Effects of short- and long-term recombinant equine growth hormone and short-term hydrocortisone administration on tissue sensitivity to insulin in horses

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Objective—To determine the effects of short-term IV administration of hydrocortisone or equine growth hormone (eGH) or long-term IM administration of eGH to horses on tissue sensitivity to exogenous insulin.

Animals—5 Standardbreds and 4 Dutch Warmblood horses.

Procedure—The euglycemic-hyperinsulinemic clamp technique was used to examine sensitivity of peripheral tissues to exogenous insulin 24 hours after administration of a single dose of hydrocortisone (0.06 mg/kg), eGH (20 µg/kg), or saline (0.9% NaCl) solution and after long-term administration (11 to 15 days) of eGH to horses. The amounts of metabolized glucose (M) and plasma insulin concentration (I) were determined.

Results—Values for M and the M-to-I ratio were significantly higher 24 hours after administration of a single dose of hydrocortisone than after single-dose administration of eGH or saline solution. After long-term administration of eGH, basal I concentration was increased and the mean M-to-I ratio was 22% lower, compared with values for horses treated with saline solution.

Conclusions and Clinical Relevance—Increases in M and the M-to-I ratio after a single dose of hydrocortisone imply that short-term hydrocortisone treatment increases glucose use by, and insulin sensitivity of, peripheral tissues. Assuming a single dose of hydrocortisone improves sensitivity of peripheral tissues to insulin, it may be an interesting candidate for use in reducing insulin resistance in peripheral tissues of horses with several disease states. In contrast, long-term administration of eGH decreased tissue sensitivity to exogenous insulin associated with hyperinsulinemia. Therefore, increased concentrations of growth hormone may contribute to insulin resistance in horses with various disease states. (*Am J Vet Res* 2005;66:1907–1913)

Insulin resistance can be defined as the metabolic state in which physiologic concentrations of insulin stimulate a lower-than-anticipated biological response (cellular glucose uptake).[1] As such, insulin resistance can be quantified by examining glucose tolerance, a variable that can be measured most accurately by use of 2 clamp techniques[2] (ie, the hyperglycemic clamp and the euglycemic-hyperinsulinemic clamp technique), both of which have been validated for use in horses.[3,a] The hyperglycemic clamp technique is primarily a method for quantifying the sensitivity of the pancreas to exogenous glucose, whereas the eu-glycemic-hyperinsulinemic clamp technique is a method for quantifying tissue sensitivity to insulin.

Insulin resistance in horses is of interest because it has been associated with several disease states, including obesity,[4,5] hyperadrenocorticism,[6] equine metabolic syndrome or peripheral hyperadrenocorticism,[7] laminitis,[4,8] hyperlipemia,[4,8] and excessive amounts of **growth hormone (GH)**.[9] Although it has been suggested that both cortisol[6] and GH[9,b,c] are responsible for increased insulin resistance in horses with some of these conditions, the exact cause of insulin resistance has not been documented for any of them, primarily because insulin resistance in most studies[6,9,b,c] was only estimated by use of indirect measurements, such as a single plasma glucose or insulin concentration, oral or IV glucose tolerance tests, or an insulin tolerance test. A more precise investigation of the possible cause or causes of increased insulin resistance can be conducted by measuring the effect of proposed causal agents on tissue sensitivity to exogenous insulin by use of the euglycemic-hyperinsulinemic clamp technique. During the steady-state condition of the euglycemic-hyperinsulinemic clamp technique, endogenous glucose production by the liver and endogenous insulin production by the pancreas are suppressed, and it is therefore possible to directly quantify the metabolism of glucose by peripheral tissues.[10]

The objective of the study reported here was to use the euglycemic-hyperinsulinemic clamp technique to assess the effects of short-term administration of hydrocortisone or **equine GH** (**eGH**; experiment 1) or long-term administration of eGH (experiment 2) to horses on the sensitivity of peripheral tissues to exogenous insulin. The testable hypotheses were that eGH and hydrocortisone would be completely inert and have exactly the same effect as physiologic saline (0.9% NaCl) solution on insulin resistance of peripheral tissues. To our knowl-

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edge, the relationships between eGH and insulin sensitivity of peripheral tissues as well as between hydrocortisone and insulin resistance of peripheral tissues have not been examined in horses by use of the euglycemic-hyperinsulinemic clamp technique.

Materials and Methods

Animals—Nine horses were used in the 2 experiments of the study reported here. Five healthy Standardbreds (4 mares and 1 gelding) were used in experiment 1. Horses ranged from 3 to 13 years of age (mean ± SD, 5.6 ± 4.2 years) and weighed between 411 and 457 kg (mean ± SD, 435 ± 14 kg). The horses were housed in stables all day except for 1 hour of exercise (ie, walking controlled by use of a walking machine).

For experiment 2, we used 2 of the Standardbreds (mares) from the first experiment and 4 Dutch Warmblood mares. Horses ranged from 4 to 13 years of age (mean ± SD, 8.3 ± 3.7 years) and weighed between 447 and 736 kg (mean ± SD, 557 ± 94 kg). Body condition score of the horses ranged from 4 to 5 (mean ± SD, 4.5 ± 0.55). The horses were maintained on pasture all day and used for light riding.

The medical history for each horse was collected. A clinical examination and hematologic evaluation were performed, with emphasis on body condition score and indications of insulin resistance (basal plasma glucose and insulin concentrations). Horses were excluded from the study on the basis of a body condition score < 4 or > 5,[11] hyperglycemia, hyperinsulinemia, or clinical evidence of systemic disease. Horses were also excluded when they had received medications within the month preceding onset of the study. The experiments were approved by the Committee for Animal Welfare at the Faculty of Veterinary Medicine, Utrecht University.

Experiment 1—The effects of a single dose of eGH, hydrocortisone, or saline (control) solution on the sensitivity of peripheral tissues to exogenous insulin were examined by use of a euglycemic-hyperinsulinemic clamp technique in 5 horses allotted randomly to treatment order for a 3-way crossover design. The 3 treatments were hydrocortisone[d] (0.06 mg/kg), recombinant eGH,[e] (20 µg/kg), and control solution (5 mL of saline solution). All treatments were administered IV 24 hours before measurement of variables by use of the euglycemic-hyperinsulinemic clamp technique. Successive treatments in each horse were separated by an interval of at least 7 days. In each case, food was withheld from the horses beginning 12 hours before and until completion of the euglycemic-hyperinsulinemic clamp technique.

Experiment 2—Six mares were used in an experiment in accordance with a 2 × 2 Latin-square design to investigate the effects of long-term administration of eGH on sensitivity of peripheral tissues to exogenous insulin, as measured by use of the euglycemic-hyperinsulinemic clamp technique. The 2 treatments were eGH and control solution, with horses allotted randomly to the initial treatment. As recommended in the data sheets of the manufacturer[e] for prolonged treatment with eGH, each horse was treated with 5 mg of eGH dissolved in 2 mL of saline solution for the first 2 days and thereafter with 10 mg of eGH dissolved in 4 mL of saline solution. Correspondingly, the control treatment consisted of 2 mL of saline solution for the first 2 days and 4 mL of saline solution thereafter. All treatments were administered IM once daily for a minimum of 11 days (range, 11 to 15 days; mean ± SD, 13 ± 1.7 days). Variation in duration of treatment resulted from attempts to control for effects of reproductive hormones[12] because insulin resistance has been associated with disturbances in the duration of the estrous cycle.[13] Therefore, the administration of eGH or saline solution was started on the day of ovulation in each mare and continued until the dominant follicle of the subsequent estrus reached 35 mm in diameter, which caused variation in duration of eGH or control treatments. The subsequent estrus was induced by IM administration of a prostaglandin $F_{2\alpha}$ analogue (0.375 µg of cloprostenol sodium[f]) on day 6 after the ovulation that coincided with the onset of treatment. The IM injections were rotated among 8 injection sites in each horse (ie, neck, pectoral, gluteal, and semimembranosus-semitendinosus muscles on the left and right side).

The euglycemic-hyperinsulinemic clamp technique was performed 24 to 72 hours after the last dose of eGH or saline solution. The variation in time between end of treatment and onset of testing by use of the clamp technique was primarily caused by the fact that treatment was completed concurrently in some horses and we were unable to perform measurements of all horses 24 hours after completion of treatment. To verify that eGH was still effective in horses in which measurements were performed > 24 hours after completion of treatment, basal serum concentrations of **insulin-like growth factor (IGF)**-1 and basal plasma concentrations of insulin were measured before testing by use of the clamp technique.

Horses were allowed a recovery period of at least 1 estrus cycle between the 2 courses of treatment to avoid possible carryover effects of eGH treatment. Basal **plasma insulin concentration (I)** and basal serum IGF-1 concentration measured before testing by use of the clamp technique in each horse were used to verify that there were no carryover effects.

Euglycemic-hyperinsulinemic clamp technique—In both experiments, in vivo insulin sensitivity was examined by use of the euglycemic-hyperinsulinemic clamp technique described elsewhere.[3] To perform the clamp technique, a catheter was inserted into each jugular vein. One of these catheters was subsequently used for infusion of glucose (50% solution) and insulin,[g] whereas the other catheter was used for collection of blood samples to monitor circulating glucose and insulin concentrations. The first blood sample (time 0) was used to establish the basal glucose concentration. Within 10 minutes after collection of the first blood sample, a priming dose of insulin (45 mU/kg dissolved in 50 mL of saline solution) was administered IV as a bolus injection. This bolus injection of insulin was followed immediately by constant rate infusions of insulin (6 mU/[kg × min])[3] and glucose (mean infusion rate, 8.6 µmol/[kg × min]).[3] Constant rate infusions were accomplished by use of a pump.[h]

Throughout the period of insulin and glucose infusion, blood samples were collected at 10-minute intervals into heparinized syringes. Within 2 minutes after collection, glucose concentration in each sample was measured by use of an automated analyzer.[i] Glucose was maintained at the basal concentration measured in each horse (range, 3.9 to 5.6 mmol/L). When the blood glucose concentration differed from the basal concentration, the glucose infusion rate was adjusted to compensate. A steady-state condition was presumed to exist when the plasma glucose concentration and glucose infusion rate were concurrently held constant for at least 30 minutes and the plasma glucose concentration was within the range for euglycemic values.

Values for I were determined before the clamp technique was started (time 0) and in 3 samples collected at 10-minute intervals during the euglycemic-hyperinsulinemic steady-state condition into lithium-heparinized tubes. These blood samples were centrifuged[j] for 10 minutes at 6,000 × *g*; the resulting plasma was harvested and stored at –20°C until insulin concentrations were analyzed by use of a radioimmunoassay[k] validated for use in samples obtained from horses.[14]

Calculations—For plasma glucose concentrations to exist in a steady-state condition, the amount of glucose infused

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must equal the amount of glucose being removed from the glucose space (ie, **amount of metabolized glucose [M]**), provided that endogenous glucose production is suppressed completely. However, in reality, the plasma glucose concentration is never absolutely constant,[10] and a space correction factor must be included to account for glucose that is added or removed from the glucose space by means other than metabolism. Thus, M during steady-state conditions during use of the clamp technique can be expressed by the following equation[10]:

$$M = INF - SC,$$

where INF is the amount of glucose infused and SC is the space correction factor. The space correction was calculated by use of the following equation[10]:

$$SC = ([G2 - G1] \times GS)/([t2 - t1] \times BW),$$

where G1 and G2 are the glucose concentrations at time points t1 and t2, respectively; GS is the glucose space, which is calculated as 0.19 L/kg × body weight of the horse (in kg); and BW is the body weight of the horse (in kg). In this study, values of M were calculated for 10-minute intervals.

Values for I were calculated for the 3 samples recovered during the steady-state condition. Those concentrations were used to calculate the mean M-to-I ratio, which reflects the quantity of glucose metabolized per unit of plasma insulin and is a good index of tissue sensitivity to exogenous insulin.[10]

In addition, for results of 3 clamp-technique measurements of relatively long duration performed in 3 horses, 2 steady-state conditions were identified. The M and I values for each steady state were measured as described previously.

Analysis of blood samples—For experiment 1, additional blood samples were collected into coagulant-free (ie, serum) tubes[l] before the experiment began and at the beginning of measurements by use of the clamp technique. These samples were used to measure IGF-1 concentrations. In addition, blood samples were collected into lithium-heparinized tubes[m] at the beginning of measurements obtained by use of the clamp technique and at the end of the steady state; these samples were used for analysis of **nonesterified fatty acid (NEFA)** and cortisol concentrations. For experiment 2, blood samples were collected at the beginning of measurements obtained by use of the clamp technique (for use in measuring IGF-1, NEFA, and β-hydroxybutyrate concentrations) and at the end of the steady state (for use in measuring NEFA and β-hydroxybutyrate concentrations).

Assays—Plasma cortisol concentrations were measured by use of a radioimmunoassay[n] validated for use in samples obtained from horses.[14] Serial dilutions of equine plasma with a high cortisol concentration yielded results that were parallel to the standard curve. The addition of measured amounts of cortisol to samples of plasma resulted in a mean ± SD recovery of 101 ± 7%. The detection limit of the assay was 1.1 nmol/L, and the intra-assay and interassay **coefficient of variation (CV)** was 6% and 8%, respectively.[14] Cross-reactivity for the radioimmunoassay (calculated at 50% of the initial binding) included cortisol (100%), 21-deoxycortisol (62%), corticosterone (11%), prednisolone (3%), cortisone (2%), 11-deoxy-cortisol (1.3%), dexamethasone (0.3%), deoxycorticosterone (1.3%), and 17-α-hydroxyprogesterone (0.1%).[14]

Plasma insulin concentration was measured by use of a commercial radioimmunoassay kit[k] that had been validated for use in samples obtained from horses. Curves obtained with serial dilutions were parallel to the standard curve. The detection limit of the assay was 8.6 pmol/L. The intra-assay and interassay CV was 5% and 7%, respectively.[14,15] The insulin antiserum cross-reacted with proinsulin (approx 40%) and had a particularly low cross-reactivity to compounds other than insulin and proinsulin.[14]

Serum IGF-1 concentrations were measured in samples extracted by use of acid ethanol; extraction efficiency was 85% to 90%. Concentrations were measured by use of a radioimmunoassay kit[16] that had been validated for use in samples obtained from horses.[17] Curves obtained with serial dilutions were parallel to the standard curve. The intra-assay and interassay CV was 3.2% and 15.6%, respectively.[16,17]

Plasma NEFA concentrations were measured by use of a commercial kit[o] that had been validated for use in samples obtained from horses. The intra-assay and interassay CV was 7.3% and 16.0%, respectively. Curves obtained with serial dilutions were parallel to the standard curve.

Serum β-hydroxybutyrate concentrations were measured by use of a commercial kit[p] that had been validated for use in samples obtained from horses. The intra-assay and interassay CV was 7.8% and 10.9%, respectively. Curves obtained with serial dilutions were parallel to the standard curve.

Plasma glucose concentrations were analyzed by use of an automated analyzer.[i]

Statistical analyses—All values were recorded as mean ± SD and analyzed by use of 2-way, repeated-measures ANOVAs[q] in which horses, treatment order, and treatment were the variables. Post hoc analysis was performed by use of the Bonferroni test. Paired-sample *t* tests were used to compare values for steady-state conditions reached after short versus long duration of the clamp technique. In all cases, differences were considered significant at values of $P < 0.05$.

Results

Experiment 1—Mean ± SD values for M, I, basal I, the M-to-I ratio, cortisol concentration, and IGF-1 concentrations were determined (**Table 1**). The first hour after onset of the clamp technique is considered an equilibration period; therefore, only steady states achieved at least 60 minutes after starting the insulin infusion were considered useful for comparison. In experiment 1, all clamp tests fulfilled this criterion, with the steady-state condition reached after a mean of 125 ± 55 minutes. No significant between-treatment variations in duration of the clamp technique were found.

Hydrocortisone treatment resulted in mean values for M and the M-to-I ratio that were significantly higher than for the control or eGH treatments. However, there were no significant differences found before onset of testing and at steady state during testing by use of the clamp technique in plasma hydrocortisone concentrations or serum IGF-1 concentrations. Furthermore, treatment order did not significantly affect M or the M-to-I ratio. Mean ± SD value for the space correction factor for the 3 clamp applications was 0.0 ± 0.04 L (mean CV, 1.82 ± 0.55%). Mean plasma NEFA concentrations at the beginning of measurement by use of the clamp technique did not differ significantly among treatments (hydrocortisone, 0.38 ± 0.13 mmol/L; eGH, 0.46 ± 0.08 mmol/L; and saline solution, 0.57 ± 0.17 mmol/L). However, in all groups, mean plasma NEFA concentration during the steady state was significantly lower (hydrocortisone, 0.08 ± 0.01 mmol/L; eGH, 0.08 ± 0.01 mmol/L; and saline solution, 0.08 ± 0.02 mmol/L), compared with the values before use of the clamp technique.

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Table 1—Mean ± SD values for insulin concentrations and glucose metabolism for 5 Standardbreds after IV injection of a single dose of saline (0.9% NaCl) solution, hydrocortisone, or equine growth hormone (eGH) administered 24 hours before measurements were obtained by use of a eu-glycemic-hyperinsulinemic clamp technique.

Variable	Saline solution	Hydrocortisone	eGH
M (mmol/[kg × min])	0.010 ± 0.0026[a]	0.015 ± 0.0047[b]	0.011 ± 0.0026
$I_{steady\ state}$ (pmol/L)	4,079 ± 1,010	4,925 ± 462	3,928 ± 710
M-to-I ratio ($\times 10^{-6}$)	2.5 ± 0.8[c]	3.2 ± 0.9[d]	2.6 ± 1.1
I_{basal} (pmol/L)	37.4 ± 8.9	37.8 ± 19.2	48.4 ± 32.4
$Cortisol_{basal}$ (nmol/L)	171.3 ± 50.9	183.6 ± 17.1	200.0 ± 56.0
$Cortisol_{steady\ state}$ (nmol/L)	202.0 ± 34.1	213.7 ± 38.7	206.0 ± 54.0
IGF-1_{basal} (ng/mL)	222 ± 45	214 ± 47	220 ± 60

M = Amount of metabolized glucose. $I_{steady\ state}$ = Plasma insulin concentration (I) at steady state during testing by use of the euglycemic-hyperinsulinemic clamp technique. I_{basal} = Value for I before onset of testing by use of the euglycemic-hyperinsulinemic clamp technique. $Cortisol_{basal}$ = Plasma cortisol concentration before onset of testing by use of the euglycemic-hyperinsulinemic clamp technique. $Cortisol_{steady\ state}$ = Plasma cortisol concentration at steady state during testing by use of the euglycemic-hyperinsulinemic clamp technique. IGF-1_{basal} = Serum concentration of insulin-like growth factor-1 before onset of testing by use of the euglycemic-hyperinsulinemic clamp technique.
[a–d]Within a row, values with different superscript letters differ significantly ([a,b]$P < 0.001$ and [c,d]$P = 0.009$).

Table 2—Mean ± SD values for metabolic variables for 6 horses after an IM injection of eGH or saline solution administered once daily for 11 to 15 days and tested by use of a euglycemic-hyperinsulinemic clamp technique 24 to 72 hours after the final daily injection.

Variables	Saline solution	eGH	Reference range
M (mmol/[kg × min])	0.019 ± 0.0067	0.016 ± 0.0042	NA
$I_{steady\ state}$ (pmol/L)	2,720 ± 581	2,933 ± 294	NA
M-to-I ratio ($\times 10^{-6}$)	7.3 ± 2.9[a]	5.7 ± 1.6[b]	NA
I_{basal} (pmol/L)	62.0 ± 52.7[a]	163.0 ± 77.7[b]	< 409.0
$NEFA_{basal}$ (mmol/L)	0.78 ± 0.21[A]	0.65 ± 0.11[C]	< 0.66
$NEFA_{steady\ state}$ (mmol/L)	0.11 ± 0.05[B]	0.11 ± 0.05[D]	< 0.66
IGF-1_{basal} (ng/mL)	200 ± 50[a]	420 ± 125[b]	NA
β-$Hydroxybutyrate_{basal}$ (mmol/L)	0.21 ± 0.02[E]	0.16 ± 0.06	< 0.39
β-$Hydroxybutyrate_{steady\ state}$ (mmol/L)	0.09 ± 0.05[F]	0.09 ± 0.03	< 0.39

NA = Not available. $NEFA_{basal}$ = Nonesterified fatty acid concentration before onset of testing by use of the euglycemic-hyperinsulinemic clamp technique. $NEFA_{steady\ state}$ = Nonesterified fatty acid concentration at steady state during testing by use of the euglycemic-hyperinsulinemic clamp technique. β-$Hydroxybutyrate_{basal}$ = β-Hydroxybutyrate concentration before onset of testing by use of the euglycemic-hyperinsulinemic clamp technique. β-$Hydroxybutyrate_{steady\ state}$ = β-Hydroxybutyrate concentration at steady state during testing by use of the euglycemic-hyperinsulinemic clamp technique.
[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$). [A,B]Within a column, values with different superscript letters differ significantly ($P < 0.05$). [C,D]Within a column, values with different superscript letters differ significantly ($P < 0.05$). [E,F]Within a column, values with different superscript letters differ significantly ($P < 0.05$).
See Table 1 for remainder of key.

Experiment 2—Mean ± SD values for M, I, basal I, M-to-I ratio, NEFA concentration, β-hydroxybutyrate concentration, and IGF-1 concentration for mares treated for > 11 days with eGH or saline solution were calculated (**Table 2**). Similar to experiment 1, the first hour after onset of the clamp technique was considered an equilibration period; therefore, only steady states achieved at least 60 minutes after starting the insulin infusion were considered useful for comparison. For experiment 2, all clamp tests fulfilled this criterion, with the steady-state condition reached after a mean of 149 ± 48 minutes. There were no significant between-treatment variations in duration of the clamp technique.

Mean ± SD value for the space correction factor for the 2 clamp applications was 0.0 ± 0.1 L (mean CV, 1.96 ± 0.95%). Long-term administration of eGH led to significant increases in the basal I and basal IGF-1 concentration and to a significant decrease in the mean M-to-I ratio. The horses were randomly allotted to the treatments; however, comparison of the data for horses treated with saline solution first and eGH second with that for horses treated with eGH first and saline solution second revealed a significant ($P < 0.001$) decrease in the M-to-I ratio when saline solution was used as the second treatment. No significant changes were found for M. The NEFA concentrations decreased significantly during testing by use of the clamp technique but did not differ significantly between treatments. A similar pattern was observed for β-hydroxybutyrate concentrations, although the decrease in concentrations from values before onset of the clamp technique to values at the steady-state condition was only significantly different for the control treatment.

Maximum duration of the clamp technique for experiments 1 and 2 was 310 minutes. The maximum duration of the clamp technique that provided values used for the calculations was 280 minutes, which included the steady-state period.

Mean M and the mean M-to-I ratio did not differ significantly between measurements obtained by use of the clamp technique with a short duration (steady-

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state condition achieved after 80, 80, and 120 minutes, respectively), compared with values for measurements obtained by use of the clamp technique with a relatively long duration (260, 280, and 190 minutes, respectively). Mean CV of the plasma glucose concentration of the measurements obtained by use of the clamp technique with a short duration was 2.23 ± 0.40%, whereas the corresponding value for the measurements obtained by use of the clamp technique with a relatively long duration was 2.73 ± 0.91%. Mean I for measurements obtained by use of the clamp technique with a short duration was 3,817 ± 1,014 pmol/L, which did not differ significantly from mean I for measurements obtained by use of the clamp technique with a relatively long duration (4,659 ± 590 pmol/L).

Discussion

Glucocorticoids have many effects on metabolism. Overall, however, their actions on carbohydrate metabolism are considered to be glucose-sparing effects that are typified by increased hepatic glucose production and diminished glucose use by peripheral tissues.[18] For this reason, we expected hydrocortisone administration to result in a decrease in M and the M-to-I ratio, indicating increased insulin resistance and diminished glucose use in peripheral tissues. On the contrary, a single dose of hydrocortisone administered to clinically normal Standardbred horses 24 hours before onset of testing by use of a euglycemic-hyperinsulinemic clamp technique resulted in an increase in glucose metabolism and sensitivity of peripheral tissues to exogenous insulin. This phenomenon of an apparent increase in glucose use as a result of cortisol has been reported in dogs with hyperadrenocorticism[19] and healthy humans.[20] However, despite the fact that absolute glucose use was increased in both of those reports, it was proposed that there was a relative impairment of glucose clearance because the increase was less than would have been expected on the basis of the size of the concurrent increase in circulating glucose concentrations. In short, it appeared that cortisol induced a state of hepatic insulin resistance that led to increased endogenous hepatic glucose production, with the subsequent hyperglycemia triggering hyperinsulinemia.[21] The combination of hyperglycemia and hyperinsulinemia could mask inhibitory effects of cortisol on glucose use by peripheral tissues because hyperglycemia stimulates increased glucose use by tissues and hyperinsulinemia helps overcome insulin resistance in peripheral tissues. In fact, the glucocorticoid-induced increase in insulin secretion in human patients compensates for the anti-insulin effects of the glucocorticoids and thereby helps reestablish euglycemic circulating glucose concentrations and homeostasis. In the study reported here, hyperglycemia or compensatory increases in basal I were not evident after administration of a single dose of hydrocortisone.

It must be kept in mind that insulin concentrations are supraphysiologic during testing by use of the euglycemic-hyperinsulinemic clamp technique. Therefore, it is assumed that endogenous glucose production by the liver and endogenous insulin production by the pancreas are suppressed during the steady state.[10,22] This hyperinsulinemia may have overcome any insulin resistance induced by the liver and therefore may also have suppressed any increase in endogenous glucose production by the liver. Of course, the infused insulin could also have overcome some insulin resistance in the peripheral tissues, but this could not explain the increases in glucose metabolism, compared with results for the other treatments, because the same hyperinsulinemia was induced after each of the other treatments. Thus, it is suggested that increases in glucose metabolism in the study reported here were purely a reflection of an increase in hydrocortisone-induced glucose use in peripheral tissues.

In GH-deficient humans, treatment with GH is associated with insulin resistance,[23] and GH-treated patients have an increase in the incidence of type 2 diabetes mellitus.[24] In horses, treatment with GH also is associated with hyperglycemia, hyperinsulinemia, and insulin resistance.[25] For this reason, it was expected that eGH would induce insulin resistance in peripheral tissues of horses in both experiments. In the study reported here, a single dose of eGH did not induce insulin resistance because values for M and the M-to-I ratio did not differ from those for horses when administered the control treatment. In addition, serum IGF-1 concentrations and I measured before use of the clamp technique did not differ significantly from corresponding values when the horses received the control treatment. However, after long-term eGH administration, basal I and basal serum IGF-1 concentrations were significantly increased, which indicated that long-term eGH administration had a measurable biological effect. In addition, the M-to-I ratio decreased significantly, which indicated a decrease in sensitivity of peripheral tissues to exogenous insulin. However, the absolute M was not influenced significantly by long-term eGH administration, possibly because the induced hyperinsulinemia during testing by use of the euglycemic-hyperinsulinemic clamp technique was sufficient to compensate for insulin insensitivity in the peripheral tissues. This hypothesis could be examined further by use of the hyperglycemic clamp technique (ie, hyperglycemia is induced by infusion of glucose solution) to allow measurement of pancreatic sensitivity to exogenous glucose and determine the rate of glucose metabolism when there is a lack of extreme hyperinsulinemia.

In experiment 2, there was a significant decrease in the M-to-I ratio when eGH was administered as the first treatment and saline solution was administered as the second treatment. Consequently, the effect of long-term eGH treatment obviously persisted throughout 1 estrous cycle in association with I and IGF-1 concentrations within the reference range, which illustrated the sensitivity of the euglycemic-hyperinsulinemic clamp technique. As a result, it can be suggested that the effect on tissue sensitivity may persist at the receptor or postreceptor level for at least 28 days. Additional studies will be needed to test this hypothesis.

Hyperinsulinemia that lasts more than 5 to 7 hours may enhance insulin action in itself, which could be expressed by an increase in the rate of glucose infusion.[26] However, no significant differences were found

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when mean values for M and the M-to-I ratio were compared during 2 steady-state conditions during the same test in 3 horses. This corresponds with the findings of a study[26] in humans in which investigators reported that a plateau for the glucose infusion rate at a steady state was reached between 5 and 7 hours after onset of the clamp technique at approximately 30% above the glucose infusion rate reported during the same test at only 2 hours after starting the infusions. In the study reported here, duration of the longest test by use of the clamp technique was 280 minutes, which included the duration of the steady state.

Uptake and degradation of insulin is a feature of all insulin-sensitive tissues. After the liver and kidneys, muscles play the major role in insulin removal. Removal of insulin from the circulation does not imply immediate destruction of the hormone. A substantial amount of receptor-bound insulin is released from cells and reenters the circulation. Insulin clearance rates are decreased in patients that are obese or diabetic or have increases in other hormones such as catecholamines and GH.[27] The I measured during steady-state conditions revealed an assessment of insulin clearance in horses, which may serve as an interesting basis for future studies.

Cortisol and GH both influence lipid metabolism by stimulating lipolysis,[28,29] and both trigger an increase in plasma concentrations of free fatty acids. Typically, insulin inhibits this hormone-induced lipolysis by stimulating an increase in activity of lipoprotein lipase and decreasing activity of hormone-sensitive lipase; this combination eventually clears the plasma of triglycerides. Withholding of food also enhances the rapid release of free fatty acids from adipose tissue into the circulation via the activation of hormone-sensitive lipase. Therefore, it was expected that the administration of eGH or hydrocortisone followed by a 12-hour period of food withholding would result in a higher mean plasma NEFA concentration before testing by use of the clamp technique, compared with the concentration recorded when horses received the control treatment. However, in both experiments, mean plasma NEFA concentrations did not differ significantly among hydrocortisone, eGH, or saline solution treatments. In experiment 2, this could be explained by inhibition of lipolysis as a result of secondary hyperinsulinemia induced by long-term administration of eGH.

In experiment 1, a single dose of eGH did not induce an increase in NEFA or IGF-1 concentrations or substantially increase hyperinsulinemia, which suggested that a single dose of eGH is not sufficient to induce a measurable biological effect. The failure of administration of a single dose of hydrocortisone to induce an increase in NEFA concentrations indicative of lipolysis has been described elsewhere.[28] Finally, the decrease in mean plasma NEFA and mean serum β-hydroxybutyrate concentrations following the onset of infusions during the clamp technique in both experiments was presumably a result of the antilipolytic action of the infused insulin, as has been described elsewhere.[a]

In the study reported here, we determined that a single dose of hydrocortisone in Standardbreds increased glucose metabolism and increased insulin sensitivity of peripheral tissues to exogenous insulin, whereas a single dose of eGH did not affect either of those variables. In contrast, long-term eGH administration increased basal I and basal IGF-1 concentrations and decreased tissue sensitivity to exogenous insulin without increasing plasma concentrations of NEFA and β-hydroxybutyrate. Furthermore, use of the euglycemic-hyperinsulinemic clamp technique appeared to suppress lipolysis and ketogenesis, which was reflected in decreases in plasma concentrations of NEFA and β-hydroxybutyrate. These findings could help investigators identify underlying mechanisms for disorders associated with insulin resistance and aid in the development of appropriate treatments for animals with these disorders (eg, horses with laminitis).

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e. EquiGen (10 mg eST), BresaGen, Adelaide, Australia.
f. Estrumate, Schering-Plough Animal Health, Brussels, Belgium.
g. Actrapid recombinant human insulin (100 U/mL), Novo Nordisk A/S, Bagsvaerd, Denmark.
h. Volumetric pump, model Argus 414, Argus Medical AG, Heimberg, Switzerland.
i. ABL-605, Radiometer Copenhagen, Westlake, Ohio.
j. Rotina 48R, Hettich zentrifugen, Tuttlingen, Germany.
k. Coat-A-Count TKIN2 836, Diagnostic Products Corp, Los Angeles, Calif.
l. Vacuette, Z serum separator clot activator, Greiner Bio-One GmbH, Kremsmünster, Austria.
m. Vacuette, lithium heparin, Greiner Bio-One GmbH, Kremsmünster, Austria.
n. Coat-A-Count, Diagnostic Products Corp, Los Angeles, Calif.
o. Randox kit NEFA, FA 115, Randox Laboratories Ltd, Antrim, UK.
p. Randox kit Ranbut, RB1007, Randox Laboratories Ltd, Antrim, UK.
q. SPSS, version 10.0 for Windows, SPSS Inc, Chicago, Ill.

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