain values for adiposity measurements and minimal model parameters were observed, including pre–weight gain BCS and SI (r = –0.33 [P = 0.27]).

Discussion

In the present study, 16 weeks of overfeeding that resulted in 88 ± 11 kg of body weight gain (20% increase) and an overweight or obese state (BCS ≥ 7) was associated with a concurrent decrease in SI that was effectively compensated for by an increase in AIRg. Circulating insulin and leptin concentrations increased, whereas NEFA, triglyceride, and glucose concentrations did not differ with the induction of obesity.

Insulin-mediated glucose disposal (SI) was 71 ± 28% lower than period 1 values after the induction of obesity. The decrease in SI was accompanied by a 408 ± 201% increase in AIRg, representing the endogenous insulin secretion in response to the glucose dose. This resulted in a similar (P ≥ 0.05) DI in periods 1 and 5, indicating that as the degree of insulin resistance increased, there was a proportionate increase in insulin

![Figure 4—Minimal model parameters of SI (A) and AIRg (B) calculated from glucose and insulin curves of FSIGTT performed during the final week of periods 1 and 5 in a study of 13 horses. P values represent differences between period 1 and 5 values.](image1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 4</th>
<th>Period 5</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI (× 10^-4 [L·min^-1·mU^-1])</td>
<td>2.07 ± 1.76b (0.50 to 6.05)</td>
<td>0.62 ± 0.33a (0.27 to 1.41)</td>
<td>0.39 ± 0.27b (0.11 to 1.01)</td>
<td>−1.68 ± 1.73 (−5.98 to 0.01)</td>
</tr>
<tr>
<td>Sg (%/min)</td>
<td>1.07 ± 0.52 (0.24 to 2.05)</td>
<td>1.29 ± 0.55 (0.58 to 2.35)</td>
<td>1.17 ± 0.73 (0.35 to 2.74)</td>
<td>NS</td>
</tr>
<tr>
<td>AIRg (mU·min·L^-1)</td>
<td>206 ± 88b (89 to 403)</td>
<td>804 ± 265b (441 to 1,314)</td>
<td>973 ± 353b (487 to 1,864)</td>
<td>767 ± 332 (397 to 1,461)</td>
</tr>
<tr>
<td>DI (× 10^-3)</td>
<td>3.70 ± 2.38 (0.91 to 8.41)</td>
<td>5.30 ± 2.96 (2.21 to 12.77)</td>
<td>3.63 ± 2.60 (0.87 to 9.47)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SD (range).

a,b Means in the same row with different superscripts differ significantly (P < 0.05) in pairwise comparisons.

NS = No significant (P ≥ 0.05) differences between periods; therefore, change was not calculated.
 secretion to compensate for the decrease in insulin sensitivity. Additionally, Sg or glucose-mediated glucose disposal was not affected by weight gain. Collectively, these results indicated that changes in glucose dynamics occurred through insulin-dependent rather than insulin-independent pathways. Previously, Hoffman et al. similarly observed compensated insulin resistance in obese Thoroughbred geldings, compared with lean Thoroughbred geldings. However, that study also found greater insulin-independent glucose uptake (higher Sg) in obese horses, which is in contrast to the results of the present study. Taken together, these results indicate that an upregulation of insulin-independent pathways may be an inherent characteristic of horses predisposed to obesity, rather than a consequence of obesity.

Because no comparable control group was assessed in the present study, it cannot be excluded that changes in variables were a result of factors other than an increase in adiposity. In previous studies, adaptation to a high nonstructural carbohydrate diet decreased SI when compared with a high fiber and fat diet. In Thoroughbred weanlings and mature geldings, horses adapted to a diet high in nonstructural carbohydrates had 36% or 38% lower SI, respectively, compared with horses adapted to a diet high in fat and fiber. Although no other minimal model parameters differed between groups in the weanlings, AIRg and DI were lower in high nonstructural carbohydrate–fed mature geldings. In the present study, SI decreased with weight gain on a high nonstructural carbohydrate diet; however, in contrast to results of previous dietary studies, there was also a concurrent increase in AIRg values. Additionally, SI and AIRg values remained lower and higher, respectively, than pre–weight gain values after 5 weeks of low nonstructural carbohydrate hay feeding. Therefore, it is unlikely that changes in glucose and insulin dynamics in the present study were primarily a result of dietary adaptation.

Results of the present study were comparable to results from studies of diet-induced obesity in other species. Induction of weight gain by overfeeding decreases insulin sensitivity in humans, mice, dogs, and rabbits. Various mechanisms by which obesity may contribute to insulin resistance include an increase in inflammatory cytokines, accumulation of intramyocellular triglycerides, or increase in circulating NEFAs. Adipose tissue in obesity releases increased amounts of inflammatory cytokines, including tumor necrosis factor-α and interleukin-6, which may then directly interfere with insulin signaling. Additionally, increases in fatty acids in circulation or within myocytes may directly interfere with insulin signaling pathways or indirectly affect insulin sensitivity through stimulation of inflammatory pathways.

Although previous studies that used cross-sectional study designs have detected lower insulin sensitivity in obese horses, compared with lean horses, leading to the hypothesis that obesity causes insulin resistance, the present study was the first to detect a decrease in insulin sensitivity concurrent with the induction of obesity by use of specific quantitative methods of FSIGTT followed by minimal model analysis. Previous studies correlating metabolic variables over a range of BCS included a large number of horses (n = 71 and 60, respectively) covering a wide range of body conditions (6-unit BCS range) to determine significant correlations. The present study revealed that a < 2-unit increase in BCS inducing an overweight or obese state was associated with concurrent significant changes in metabolic variables in a small population of horses.

Few significant correlations were observed between adiposity measures and minimal model parameters, likely because of the small sample population (n = 13), the narrow range of adiposity values, and the small changes in values before and after weight gain. Nonetheless, it was evident that SI decreased in 12 of 13 horses and AIRg increased in all 13 horses, indicating that the direction of change in minimal model parameters was consistent. However, the magnitude of the change in SI and AIRg could not be predicted on the basis of the extent of change in adiposity. The single horse that did not have a decrease in SI with weight gain had the lowest SI to begin with (0.5 X 10^4 L/min/mU), suggesting an alternative mechanism for insulin resistance before the induction of obesity. Although adiposity measurements were not correlated to changes in SI, period 1 SI values were highly correlated to changes during the analysis.

Because insulin clearance was not measured in this study, it cannot be excluded that changes in AIRg and basal insulin concentration were partially attributable to decreased insulin clearance by the liver rather than an increase in insulin secretion from the pancreas. In a study of diet-induced weight gain in humans, changes in insulin concentrations after orally or IV administered glucose doses were fully attributable to decreased insulin clearance after weight gain. Similarly, increases in basal insulin concentrations after weight gain were attributable to changes in both insulin secretion and clearance, with a greater contribution of reduced insulin secretion than increased insulin clearance. In a study of diet-induced weight gain in humans, changes in insulin concentrations after orally or IV administered glucose doses were fully attributable to decreased insulin clearance after weight gain. Similarly, increases in basal insulin concentrations after weight gain were attributable to changes in both insulin secretion and clearance, with a greater contribution of reduced insulin secretion than increased insulin clearance. In a study of diet-induced weight gain in humans, changes in insulin concentrations after orally or IV administered glucose doses were fully attributable to decreased insulin clearance after weight gain. Similarly, increases in basal insulin concentrations after weight gain were attributable to changes in both insulin secretion and clearance, with a greater contribution of reduced insulin secretion than increased insulin clearance.
hyperinsulinemia has been observed in insulin-resistant ponies on a high nonstructural carbohydrate diet, compared with a low nonstructural carbohydrate diet. Collectively, the results of these studies and the present study indicate that basal hyperinsulinemia results from an interaction of degree of insulin resistance and concentration of nonstructural carbohydrates in the diet.

In the presence of increased insulin concentrations, euglycemia was maintained throughout the study. In addition to similar DI values before (period 1) and after (period 5) weight gain, maintenance of euglycemia provides further support that the decrease in insulin sensitivity was effectively compensated for by an increase in insulin secretion. Although, in the present study, relatively short-term obesity resulted in compensated insulin resistance, chronic obesity may eventually lead to uncompensated insulin resistance through pancreatic β-cell exhaustion. The inability of the pancreas to secrete sufficient insulin to maintain euglycemia, despite persistently increased circulating insulin concentrations, has been associated with clinical laminitis in ponies.

The effect of feed composition on lipid metabolism was greater than any effect of increased adiposity as evidenced by concentrations of NEFAs and triglyceride that were similar before and after weight gain when fed the forage diet but lower during periods of high concentrate feeding. The high concentrate diet had higher starch concentrations and higher fat concentrations than the forage diet (Table 1), both of which could potentially cause changes in plasma lipid concentrations. It was unexpected that weight gain would have no effect on NEFA concentrations because groups of obese, insulin-resistant horses have higher NEFA concentrations than lean horses. This indicates that abnormal lipid metabolism may be an inherent characteristic of obesity-prone equids or that chronic insulin resistance is necessary for changes in lipid metabolism.

Leptin concentrations after weight gain were comparable to reported concentrations in obese, insulin-resistant horses. Leptin is primarily produced and secreted from adipose tissue at concentrations that are proportional to fat mass, and its secretion is stimulated by insulin and inversely related to insulin sensitivity in obese horses. In the present study, leptin concentration increased through week 24, corresponding to increases in feed intake, adiposity, and insulin concentration. A previous study revealed a positive correlation between leptin concentration and BCS in a large population of horses (n = 71) and across a wide range of scores (6-unit range; BCS, 3 to 8). However, a < 2-unit change in BCS during a decrease in condition of overweight (BCS ≥ 6) ponies or an increase in condition of thin (BCS ≤ 5) ponies did not result in significant changes in leptin concentrations. Conversely, during the present study, a 1.7 ± 0.5-unit increase of BCS into an overweight or obese state resulted in a 3-times increase in leptin concentration from 3.3 ± 0.5 ng/mL during week 3 to 9.8 ± 0.9 ng/mL during week 30.

Leptin also signals satiety and energy status, and although overfeeding greater than maintenance requirements increased leptin concentrations, underfeeding less than maintenance requirements decreased leptin concentrations in horses. In the present study, leptin concentration decreased from week 24 to 26, corresponding to decreased energy intake and insulin concentration. During period 5, horses were initially fed 100% of their calculated DE requirements for maintenance, which was then incrementally increased (for a mean of 133% of DE requirements for period 5) because the initial amount provided was not enough to maintain body weight. This initial negative energy balance may have contributed to the decrease in leptin or insulin during the first weeks of period 5.

Three horses had mild signs of laminitis during the present study; however, these cases were not confirmed radiographically and laminitis status was not evaluated prior to study initiation. Laminitic episodes were observed during weeks of the highest basal insulin concentrations and dissipated in association with significant decreases in insulin concentrations between weeks 24 and 27. Low insulin sensitivity, high insulin concentrations, or both have been associated with laminitic predisposition in ponies and experimental induction of prolonged hyperinsulinemia (> 1,000 mU/L) while maintaining euglycemia-induced laminitis in healthy ponies. Risk factors for laminitis determined in ponies, including generalized obesity (BCS ≥ 7), regional obesity (CNS ≥ 4), hyperinsulinemia (> 32 mU/L), and hyperleptinemia (> 7.5 ng/mL), were applied to the horses that developed laminitis during the present study. These horses had none of the risk factors prior to weight gain; however, after weight gain (weeks 24 and 30), 2 of the horses had 3 risk factors and 1 horse had 2 risk factors.

Although the 3 horses that developed laminitis did not have any outlying values for insulin concentration or minimal model parameters, individual susceptibility may have been greater in those horses because of factors not measured, such as inflammatory mediators. Additionally, as speculated with experimental models of laminitis induced by carbohydrate overload, gastrointestinal tract disturbances, such as changes in hindgut bacteria, the release of endotoxins, or increased intestinal mucosal permeability, may have been initiating factors of the laminitic episodes.

In the present study, a 1.7 ± 0.5-unit increase of BCS into an overweight or obese state had profound effects on plasma hormone concentrations and insulin sensitivity, including 3-times increases in plasma leptin and insulin concentrations, 71 ± 28% decrease in SI, and 408 ± 201% increase in AIRg. Disposition index and plasma glucose concentrations were unchanged, suggesting that insulin resistance concurrent with an acute increase in adiposity was effectively compensated for by an increase in insulin secretory response. Additionally, Sg was not altered, indicating that changes in glucose dynamics occurred through insulin-dependent rather than insulin-independent pathways. Plasma lipid concentrations were not affected by increased adiposity; however, they were decreased by feeding a high concentrate diet. Results suggested that preventing weight gain and an overweight or obese state is 1 potential strategy to help minimize insulin resistance, hyperinsulinemia, and hyperleptinemia in horses. This avoidance may then reduce risk for laminitis and help maintain a healthy metabolic state.
References


